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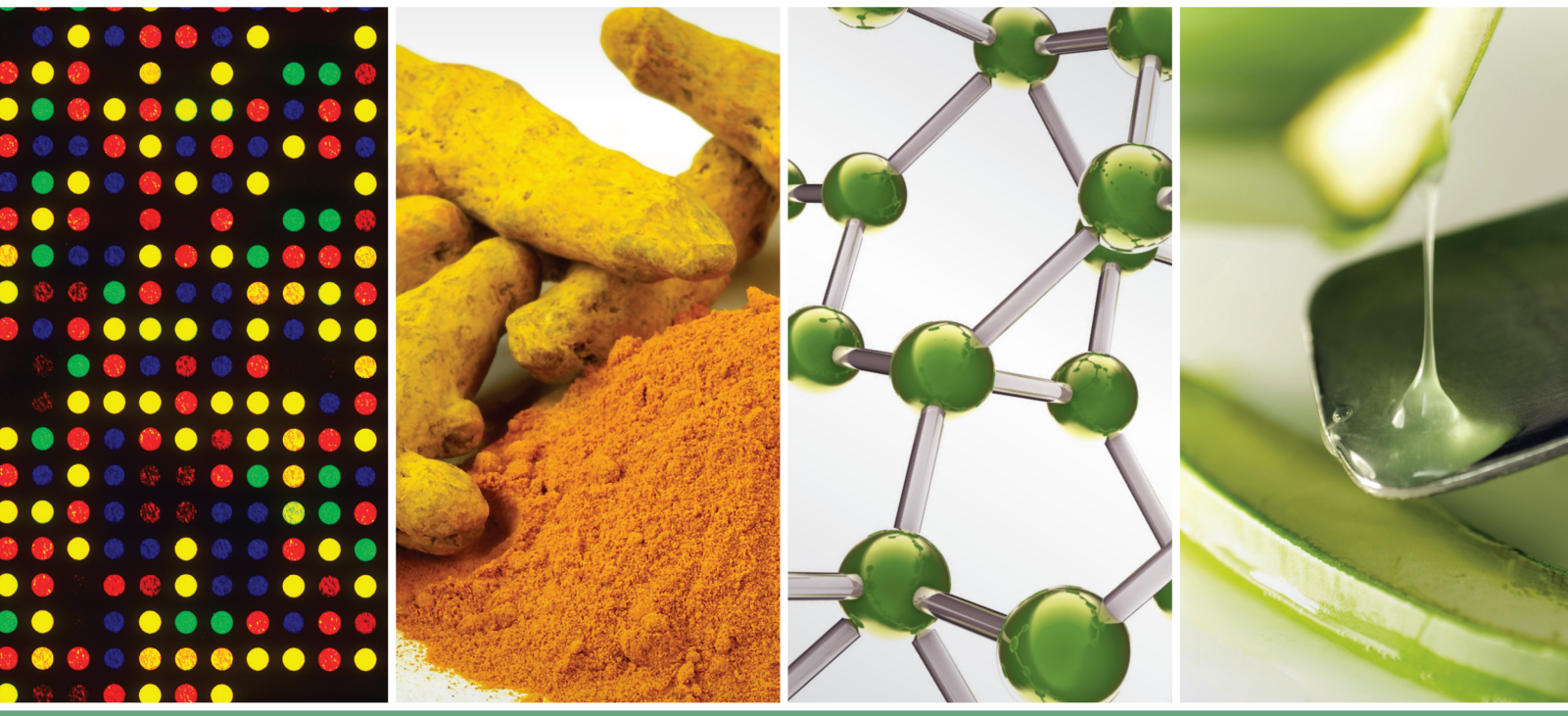
The Potential Benefit of Complementary/Alternative Medicine in Cardiovascular Diseases

Guest Editors: Ke-ji Chen, Ka Kit Hui, Myeong Soo Lee, and Hao Xu



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Editorial

The Potential Benefit of Complementary/Alternative Medicine in Cardiovascular Diseases

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Cardiovascular diseases (CVDs) prevalence continues to increase, and it is still the number one killer so far. In 2002, nearly 17 million deaths all over the world were attributable to CVDs, which accounted for almost 30% of the total deaths. Despite treatment with percutaneous coronary intervention (PCI) and many other conventional medicines, CVDs patients are still confronted with certain risk of recurrent acute cardiovascular events, readmission to the hospital, and unfavorable quality of life. In recent years, more and more clinicians have successfully applied complementary/alternative medicine (CAM) in CVDs prevention and treatment based on standardized conventional therapy. Nevertheless, the role of CAM in CVDs still needs more clinical evidence and definite mechanism of actions.

In this issue, a collection of several original research articles and reviews are presented that address the clinical application and the mechanism of action of CAM in the treatment of CVDs. These works were submitted by researchers from different parts of the world, including China, Japan, South Korea, Australia, and Sweden. In these studies, the effectiveness of Chinese medicine and some other alternative therapeutic methods in improving symptoms was demonstrated in patients with hypertension, chronic stable coronary artery disease, chronic heart failure, and so forth. Specifically, the use of Chinese herbal medicines was reviewed for the prevention of in-stent coronary restenosis after PCI. The study of Tanshinone IIA, a diterpene quinine extracted from the root of salvia miltiorrhiza, a Chinese traditional herb, was presented as a promising cardioprotective agent. The positive effect of Chinese food and herbal medicines in improving certain moderate dyslipidemias was described. The usefulness of Xuezhikang, an extract from Red Yeast Rice, was reviewed in the treatment of coronary heart disease complicated by dyslipidemia. A pharmacological and mechanistic study showed Naoxintong's effect on cytochrome P450 2C19. Further, one study showed the effect of berberine on improving insulin sensitivity by inhibiting fat store and adjusting adipokines profile in human preadipocytes and metabolic syndrome patients.

In the authors' opinion, the clinical research of Chinese medicine and other CAMs for CVDs still faces some major challenges. Issues such as overall quality of medical service and the unmet medical needs in the contemporary society are common to these medicines. A general guideline is required for practicing Chinese medicine and other CAMs, which should be developed based on solid evidence from well-designed and well-executed clinical studies. Such is the direction that the research of Chinese Medicine and other CAM should follow.

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Research Article

Impact of Transcendental Meditation on Left Ventricular Mass in African American Adolescents

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Background. An early sign of ventricular remodeling is increased left ventricular mass (LVM) which over time may lead to left ventricular hypertrophy, the strongest predictor of cardiovascular morbidity and mortality, other than advancing age. *Methods*. 62 (30 TM; 32 CTL) African American adolescents (age 16.2 ± 1.3 years) with high normal systolic BP were randomly assigned to either 4-month Transcendental Meditation (TM) or health education control groups. The echocardiographic-derived measure of LVM index (LVMI = LVM/ht$^{2.7}$) was measured before and after the 4-month TM study and at 4-month followup. 2D-guided M-mode echocardiography using a Hewlett Packard 5500 echosonograph was used to determine LVMI. *Results*. The TM group exhibited a greater decrease in LVMI at 4-month followup compared to the CTL group (−2.6 versus +0.3 gm/ht$^{2.7}$, $P < 0.04$). The TM group exhibited a lesser increase in BMI at 4-month follow-up compared to the CTL group (0.2 ± 1.6 versus 1.1 ± 1.4, $P < 0.03$). *Conclusion*. These findings indicate that among a group of prehypertensive African American adolescents, 4 months of TM compared to heath education resulted in a significant decrease in LVMI, and these changes were maintained at 4-month follow-up.

1. Introduction

Increased left ventricular mass (LVM) has long been known to increase the risk for coronary artery disease (CAD), congestive heart failure, stroke, cardiac arrhythmias, and sudden death [1, 2]. Left ventricular mass may be reduced with BP reduction [3], and findings also suggest that lifestyle changes, such as moderate sodium restriction [4] as well as pharmacologic therapy [5], decrease LVM in both youth and adults. Longitudinal studies in sedentary subjects suggest exercise results in enlargement of LVM following training [6]. Prehypertension is associated with increased LVM in adolescents and young adults [7], and resting heart rate, systolic BP, gender, hemodynamic responses to stress, and adiposity were seen to be early determinants of LVM in children [8]. Other factors that can strain the workload on the heart are cardiovascular reactivity [9] and chronic stress (prolonged hyperactivation of the sympathetic nervous system) which favor increase in LVM [10].

Studies have shown that Transcendental Meditation (TM) lowers indicators of psychosocial stress such as anger, hostility, and depression [11, 12]. TM has shown promise as a method for prevention and treatment of CVD and reducing CVD risk [13]. Among hypertensive patients, TM compared to standard treatment or health education results in greater reductions in systolic (SBP) and diastolic blood pressures (DBPs) [14, 15] and reduces CV mortality [16, 17]. In a prospective, single-blind, controlled study, Zamarra et al. reported that the TM program was useful in reducing exercise-induced myocardial ischemia in patients with coronary artery disease [18]. Castillo-Richmond et al. found that over a period of six to nine months among a group of hypertensive African American (AA) adults, the TM program resulted in significant reduction in carotid intima-medial thickness (IMT) compared to a slight increase in the control group [19]. Another study of long-term TM practitioners showed acute reductions in BP during TM which were suggested to be a result of reductions in

vasomotor tone (i.e., total peripheral resistance, a possible mechanism for BP reduction) [20]. These findings in adults have important implications for inclusion of TM in efforts to prevent and treat cardiovascular diseases and its clinical consequences [21] and have extended into the prehypertensive adolescent population, with TM decreasing resting SBP [22], ambulatory BP [23], and heart rate (HR) and cardiac output reactivity to behavioral stressors [22].

TM was shown to reduce LVM in a sample of hypertensive AA adults [24]. In another hypertensive AA sample ($N = 102$), TM practice did not change LVM after 7 months, whereas health education controls showed a significant increase, with a between-groups change score of 5.6 g/m^2 [25]. These findings warrant further research to study the impact of meditation on LVM. To date, the impact of TM upon LVM in prehypertensive youth has not been examined. The present study examined the impact of TM on LVM in African American youth at increased risk for development of CVD. Collectively, based on the findings that TM beneficially lowers BP [23], we hypothesized that TM would decrease LVM.

2. Methods

2.1. Subjects. Voluntary BP screenings were conducted on approximately 5000 African American youth at five inner-city high schools in Augusta, GA. Parents were notified in advance of the health screening via a memo sent home by the school principal which resulted in a high rate of participation (99%). One hundred fifty-six subjects found to exhibit resting SBP in the ≥85th and ≤95th percentile for their age, sex, and height [26] on three consecutive days were invited to participate in the study on the basis of having high normal BP. Exclusion criteria included resting SBP >95th percentile, current involvement in a health promotion program, unwillingness to accept randomization into either study group, self-reported pregnancy, parental report of subject's history of congenital heart defect, diabetes, any chronic illness that required regular pharmacological intervention, or use of medications that may affect BP. 156 subjects were pretested following informed written consent and were randomly assigned to either 4-month TM or health education control (CTL) groups. The study was conducted over 4 years (eight 5-month semesters) with 4-month interventions (TM and CTL) each semester. Order of interventions was randomly counterbalanced between schools. 141 completed the posttest and 110 completed the 4-month follow-up evaluations (see Figure 1).

Data were missing for 48 subjects who had moved or otherwise could not be scheduled or due to technical difficulties (e.g., for 16 subjects, there were technical difficulties, that is, poor echocardiographic images, videotape problems, and subjects who could not be measured due to obesity) leaving 62 subjects for the analyses. Thirty TM (21 male subjects) and 32 CTL (24 male subjects) with data for all three visits were used in the final analyses.

2.2. Procedures. All subjects abstained from exercise, smoking, and caffeinated beverages for 5 hours prior to testing.

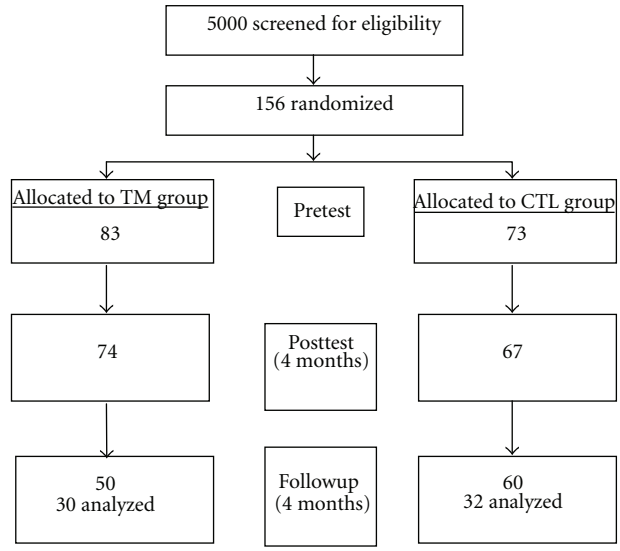

Figure 1: Flow of study recruitment.

Height (with a stadiometer), weight (with a Detecto scale, Cardinal Scale Co, Webb City, MO), and skinfolds (triceps, subscapular and suprailiac crest, which served as surrogate indicators of changes in diet and/or physical activity) were recorded using established protocols [27] at pretest, posttest, and 4-month followup. Skinfolds were measured three times on the right side of the body with Lange calipers, and the readings were averaged. From these primary measures, the sum of the three skinfolds was calculated as a measure of body fat and body mass index (BMI; weight/height^2) as a measure of general adiposity. After anthropometric measurements were obtained, supine resting hemodynamics were evaluated during minutes 10, 12, and 14. An appropriately sized blood pressure (BP) cuff was placed on the right arm for measurement of SBP, diastolic BP (DBP), and heart rate (HR) using a Dinamap Vital Signs Monitor (Model 1846SX, Critikon Incorporated, Tampa, FL).

The echocardiographic-derived measure of LVM was obtained using 2D-guided M-mode echocardiography with a Hewlett Packard 5500. M-mode was used to determine interventricular septal thickness, LV cavity dimension, and LV free (posterior) wall thickness in triplicate in accordance with the American Society of Echocardiography (ASE) convention as described by Devereux et al. [28]. LVM was determined using the following validated formula: $\text{LVM} = 0.8\ [1.04\times((\text{IVS}_\text{d} + \text{LVED}_\text{d} + \text{LVPW}_\text{d})^3 - \text{LVED}_\text{d}^3)] + 0.6$. LVM values were taken at the same time of day at pretest, posttest, and at 4-month follow-up. Technicians collecting the data were blinded as to study group affiliation. LVM was indexed to height$^{2.7}$ ($\text{LVMI} = \text{LVM}/\text{ht}^{2.7}$).

2.3. Interventions. The TM technique is a simple mental procedure practiced for 15 minutes while sitting comfortably with eyes closed [29]. During the TM technique, it has been reported that the ordinary thinking process settles down, and a distinctive "wakeful hypometabolic state" is gained [30]. The format of instruction in the standard TM course

includes introductory and preparatory sessions to outline the benefits and mechanics of the TM technique, a brief personal interview, a session of personal instruction, and three follow-up group sessions taking place over three consecutive days [31]. In the present study, after personal instruction, the TM group engaged in 15 min individual sessions at home and 15 min group sessions at school each school day, and 15 min twice daily individual home practice on weekends for 4 months. Daily group TM sessions were fitted into the school schedule without adversely impacting the academic schedule, for example, at the beginning of the school day during the "homeroom" period in a separate and secluded quiet location. Subjects were encouraged to continue daily home TM practice after the 4-month intervention was completed, but were not allowed to continue group sessions at school.

The control (CTL) group was presented a 4-month didactic series of 15 min lifestyle education sessions each day based in part on National Institutes of Health guidelines on lowering BP through weight management, diet (increasing fruit and vegetable consumption and reducing caloric, fat and sodium intake), and increasing physical activity [26]. Identification of major sources of high-calorie foods and making appropriate substitutions were covered. Participants learned the value of mild-to-moderate intensity of daily physical activity. In order to control for bias, the same instructor was used for the CTL and TM group sessions. CTL group sessions were intended to provide instruction time and attention comparable to the TM group, but CTL group did not receive instructions for any stress reduction or relaxation techniques. Attendance was taken for all TM and CTL sessions at school, and self-report compliance records were kept for TM practice at home.

2.4. Data Analysis. The statistical analysis was conducted on the SAS software package for analysis of variance and covariance (ANOVA/MANOVA) [32]. Differences between treatment groups in change in LVMI and other CV variables over the 8-month intervention period were assessed by MANCOVA using change scores in LVMI, covarying for pretest LVMI. The potential effects of SBP, DBP, HR, and BMI, on change in LVMI, were studied by entering them as covariates. Changes in secondary outcome variables, SBP, DBP, HR, and BMI, were assessed the same way as LVMI, that is, by ANCOVAs using the pretest levels of each variable as a covariate, and by paired t-tests to assess changes within groups. All statistical tests were two tailed.

3. Results

3.1. Anthropometric Measures. There were no statistical significant differences between treatment groups for preintervention anthropometric and hemodynamic parameters (all Ps > 0.05, see Table 1). The TM group showed a trend for higher baseline heart rate (HR, $P = 0.053$). The compliance rate for twice daily practice in the TM group at home and at school was 77%.

3.2. Changes in LVM and LVMI. The changes at posttest were not statistically significant. The TM group exhibited a greater decrease in LVM from baseline to the 4-month follow-up compared to the CTL group (-9.1 ± 20.2 versus 2.6 ± 22.7 gm, $P < 0.04$, see Table 2). The TM group exhibited a greater decrease in LVMI (LVM indexed by height$^{2.7}$) from baseline to the 4-month follow-up compared to the CTL group (-2.7 ± 4.9 versus $+0.3 \pm 5.2$ gm/ht$^{2.7}$, $P < 0.03$, see Figure 2). Pearson correlations between change in LVMI and change in SBP were 0.16 for TM and 0.12 for CTL at posttest, and 0.02 for TM and 0.05 at follow-up (all P = ns).

Table 1: Descriptive characteristics at pretest[a].

	TM ($n = 30$; 9 F, 21 M)	CTL ($n = 32$; 8F, 24 M)
Age (years)	15.7 ± 1.3	16.0 ± 1.3
Weight (kg)	79.3 ± 23.0	80.3 ± 21.1
Height (cm)	170.0 ± 9.2	171.9 ± 7.8
Body mass index (kg/m^2)	27.09 ± 6.2	27.13 ± 6.9
SBP (mmHg)	128.0 ± 9.5	124.0 ± 12.7
DBP (mmHg)	64.7 ± 7.6	61.7 ± 9.4
HR (bpm)	69.6 ± 11.2	64.5 ± 9.1*
Sum of 3 skinfolds	55.72 ± 26.9	64.0 ± 35.3
LVM (gm)	137.7 ± 40.6	142.6 ± 37.2
LVMI	32.4 ± 7.4	33.2 ± 8.7

[a]Values are means ± standard deviations. *P value <0.06.
SBP = supine resting systolic blood pressure, DBP = supine resting diastolic blood pressure. HR = supine resting heart rate. LVM = left ventricular mass. LVMI = LVM indexed by height$^{2.7}$.

3.3. Changes in BMI and Body Weight. There were no significant differences between the groups in changes across the 8-month study for sum of three skinfolds ($P > 0.05$). The TM group exhibited a decrease in BMI at posttest compared and increase in the CTL group (–0.05 ± 1.0 versus 0.9 ± 1.2, $P = 0.001$, see Table 2). The TM group exhibited a lesser increase in body weight at the posttest compared to the CTL group (0.5 ± 3.0 versus 3.8 ± 4.5, $P < 0.05$). The TM group exhibited a lesser increase in BMI at 4-month follow-up compared to the CTL group (0.2 ± 1.6 versus 1.1 ± 1.4, $P < 0.03$, see Table 2). The TM group exhibited a lesser increase in body weight at the 4-month follow-up compared to the CTL group (1.5 ± 4.6 versus 3.2 ± 3.9, $P = 0.004$). Pearson correlations between change in LVMI and change in BMI were –0.08 for TM and 0.09 for CTL at posttest, and 0.10 for TM and 0.17 at follow-up (all P = ns).

4. Discussion

This study examined the impact of the TM technique on LVM in AA adolescents with high normal BP after the 4-month intervention and at 4-month followup. The TM group exhibited a significant decrease in LVM at the 4-month follow-up compared to the CTL group. This supports a previous finding with adult hypertensive AAs that showed an LVM decrease at 12 months in the TM group [24]. In addition, the TM group showed greater control of body weight, that is, blunted the expected normal

Table 2: Comparison of changes from baseline to 4-month posttest and 4-month follow-up[b].

	(4-month posttest)	(4-month follow-up)	(4-month posttest)	(4-month follow-up)	P value
	TM		CTL		
SBP	−4.7 ± 8.5	−2.7 ± 11.0	0.8 ± 8.0	−0.03 ± 11.9	0.37
DBP	−0.05 ± 7.8	0.08 ± 9.3	1.1 ± 6.4	1.6 ± 7.9	0.50
HR	−4.2 ± 10.8	−4.5 ± 10.6	−2.3 ± 6.0	−2.8 ± 6.6	0.44
LVM	−3.2 ± 20.2	−9.1 ± 20.2	1.4 ± 18.6	2.6 ± 22.7	0.036
LVMI	−1.1 ± 4.6	−2.7 ± 4.9	0.1 ± 4.3	0.3 ± 5.2	0.024
Weight	0.5 ± 3.0	1.5 ± 4.6	3.2 ± 3.9	3.8 ± 4.5	0.047
BMI	−0.1 ± 1.0	0.2 ± 1.6	0.9 ± 1.2	1.1 ± 1.4	0.028
Sum3SF	0.03 ± 0.2	0.04 ± 11.6	0.06 ± 0.3	−3.34 ± 12.3	0.435

[b]Change scores from baseline ± standard deviation. SBP = supine resting systolic blood pressure (mmHg), DBP = supine resting diastolic blood pressure (mmHg). HR = supine resting heart rate (bpm). LVM = left ventricular mass (gm). LVMI = LVM indexed by $ht^{2.7}$. Weight (kg). BMI = body mass index. Sum 3SF = sum of three skinfolds.

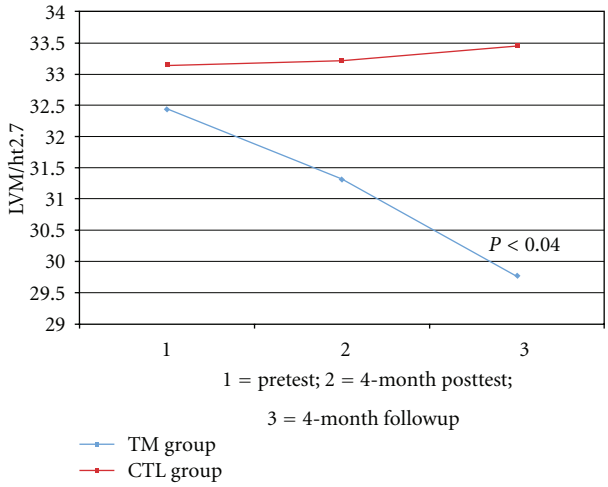

Figure 2: Changes in left ventricular mass index.

body weight/BMI growth rate, after formal cessation of the intervention, and the difference between the two groups was significant at the 4-month followup.

The underlying physiological mechanisms responsible for LVMI reduction are not completely understood but may be related to BP reduction [23]. Stress reactivity has been associated with LVM increase two years later in adolescents [33]. Mental stress is associated with increased CV risk, because of the activation of sympathetic nervous system and the renin-angiotensin-aldosterone system. TM has been found to be associated with reduced BP reactivity to behavioral stress [22] related to reduced sympathetic nervous system tone [21]. TM has also been shown to have several beneficial hormonal and endocrine effects related to decreased sympathetic nervous system stimulation [34, 35] and decreased hypothalamic-pituitary-adrenocortical axis dysregulation [36, 37], as well as decreased cortisol levels [36], and diminished beta-adrenergic receptor sensitivity [38].

The current findings should be interpreted cautiously due to the relatively small sample size. Loss to follow-up was not unexpected in this type of study, and this loss was similar for each group. Impact on the LV cavity or walls was not measured. The subjects were not screened for elevated LVM as a basis for study entry but rather were screened for elevated BP. Blinding of the sonographers to subjects' group classifications further decreased likelihood of any systematic bias in the measurements. The intervention was well received by the participants, and there were no adverse effects reported. Findings for BP changes [23] and beneficial impact upon measures of school behavior, that is, absenteeism, rule infractions, and suspension rates in the current study sample, have been published previously [39]. The TM group exhibited significantly greater decreases in ambulatory daytime systolic BP compared with little or no change in the CTL group across the 8-month study [23]. Future research should provide more precise information on changes in LV geometrical patterns linking BP and BMI [40].

5. Conclusion

To our knowledge, this is the first randomized, controlled study to demonstrate a decrease in LVM via a meditation program in prehypertensive adolescents. If this improvement is replicated among other at-risk groups and in cohorts of CVD patients, this will have important implications for inclusion of TM in the efforts to prevent and treat CVD and its clinical consequences. TM treatment may have therapeutic benefit for CVD patients and may impact favorably on prevention of vascular and myocardial complications of CVD. The decreases in LVM observed in this study, if maintained over time, have potentially important clinical significance. The successful implementation of the intervention points to the potential of school-based stress reduction programs as a means of decreasing likelihood of early onset of LVH in high-risk youth, particularly AAs.

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Review Article

Chinese Medicine Injection Qingkailing for Treatment of Acute Ischemia Stroke: A Systematic Review of Randomized Controlled Trials

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Qingkailing (QKL) injection was a famous traditional Chinese patent medicine, which was extensively used to treat the acute stages of cerebrovascular disease. The aim of this study was to assess the quantity, quality and overall strength of the evidence on QKL in the treatment of acute ischemic stroke. *Methods*. An extensive search was performed within MEDLINE, Cochrane, CNKI, Vip and Wan-Fang up to November 2011. Randomized controlled trails (RCTs) on QKL for treatment of acute stroke were collected, irrespective of languages. Study selection, data extraction, quality assessment, and data analyses were conducted according to the Cochrane standards, and RevMan5 was used for data analysis. *Results*. 7 RCTs (545 patients) were included and the methodological quality was evaluated as generally low. The pooled results showed that QKL combined with conventional treatment was more effective in effect rate, and the score of MESSS and TNF-α level compared with conventional treatment alone, but there was no significant difference in mortality of two groups. Only one trial reported routine life status. There were four trials reported adverse events, and no obvious adverse event occurred in three trials while one reported adverse events described as eruption and dizziness.

1. Introduction

Stroke is a major cause of death and disability in the world and the most important strategy for treatment of ischemic stroke is prevention and effective therapy. In China, acupuncture and traditional Chinese medicine (TCM) have been used to treat stroke patent medicine or a history over 2000 years. In recent decades, patent medicines of TCM were widely and regularly used in stroke patients in either Western medicine hospitals or traditional Chinese medicine hospitals. However, few studies have been published in English reporting the effectiveness and safety of many commonly used TCM [1, 2]. Currently, there is yet no routine effective, generally accepted, specific treatment for ischemic stroke, except for thrombolytic treatment for highly selected patients. Therefore, confirmation of the effectiveness of TCM could have a great impact on stroke management in the world.

Qingkailing (QKL) injection was originally prepared by the Beijing University of Chinese Medicine in the 1970s, by modifying a traditional Chinese medicine, Angongniuhuang pills, composed of *Radix Isatidis*, *Flos Lonicerae*, *Concha Margaritifera Usta*, baicalin, *Fructus gardeniae*, cholic acid, hyodeoxycholic acid, and *Cornu Bubali* [3]. It has been extensively used to treat the acute stages of cerebrovascular disease and has performed excellently in improving neurological function [4]. Animal experiments have shown that QKL injection can promote endothelial nitric oxide synthase expression, reduce calcium overload, regulate matrix metalloproteinase-9 expression, and inhibit inflammation in a murine model of cerebral ischemia/reperfusion [5–8]. Several clinical studies reported the effectiveness ranging from case reports and randomized clinical trials, but the evidence for its effect was not clear.

The aim of this study was to assess the quantity, quality and overall strength of the evidence on QKL in the treatment of acute ischemic stroke.

2. Methods

2.1. Database and Search Strategies. The electronic databases of MEDLINE (1982–2011), Cochrane Controlled Trials

Register ((Issue 10, 2011)), CNKI database (1974–2011), Vip database (1989–2011), and Wan-Fang database (1998–2011) were searched by using a combination of MESH subject headings of "Qingkailing" and "Stroke" or "cerebral infarction" without language limitation. Reference lists from trials selected by electronic searching and conference compilations were hand searched. All of those searches ended before November 2011.

2.2. Inclusion Criteria. Definite or possible randomized controlled trials (RCTs) were included. RCTs combined QKL injection with conventional treatment with the conventional treatment alone, and QKL injection plus conventional treatment compared with a western medicine plus conventional treatment were included. While the trials with other TCM used either in QKL or control groups were excluded. There were no restrictions on population characteristics, language, and publication type.

Trials that included patients of any age or sex with acute ischemic stroke were eligible. Ischemic stroke was defined if the patients met the World Health Organization or the similar Chinese National criteria (demanding a CT/MRI scan as confirmation) of stroke, and hemorrhagic stroke was excluded. Possible ischemic stroke in which CT/MRI were not performed was also to be included.

The primary outcome measures were death and effect rate (which was based on the neurological deficit improvement), and adverse events. The secondary outcome measures were neurological deficit scoring, infarction volume of CT, inflammation factors, and quality of life.

2.3. Data Extraction and Quality Assessment. The data was entered into an electronic database by the two reviewers (F. F. Cheng and X. Q. Wang) independently, in the case where the two entries did not match, and a third person (Y. Lu) may be involved for verification. Quality assessment of included randomized controlled trials: sequence generation, allocation concealment, blinding of participants personnel and outcome assessors, incomplete outcome data, selective outcome reporting, and other sources of bias [9].

2.4. Data Synthesis. The statistical package (RevMan 5) was used for data analyses, which was provided by the Cochrane Collaboration. Dichotomous data were presented as risk ratio (RR) and continuous outcomes as mean difference (MD), both with 95% confidence interval (CI). Heterogeneity between trials results was tested, and heterogeneity was presented as significant when I^2 is over 50% or $P < 0.1$. Random effect model was used for the meta-analysis if there was significant heterogeneity and fixed effect model was used when the heterogeneity was not significant [9]. Publication bias was explored via a funnel-plot analysis.

3. Result

3.1. Description of Included Trials. After primary search of 5 databases, 254 trials were screened out from electronic and manual searches (Figure 1), and the majority were excluded due to being found from more than one database or obvious ineligibility which including irrelevant titles and abstract. 6 trials were excluded because of duplicated publication, 62 trials were excluded due to the animal studies, the rest 41 trials were noncontrolled clinical trials including case report, case series, or review. 145 full text papers were retrieved and 138 trials were excluded based on the inclusion criteria. The most frequent reasons for exclusion were other types of intervention examined (78), either no proper outcomes reported (29), or not a randomized trial (24), while 7 RCTs including cerebral hemorrhage were excluded. In the end, 7 RCTs (545 participants) [10–16] were reviewed. All studies were published in Chinese. The bibliographic details of these trials are given in Table 1.

The age of patients in the included studies ranged from 33 to 78 years old. All trials included more males (60% to 70%) than females. All included trials applied standard medicine diagnostic criteria for ischemia stroke, and all of them reported the need for all patients to have had CT/MRI scanning to confirm the diagnosis. Only QKL injection was studied in these included trials. The dose range of QKL injection was from 30 mL to 80 mL and dose route was intravenous injection in all included studies. Most of included trials were designed to compared QKL injection plus conventional treatment with the conventional treatment alone, the later including basic therapy, and urokinase, and only one trail [10] compared QKL injection plus conventional treatment with nimodipine plus conventional treatment (Table 1). None of the trials was randomized, double-blind, placebo controlled. The timing of the start of treatment after stroke onset was within 72 hours, and the total duration of treatment varied from 7 to 20 days.

All the including trials reported the effect rate, but only two mentioned the death rate. Only one trial [11] had undergone assessment of patients' routine living ability after treatment. Three trials [11–13] reported neurological deficit, using the Modified Edinburgh-Scandinavian Stroke Scale (MESSS), which was recommended at the Second and revised at the Fourth National Cerebrovascular Diseases Conference in China. And one trial [12] measured infarction volume of CT. Tan et al. [10] and Wu et al. [14] reported inflammation factor in blood serum, both including TNF-α and IL-6. Yang et al. [12] reported the effect of QKL injection in treating coma induced by stroke.

3.2. Methodological Quality of Included Trials. The methodological quality of most included trials was generally "poor." The sample size of including trials varied from 40 to 150 patients. None of the 26 trials reported sample size calculation. None of the trials was properly randomized, double blinded, placebo controlled, and no trials employed a blinding procedure, yet only one trial reported the method of randomization (random number table) [10]. None of the trials had grade A level of adequate concealment of randomization. None of the trials reported the number of patients that were lost to followup and whether they had used intention-to-treat analysis (Table 2).

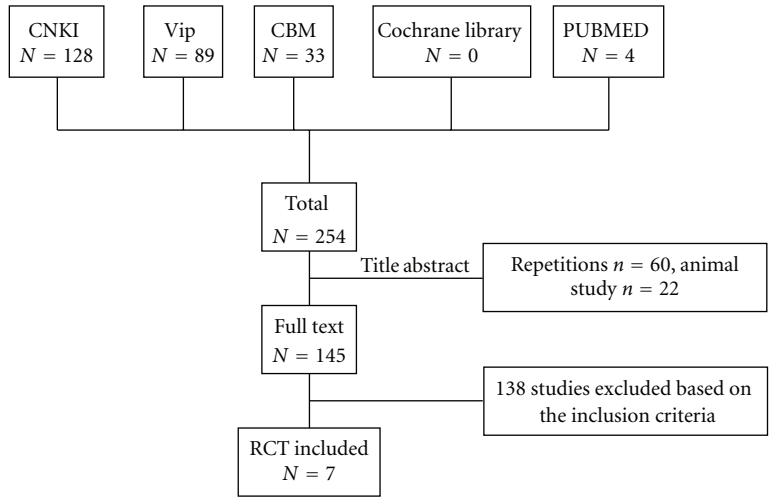

Figure 1: Study selection process.

Table 1: Characteristics and methodological quality of included studies.

Study ID	Sample	Time of onset	CT/MRI	Intervention in control group	QKL injection dose/day	Course	Followup (month)	Death	Adverse effect
Liang 2000 [15]	80	<72 h	Yes	Conventional treatment	30 mL	14 d	1	No	Unclear
Tan et al. 2003 [10]	65	<72 h	Yes	Conventional treatment	40 mL	14 d	No	2/2	2 eruption and 1 dizziness
Wu et al. 2007 [14]	88	<72 h	Yes	Conventional treatment	1200 mg (freeze-drying agent, roughly equivalent to 60 mL)	14 d	No	No	Unclear
Yan and Li 2010 [13]	150	<6 h	Yes	Urokinase + conventional treatment	40 mL	7 d	No	No	No
Yang et al. 2003 [12]	58	<72 h	Yes	Conventional treatment	80 mL	20 d	No	No	No
Zeng and Feng 2003 [11]	64	<24 h	Yes	Conventional treatment	60 mL	20 d	No	No	Unclear
Yu and Liao 1999 [16]	40	<72 h	Yes	Conventional treatment	50 mL	14 d	1	1/7	No

Conventional medicine treatment includes mannital, dextran, nimodipine, aspirin, and so on.

3.3. Effect of the Interventions

3.3.1. Primary Outcomes

Effect Rate. All the seven trials reported clinical effect rate to evaluate the outcome, which was based on the reduction of neurological deficit scoring [18]. Four trails [11–14] used the percentage of MESSS scores reduced rate to measure the outcome: cure (MESSS scores reduced rate from 91 to 100%), significantly effective (MESSS scores reduced rate more from 46 to 90%), effective (MESSS scores reduced rate from 18 to 46%), and ineffective (MESSS scores reduced rate less than 18%). Other three studies [10, 15, 16] used similar evaluation standards at the study time. We put these two different kinds of measurements together to evaluate the general effectiveness. Total effective rate is the combination of cure, significant effective and effective rate. The meta-analysis showed significant difference between groups of QKL injection plus conventional treatment and conventional treatment alone on total effective rate (RR: 1.12 [1.04, 1.19]; $P < 0.01$) (Figure 2).

Death. Two trials [10, 16] out of seven reported death. Tan et al. [10] assessed death at the end of 2-week therapeutic course, and Yu and Liao [16] reported it 1 month after stroke onset. Total number of death was 12 out of 105 patients. There was no statistically significant difference between two groups (RR, 0.39; 95% CI, 0.10 to 1.55; $P = 0.18$) (Figure 3).

Adverse Effect. Four trials [10, 12, 13, 16] reported adverse events, while the other three did not mention it. No obvious

Table 2: Quality assessment of included randomized controlled trials.

Study ID	Sequence generation	Allocation concealment	Incomplete outcome data	Blinding	Other source of bias	Selective outcome reporting	Risk of bias
Liang 2000 [15]	Unclear	Unclear	No	Unclear	Unclear	No	Unclear
Tan et al. 2003 [10]	Table of random number	Unclear	Yes	Unclear	Unclear	No	Unclear
Wu et al. 2007 [17]	Unclear	Unclear	No	Unclear	Unclear	No	Unclear
Yan and Li 2010 [13]	Unclear	Unclear	Yes	Unclear	Unclear	Yes	Unclear
Yang et al. 2003 [12]	Unclear	Unclear	No	Unclear	Unclear	No	Unclear
Zeng and Feng 2003 [11]	Unclear	Unclear	No	Unclear	Unclear	No	Unclear
Yu and Liao 1999 [16]	Unclear	Unclear	Yes	Unclear	Unclear	No	Unclear

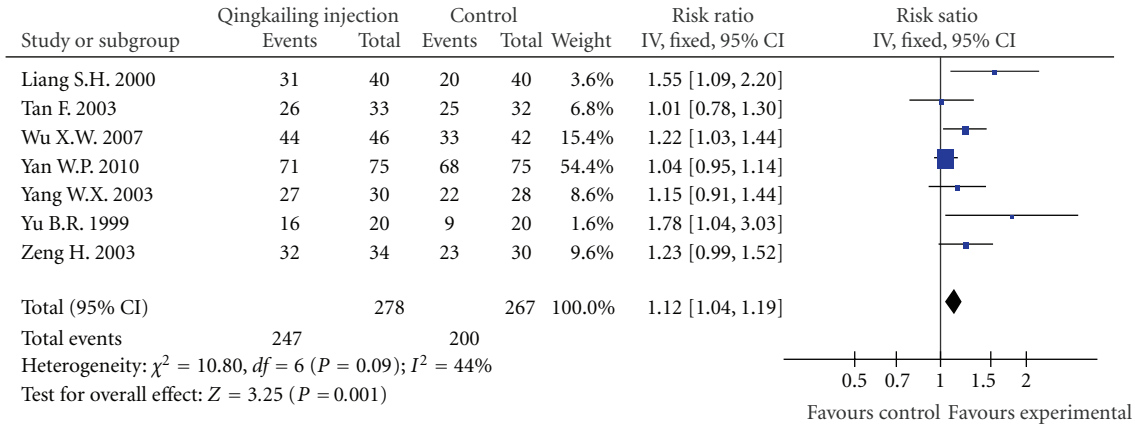

Figure 2: Forest plot of comparison: effect rate.

adverse events occurred in three trials, and one trial [10] reported adverse events described as eruption and dizziness; however, eruption also appeared in control group. The duration of treatment in all included studies was short (7 to 20 days). Outcomes were measured within 1 month after stroke onset, without longer followup. Although few related information about adverse effects was recorded, potential or long-time adverse effects in the included trials could not be excluded (Table 1).

3.3.2. Secondary Outcomes

MESSS Scoring. Three trials (272 patients) [11–13] reported detailed neurological deficit scoring (MESSS). The scoring performed no significant difference between two groups before treatment, and meta-analysis of data on MESSS scoring at the end of duration of treatment showed that QKL injection plus conventional treatment had more benefit compared with conventional treatment alone (WMD: −3.49; 95% CI, −4.70 to −2.28; $P < 0.01$) (Figure 4).

Infarction Volume. One trial [12] assessed infarction volume of CT, before treatment and at the end of 21 days of treatment, respectively, using the formula of quantitative estimation on hematoma. The baseline of infarction volume had no significant difference between two groups and the infarction volume of QKL group at endpoint reduced significantly more than control (WMD, −1.85; 95% CI, −3.40 to −0.30; $P = 0.02$).

Inflammation Factor. Two trials [10, 14] provided data of inflammation factors in blood serum. They both reported TNF-α and IL-6, so we make meta-analysis of them. At the end of treatment, results showed there was significant difference between QKL treatment and routine treatment groups for TNF-α level (WMD −5.56; 95% CI, −9.23 to −1.90; $P < 0.01$), but no statistic significance for IL-6 (WMD −22.61; 95% CI, −53.91 to 8.68; $P = 0.16$) (Figure 5).

Revival of Coma. Yang et al. [12] reported the effect of QKL injection in reviving coma induced by stroke. In QKL

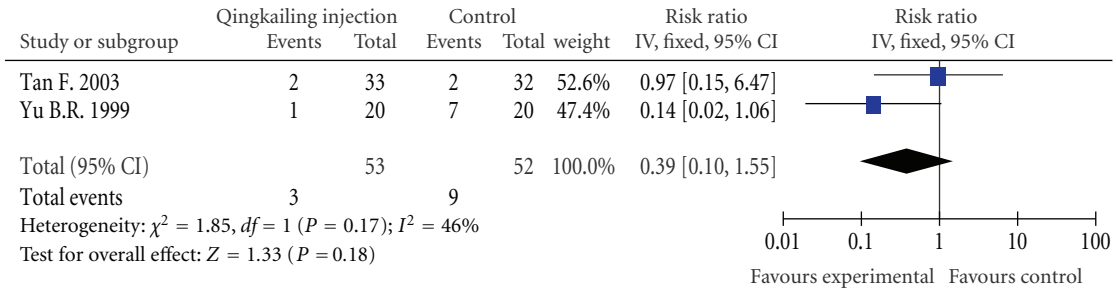

Figure 3: Forest plot of comparison: death.

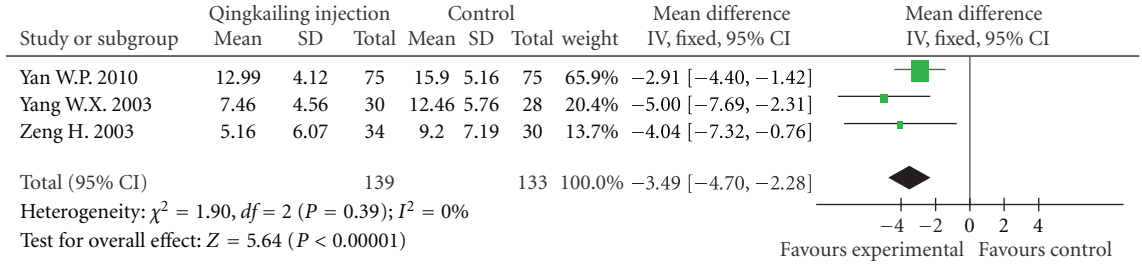

Figure 4: Forest plot of comparison: MESSS scoring.

injection treatment group, 11 coma cases out of 13 revived within 7 days, while in control group that was only 4 out of 10 (RR, 2.12; 95% CI, 0.96 to 4.68; $P = 0.06$).

3.3.3. Quality of Life. Only one trial [11] reported routine life status using scoring recommended at the Second and revised at the Fourth National Cerebrovascular Diseases Conference in China [18]. At the end of treatment, QKL treatment significantly improved routine living ability than control group (WMD, −0.96; 95% CI, −1.84 to 0.08; $P = 0.03$).

3.4. Publication Bias. Although we conducted comprehensive searches and tried to avoid bias, since all trials were published in Chinese, we could not exclude potential publication bias.

4. Discussion

This review of randomized trials showed the current evidence in QKL injection for acute ischemia stroke. In many years, Western medicine has made tremendous progress and had become the dominating medical treatment worldwide. However, it has been increasingly recognized that Western medicine may sometimes fail to treat an illness, whereas such illness is reportedly improved by the so-called complementary medicine based on a different theory [19, 20]. Traditional Chinese patent medicine (TCPM) for stroke is very popular in China [1, 2, 17]. Currently, there are more than 100 TCPM used for stroke and approved by the Chinese State Food and Drug Administration [17]. QKL injection has been applied for more than 30 years. However, few relevant articles on QKL injection for stroke have been published in the English medical journals. One systemic review about TCPM for ischemic stroke published in 2007 included only 2 trials of QKL injection [17]. One of them was included in this study, while the other excluded for not meeting inclusion criteria.

The data from the 7 RCTs that were analyzed demonstrated that, QKL injection plus conventional medication maybe more effective than conventional medication alone for acute ischemia stroke. With evaluating the improvement of classification of neurological function using MESSS scoring (RR, 1.12; 95% CI, 1.04 to 1.19), the effect rate of QKL plus conventional medicine treatment group was, on average, 14 percent more than control group using conventional medicine alone. In consistency with it, meta-analysis of detailed neurological deficit scoring (MESSS) in three trails also showed QKL group had more benefit in improving neurological function. These results were positively encouraging and promising of combining QKL injection with conventional treatment, which might be beneficial to acute ischemic stroke.

However, there were several limitations in this paper. No multicenter, large sample and cooperative studies were found and most of the existing trials were of small size, yet no trials estimated the sample size. Although all trials claimed randomization, most of them failed to provide enough information to judge whether the randomization procedures had been carried out properly. And inadequate reporting of allocation concealment, blinding, intention to treat analysis, and dropouts account in all the trials may have created potential performance biases and detection biases, as patients and researchers might have been aware of the therapeutic interventions. So every trial had an unclear risk of bias or a high risk of bias. Although we conducted comprehensive searches and tried to avoid bias, since all

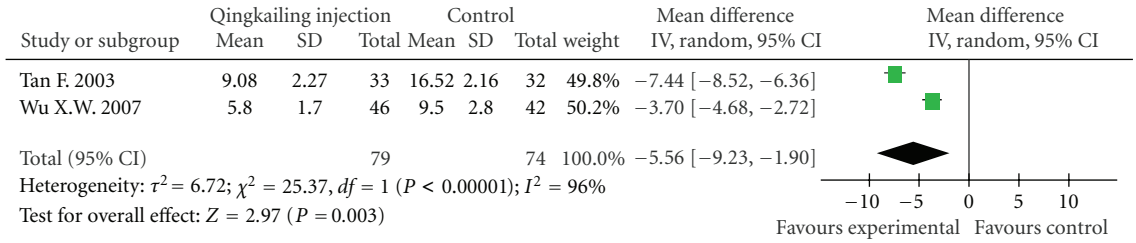

Figure 5: Forest plot of comparison: TNF-α.

trials were published in Chinese, there remained the possible existence of publication bias.

This paper showed a very low case fatality rate (12 deaths out of total 547 patients). There were several possible explanations for the striking finding: truly low case fatality rate for stroke in China; severe stroke patients were not sent to hospitals; severe stroke cases were excluded by Chinese stroke physicians in research studies; failure to report major outcome events; or did not assess long-term outcome after stroke onset. Those all resulted in bias. However, in the two trials reporting death with limited sample size, there was a tendency of reducing fatality rate in QKL injection group without significant difference.

For secondary outcomes, infarction volumes of CT and inflammation factors in blood serum were reported in a few trials. These results supported QKL injection's efficiency, and the later outcome was consistent with animal results about the mechanism of QKL in ischemic model [6, 7]. Except for one trial, most of trials did not mention quality of life, which possibly was special advantage of TCM. We suggest future trials for QKL to pay more attention to outcome of life quality and comply with international standards in the evaluation of life status. One trial reported effect in reviving stroke-induced coma and result showed more coma cases in QKL group regained consciousness within one week; however, with no statistic significance because of limited sample. It indicated that QKL may have excellent effect in restoring consciousness, which was called consciousness restoring and obstruction clearing (Xingnaokaiqiao) in TCM theory. Angongniuhuang pill was well known about its excellent consciousness restoring and obstruction clearing effect, and QKL also was widely used to treat coma. So we advise future RCTs to assess QKL effect for subgroup of severe stroke patients combined with coma.

Adverse effects were reported by Tan et al. [10] as eruption and dizziness. No obvious adverse events occurred in three trials, while other three trials did not report adverse effects. However, the concrete conclusion regarding safety cannot be determined from this paper due to the limited evidence provided by the eligible trials. Another paper about the safety of QKL concluded that, although some cases of adverse effects for QKL were reported, QKL carries a low risk of adverse drug reactions and adverse events, and some adverse events that do occur may be ascribed to improper use of the drug [21]. In order to proper assess the safety of QKL injection, large-scale clinical trials with long-term followup are required.

In conclusion, a definite conclusion on efficacy and adverse events associated with QKL injection cannot be drawn from this paper because of the unclear methodological quality of these included trials. The general lack of reporting of methodology in these trials publication was not consistent with the CONSORT statement on the reporting of the results of randomized trials (http://www.consort-statement.org/), which is being highlighted in many journals around the world. Future trials should overcome the limitations of the trials presented in this paper; particularly, they should assure adequate concealment of allocation and blinding of outcome assessors and report both of fatality rate and effect rate as the primary outcomes at long-term followup, and evaluate routine living ability using international standards. If the positive effects of QKL injection were confirmed by comprehensive clinical trials, it would lead to many promising treatments for acute ischemic stroke and could benefit patients all over the world.

Authors' Contibution

F. Cheng and X. Wang equally contributed to this paper.

Conflicts of Interests

The authors declare that there is no conflict of interests.

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Research Article

Effect of Keishibukuryogan on Endothelial Function in Patients with at Least One Component of the Diagnostic Criteria for Metabolic Syndrome: A Controlled Clinical Trial with Crossover Design

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We evaluated the effect of keishibukuryogan (KBG; Guizhi-Fuling-Wan), a traditional Japanese (Kampo) formula, on endothelial function assessed by reactive hyperemia peripheral arterial tonometry (Endo-PAT2000) in patients with metabolic syndrome-related factors by controlled clinical trial with crossover design. Ninety-two patients were assigned to group A (first KBG-treatment period, then control period; each lasting 4 weeks, with about one-year interval) or group B (first control, then KBG-treatment). In forty-nine (27, group A; 22, group B) patients completing all tests, the mean value of the natural logarithmic-scaled reactive hyperemia index (L_RHI) increased and those of serum nonesterified fatty acid (NEFA), malondialdehyde, and soluble vascular cell adhesion molecule 1 decreased significantly during the KBG-treatment period, but not during the control period, and 4-week changes of L_RHI, NEFA, and malondialdehyde between the 2 periods showed significance. These results suggest that KBG has beneficial effect on endothelial function in patients with metabolic syndrome-related factors.

1. Introduction

In Japan, the incidence of cardiovascular events has been increasing on account of the westernization of lifestyle and increases in the prevalence of overweight and metabolic syndrome [1, 2]. Recently, endothelial dysfunction has been recognized as a crucial pathogenesis in the early stage of arteriosclerosis [3, 4]. Traditional risk factors such as hypertension, dyslipidemia, and hyperglycemia are associated with endothelial dysfunction [4], and endothelial dysfunction itself is also reported to be an independent risk factor in the development of cardiovascular events [5]. Endothelial dysfunction is reversible, and its improvement can prevent the development of arteriosclerosis.

Flow-mediated dilatation (FMD) has been used for the measurement of endothelial function, and by this method the change of forearm vascular diameter is evaluated by ultrasonic apparatus [6]. On the other hand, reactive hyperemia peripheral arterial tonometry (RH-PAT), a noninvasive apparatus developed recently, allows easier measurement compared to FMD [7, 8]. It requires a less-operator-dependent technique, and the influence of sympathetic nervous activity in RH-PAT is less than that in FMD. Therefore, it makes possible the comparison of measured values from different devices.

Keishibukuryogan (KBG; Guizhi-Fuling-Wan) is a traditional Japanese (Kampo) formula used to prevent the development of atherosclerosis. In recent years, we reported the protective effects of KBG on endothelial function in cholesterol-fed rabbits and spontaneously diabetic rats [9, 10]. Further, we revealed that KBG actually inhibits the progression of atherosclerosis in cholesterol-fed rabbits [11, 12]. However, it has not been assessed whether KBG prevents the progression of atherosclerosis clinically in human subjects,

and long-term study is not easy to conduct. Therefore, this time we set out to evaluate the effects of KBG on endothelial function using RH-PAT in patients with metabolic syndrome-related factors by a controlled clinical trial with crossover design.

2. Methods

2.1. Patients. The diagnostic criteria for metabolic syndrome adopted in the study were defined by the Examination Committee for Criteria of metabolic syndrome in Japan, that is, (1) waist circumference ≥85 cm in men and ≥90 cm in women; in addition 2 or more of the following 3 components: (2) triglyceride (TG) ≥ 150 mg/dL and/or high-density lipoprotein (HDL) cholesterol < 40 mg/dL, (3) systolic blood pressure (SBP) ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 85 mmHg, and (4) fasting plasma glucose (FPG) ≥ 110 mg/dL [13]. We recruited patients aged 40–80 years who were consulting the Department of Japanese Oriental Medicine, Toyama University Hospital, for treatment of various diseases or symptoms, and having 1 or more components of the above diagnostic criteria between June 2008 and August 2011. Patients with serious liver or kidney disease, infectious disease, malignancy, previous stroke or myocardial infarction, or other diseases considered to possibly disturb the implementation of this trial, were excluded from enrollment.

2.2. KBG. KBG consists of 5 dried herbal medicines: Cinnamomi Cortex, Paeoniae Radix, Moutan Cortex, Persicae Semen, and Hoelen. These herbal powders were mixed with boiled honey at the ratio shown in Table 1 and rolled up into balls (2 g each). All these herbal medicines and honey were purchased from Uchida Wakanyaku Co. (Tokyo, Japan).

2.3. Study Design. The study was a controlled clinical trial with crossover design consisting of a 4-week KBG-treatment period and a 4-week control period. The patients were randomly assigned into group A (KBG-treatment, period I; control, period II) or group B (control, period I; KBG-treatment, period II). The Framingham heart study using the FMD technique revealed that flow-mediated vascular dilatation is influenced by season or temperature and is highest in summer and lowest in winter [14]. Therefore, we set both period I and period II in the same season, and the interval between the 2 periods at about one year. Patients were evaluated at most 4 times (test 1, beginning of period I; test 2, end of period I; test 3, beginning of period II; test 4, end of period II). KBG (6 g per day) was administered three times a day after meals in addition to their usual prescribed drugs for 4 weeks in period I in group A or period II in group B. These concomitant drugs had not been changed for at least 3 months before the beginning point of and during period I and period II (Figure 1). This study was an open-label study, as it was impossible to prepare a suitable placebo due to the unique flavor and taste of KBG.

The study design was approved by the Ethics Committee, University of Toyama. All patients provided written informed consent in accordance with the ethical guidelines set forth in the 1975 Declaration of Helsinki.

Table 1: Herbal medicines composing KBG and their ratio.

	Herbal medicine		Ratio (g)
Cinnamomi Cortex	*Cinnamomum cassia* BLUME	Guizhi	0.2
Paeoniae Radix	*Paeonia lactiflora* PALLAS	Shaoyao	0.2
Moutan Cortex	*Paeonia suffruticosa* ANDREWS	Mudanpi	0.2
Persicae Semen	*Prunus persica* BATSCH	Taoren	0.2
Hoelen	*Poria cocos* WOLF	Fuling	0.2

KBG: keishibukuryogan.
These 5 herbal powders were mixed with boiled honey (1 g) and rolled up into balls (2 g each).

2.4. RH-PAT. Endothelial function was evaluated by Endo-PAT2000 (Itamar Medical, Caesarea, Israel). The principle of RH-PAT has been described previously [7, 8, 15]. Briefly, a blood pressure cuff was placed on one upper arm, while the contralateral arm served as control. PAT probes were placed on one finger (finger II or III) of each hand (same finger on both hands). After a 5-minute equilibration period, the cuff was inflated to the higher of either 60 mmHg above systolic pressure or 200 mmHg for 5 min and then deflated to induce reactive hyperemia. PAT signals were recorded electronically in both fingers. During these procedures, the subject was requested to remain quiet and still, and the room temperature was controlled at 21–24°C. The RH-PAT data were analyzed by computer with Endo-PAT2000 software version 3.1.2 in an operator-independent manner. The PH-PAT index reflects the extent of reactive hyperemia and was calculated as the ratio of the average amplitude of PAT signal over 1 min starting 1.5 min after cuff deflation (hyperemic finger, A; control finger, C) divided by the average amplitude of the PAT signal of a 2.5-minute duration before cuff inflation (baseline, hyperemic finger, B; control finger, D). Then, the reactive hyperthermia index (RHI) was obtained from this equation: RHI = (A/B)/(C/D) × baseline correction factor. Recently, the natural logarithmic scaled RHI (L_RHI) has often been used instead of RHI [4, 15, 16], and we also used it in this study (Figure 2).

2.5. Physical Findings and Laboratory Data. Immediately before the examination of RH-PAT, patients were evaluated for physical findings, such as height (HT), body weight (BW), waist circumference, and SBP and DBP after 5-minute rest at supine position. Body mass index (BMI) was also calculated (BMI = BW (kg)/HT $(m)^2$).

Blood was collected from the cubital vein after overnight fasting immediately after the RH-PAT examination, and partially separated serum was frozen at −80°C immediately and stored until assay. Routine clinical laboratory data, such as TG, HDL-cholesterol, low-density lipoprotein (LDL) cholesterol, FPG, immunoreactive insulin (IRI), creatinine, and high-sensitive C-reactive protein (hs-CRP), were measured

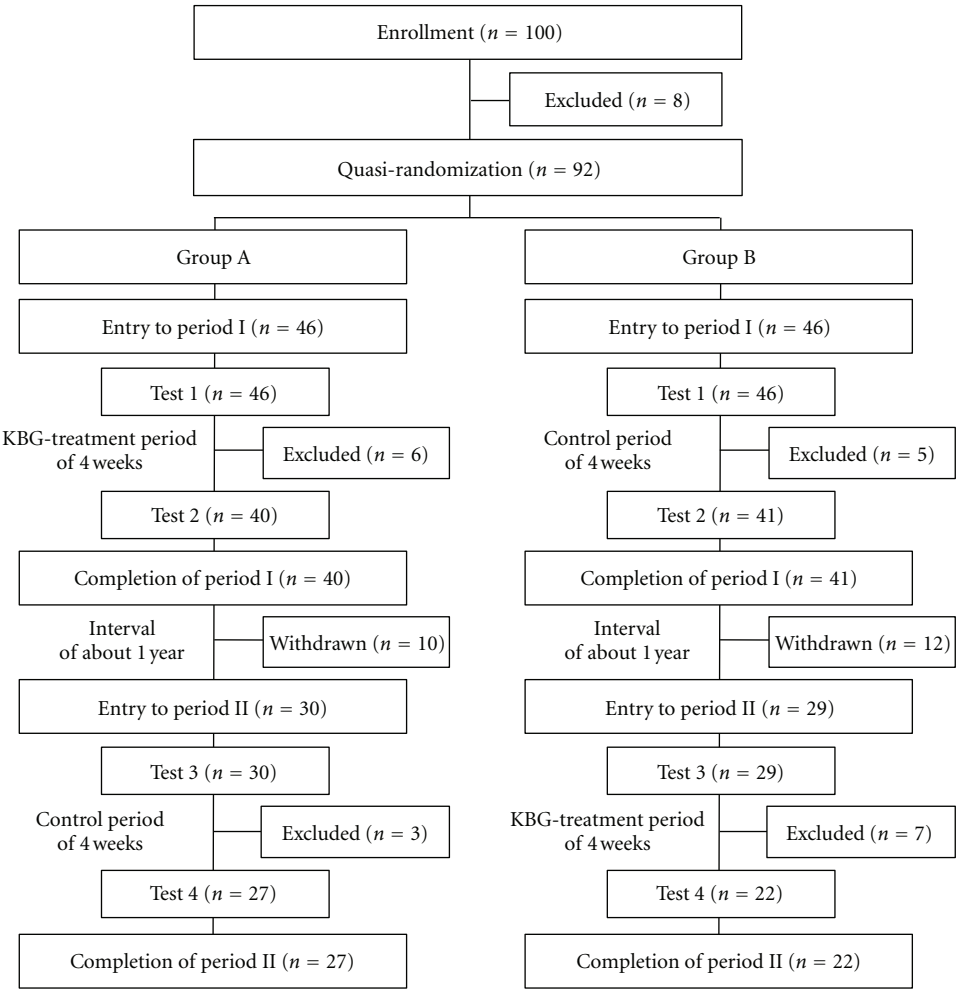

Figure 1: Flow chart of patients enrolled in the study.

by standard laboratory techniques in our hospital. Homeostasis model assessment as an index of insulin resistance (HOMA-IR) was performed for the evaluation of insulin resistance: [HOMA-IR = IRI (μU/mL) $\times$ FPG (mg/dL)/405] [17]. Nonesterified fatty acid (NEFA; free fatty acid) was measured by enzyme method at Mitsubishi Chemical Medience Co., Tokyo, Japan. We measured malondialdehyde (MDA), a marker of oxidative stress, by thiobarbituric acid reactive substance (TBARS) assay kit (OXI-TEK TBARS Assay Kit; ZeptoMetrix Co., New York, USA), and soluble vascular cell adhesion molecule 1 (sVCAM-1) by Human sVCAM-1 Quantikine ELISA Kit (R&D Systems Inc., Minneapolis, USA) in our laboratory.

2.6. Statistical Analysis. Statistical analysis was performed with JMP 9 (SAS Institute Japan, Tokyo). Data were expressed as mean $\pm$ S.E. Either Wilcoxon test or Pearson's chi-square test was used for statistical analysis of the patient's characteristics. The difference between the data at week 0 and week 4 was analyzed using the Wilcoxon matched-pairs signed-ranks test. Comparison between the change of values in KBG-treatment period and control period was performed by MANOVA test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Patients. In total, 100 patients were initially enrolled, but 8 were then excluded (6, deviation from inclusion criteria; 2, consent withdrawn), and 92 patients were finally quasi-randomized to group A (n = 46) and group B (n = 46), and entered into period I (Figure 1).

In group A, after test 1, 6 patients were excluded [3, refused intake of KBG; 1, adverse effect (glossalgia); 1, change of concomitant drug; 1, eating before test], and after the 4-week KBG-treatment period the remaining 40 patients underwent test 2 and completed period I. During the interval of about 1 year between periods I and II, 10 patients dropped out (7, refused to participate; 2, onset of other disease; 1, discontinued hospital visit). The other 30 patients entered into period II and underwent test 3. After that, 3 patients were excluded (1, onset of infectious disease; 2, change of concomitant drug), and after the 4-week control period, the remaining 27 patients underwent test 4 and completed period II.

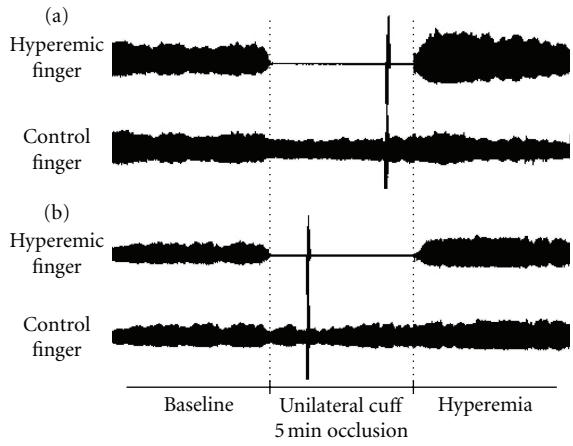

Figure 2: Representative signals of reactive hyperemia peripheral arterial tonometry (RH-PAT) with (a) normal and (b) low-reactive hyperemic response. Normal response characterized by a distinct increase in the signal amplitude after cuff release compared with baseline.

In group B, after test 1, 5 patients were excluded (3, refused to participate; 1, onset of infectious disease; 1, eating before test), and after the 4-week control period, the remaining 41 patients underwent test 2 and completed period I. During the interval between periods I and II, 12 patients dropped out (7, refused to participate; 1, onset of other disease; 4, discontinued hospital visit), and the other 29 patients entered into period II and underwent test 3. After that, 7 patients were excluded (2, adverse effects (diarrhea, mouth bitterness); 4, change of concomitant drug; 1, eating before test), and after the 4-week KBG-treatment period, the remaining 22 patients underwent test 4 and completed period II.

3.2. Patient Characteristics. The baseline characteristics of the patients who completed all the tests are shown in Table 2. A statistical difference between the 2 groups was seen in male waist circumference, but not in any of the other factors.

3.3. Effects of KBG. Comparison between various parameters at week 0 and week 4 in both the KBG-treatment period and control period and that of 4-week changes between the KBG-treatment period and control period in patients completing all tests (49 patients in group A + B, 27 in group A plus 22 in group B) are shown in Table 3. The mean value of L_RHI in the KBG-treatment period increased significantly, but not in the control period, and the 4-week changes between KBG-treatment and control periods showed statistical significance. Further, the mean values of NEFA, MDA, and sVCAM-1 in the KBG-treatment period decreased significantly, and 4-week changes of the former 2 parameters between KBG-treatment and control periods were statistically significant.

Separate data of group A (Table 4) and group B (Table 5) are also shown. In group A, the mean value of L_RHI in the KBG-treatment period increased significantly, but not in the control period, and the 4-week changes between the KBG-treatment and control periods showed statistical significance. The mean value of NEFA in the KBG-treatment period decreased significantly, and the 4-week changes between the KBG-treatment and control periods showed statistical significance. Further, the levels of MDA and sVCAM-1 in the KBG-treatment period decreased significantly. Similarly in group B, the mean value of L_RHI in the KBG-treatment period increased significantly. The levels of NEFA and MDA in the KBG-treatment period decreased significantly, and the 4-week changes of MDA levels between the KBG-treatment and control periods showed statistical significance.

4. Discussion

The risk factors of cardiovascular diseases, such as diabetes mellitus, hypertension, and dyslipidemia, often accumulate in the same person and increase the incidence of cardiovascular events synergistically. The concept of metabolic syndrome arose from a global movement to unify such high-risk pathological conditions. In Japan also, clinical diagnostic criteria of metabolic syndrome were announced in 2005 [13]. Endothelial dysfunction is often seen in patients with metabolic syndrome, and it is recognized as a primary pathogenic factor of atherosclerosis [4, 18]. Metabolic syndrome is an independent risk factor for cardiovascular events, and if it is combined with endothelial dysfunction, the risk is elevated further [19].

Several methods have been developed to evaluate endothelial function, and they enable us to perform the examination even for outpatients. Evaluation of endothelial function using FMD or PAT is useful for the early detection of arteriosclerosis. The RHI calculated using the PAT signal is applied to a parameter of endothelial function. Endothelial function in the brachial circulation correlates with that in the coronary circulation, and low RHI is useful for identifying a patient with coronary endothelial dysfunction [7]. Thus, PAT is considered to be a useful, noninvasive examination for the prediction of later cardiovascular events [16]. A value of RHI $\leq$ 1.67 (L_RHI $\leq$ 0.51) measured by Endo-PAT2000 is considered to be endothelial dysfunction, is determined in the population with a risk for ischemic heart disease, and is recognized as a cut-off value [20]. The relationship between RHI and metabolic syndrome-related components had been reported [4]. A specific characteristic of endothelial function is its reversibility, and it can be a novel therapeutic target. Studies concerning the effect of drug or supplement on endothelial function using RHI have been reported on the basis of its sensitive reaction to treatment [21, 22].

In the present study, we evaluated the endothelial function of patients with metabolic syndrome-related factors using RHI by controlled clinical trial with crossover design and revealed that KBG has the potential to improve endothelial function. That is, L_RHI increased significantly in the KBG-treatment period, but not in the control period. It was reported that patients with lower RHI had a higher incidence of cardiovascular events during the followup period [16], and improvement of impaired endothelial function is important for the prevention of the development of arteriosclerosis [3]. Therefore, KBG might be useful for preventing the progression of endothelial dysfunction and arteriosclerosis. All the

Table 2: Patient characteristics.

	Group A ($n = 27$)	Group B ($n = 22$)	P-value
Age (year)	63.5 ± 1.6	62.0 ± 1.7	0.3925[a]
Sex (male/female)	11/16	9/13	0.9905[b]
Smoking (yes/no)	2/25	1/21	0.6740[b]
Alcohol intake (yes/no)	12/15	11/11	0.6983[b]
BMI (kg/m^2)	24.1 ± 0.6	22.8 ± 0.6	0.3149[a]
Waist circumference (cm)			
Male	91.5 ± 3.1	83.0 ± 1.6	0.0435*[a]
Female	81.6 ± 1.7	82.5 ± 2.7	0.9649[a]
SBP (mmHg)	126.3 ± 3.2	123.8 ± 2.6	0.7096[a]
DBP (mmHg)	76.3 ± 2.5	75.7 ± 2.2	0.5938[a]
TG (mg/dL)	129.2 ± 14.6	128.3 ± 14.7	0.6802[a]
HDL-cholesterol (mg/dL)	65.4 ± 3.8	60.4 ± 3.2	0.2515[a]
FPG (mg/dL)	103.7 ± 3.3	101.4 ± 2.6	0.9278[a]
Central obesity (yes/no)	11/16	7/15	0.5193[b]
High blood pressure (yes/no)	16/11	13/9	0.9905[b]
Dyslipidemia (yes/no)	12/15	11/11	0.6983[b]
Hyperglycemia (yes/no)	8/19	7/15	0.8687[b]
Concomitant drugs			
Calcium channel blocker (yes/no)	8/19	7/15	0.8687[b]
ARB or ACE inhibitor (yes/no)	3/24	4/18	0.4817[b]
α or β blocker (yes/no)	3/24	1/21	0.4038[b]
Statin (yes/no)	11/16	8/14	0.7544[b]
Fibrate (yes/no)	1/26	0/22	0.3618[b]
Sulfonylurea (yes/no)	3/24	1/21	0.4038[b]
Thiazolidine analog (yes/no)	3/24	0/22	0.1066[b]
α-glucosidase inhibitor (yes/no)	2/25	1/21	0.6777[b]
Antiplatelet drug (yes/no)	1/26	0/22	0.3618[b]
Season test 1 performed (Spring/Summer/Fall/Winter)	6/14/5/2	9/5/8/0	0.0682[b]

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TG: triglyceride; HDL: high-density lipoprotein; FPG: fasting plasma glucose; central obesity, waist circumference ≥ 85 cm (male), ≥90 cm (female); high blood pressure, SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg; dyslipidemia, TG ≥ 150 mg/dL and/or HDL cholesterol < 40 mg/dL; hyperglycemia, FPG ≥ 110 mg/dL; ARB: angiotensin II receptor blocker; ACE: angiotensin-converting enzyme; Spring: March 21 to June 20; Summer: June 21 to September 21; Fall: September 21 to December 20; Winter: December 21 to March 20.

[a]Comparison between group A and group B by Wilcoxon test.

[b]Comparison between group A and group B by Pearson's chi-square test.

Data are expressed as mean ± S.E., $^*P < 0.05$.

patients enrolled in this study were free of obvious findings of arteriosclerosis, but endothelial dysfunction (L_RHI ≤ 0.51 at test 1) was seen in 37.0% (34/92) of them. Most of the conventional methods for assessing arteriosclerosis evaluate its advanced stage. Endothelial dysfunction is observed even in the initial and early stages of the progression of atherosclerosis. Therefore, an intervention for endothelial dysfunction by KBG in an as yet latent stage of arteriosclerosis is considered useful for the prevention of eventual cardiovascular events.

We have previously reported that KBG improves microcirculation evaluated by erythrocyte aggregability and deformability in patients with multiple old lacunar infarction [23, 24]. In experimental animal models, we have also demonstrated that KBG has protective effects against endothelial dysfunction in cholesterol-fed rabbits and spontaneously diabetic rats [9, 10], in addition to its inhibitory effect against plaque formation [11, 12]. KBG is composed of 5 herbal medicines: Cinnamomi Cortex, Paeoniae Radix, Moutan Cortex, Persicae Semen, and Hoelen. We have

Table 3: Effects of KBG on various parameters in A + B group ($n = 49$).

	Period	Week 0	Week 4	P-value[a]	P-value[b]
BMI (kg/m^2)	KBG	23.5 ± 0.4	23.5 ± 0.5	0.3976	0.3034
	Control	23.5 ± 0.5	23.5 ± 0.5	0.9700	
Waist circumference (cm)	KBG	85.0 ± 1.2	85.0 ± 1.2	0.9280	0.9136
	Control	85.2 ± 1.3	85.3 ± 1.3	0.9462	
SBP (mmHg)	KBG	125.0 ± 2.3	121.0 ± 2.1	0.0321*	0.2591
	Control	123.1 ± 1.9	122.0 ± 1.9	0.3629	
DBP (mmHg)	KBG	74.6 ± 1.6	73.9 ± 1.4	0.1297	0.4088
	Control	73.2 ± 1.4	74.1 ± 1.2	0.5626	
L_RHI	KBG	0.58 ± 0.03	0.70 ± 0.04	0.0003**	0.0034**
	Control	0.64 ± 0.04	0.60 ± 0.03	0.7279	
TG (mg/dL)	KBG	124.3 ± 8.9	123.2 ± 7.8	0.9453	0.5339
	Control	125.6 ± 8.9	119.1 ± 6.2	0.6347	
HDL-cholesterol (mg/dL)	KBG	62.0 ± 2.6	62.0 ± 2.5	0.6699	0.3016
	Control	62.6 ± 2.1	61.4 ± 2.0	0.1810	
LDL-cholesterol (mg/dL)	KBG	132.7 ± 4.8	133.8 ± 5.0	0.5803	0.1155
	Control	131.4 ± 4.6	127.7 ± 5.0	0.0435*	
NEFA (μEq/L)	KBG	532.9 ± 32.9	450.9 ± 26.0	0.0024**	0.0113*
	Control	507.6 ± 31.9	529.6 ± 29.0	0.3273	
FPG (mg/dL)	KBG	101.0 ± 2.3	101.5 ± 2.8	0.9159	0.2087
	Control	105.5 ± 3.0	103.1 ± 3.1	0.1297	
IRI (μU/mL)	KBG	5.73 ± 0.42	5.47 ± 0.39	0.2609	0.5396
	Control	6.26 ± 0.52	6.26 ± 0.52	0.9156	
HOMA-IR	KBG	1.46 ± 0.12	1.42 ± 0.12	0.3842	0.8010
	Control	1.69 ± 0.17	1.68 ± 0.19	0.8639	
Creatinine (mg/dL)	KBG	0.71 ± 0.02	0.72 ± 0.03	0.8238	0.6513
	Control	0.71 ± 0.02	0.71 ± 0.02	1.0000	
hs-CRP (mg/dL)	KBG	0.13 ± 0.05	0.13 ± 0.04	0.3721	0.7501
	Control	0.11 ± 0.03	0.10 ± 0.02	0.3173	
MDA (nmol/mL)	KBG	11.8 ± 1.1	9.5 ± 1.0	<0.0001**	0.0424*
	Control	12.9 ± 1.9	12.3 ± 1.9	0.2804	
sVCAM-1 (ng/mL)	KBG	723.1 ± 43.4	677.7 ± 40.9	0.0126*	0.1659
	Control	724.3 ± 36.0	714.0 ± 36.3	0.8107	

KBG: keishibukuryogan; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; L_RHI: natural logarithmic scaled reactive hyperemia; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein; NEFA: nonesterified fatty acid; FPG: fasting plasma glucose; IRI: immunoreactive insulin; HOMA-IR: homeostasis model assessment as an index of insulin resistance; hs-CRP: high-sensitive C-reactive protein; MDA: malondialdehyde; sVCAM-1: soluble vascular cell adhesion molecule 1.

[a]Comparison between week 0 and week 4 by Wilcoxon matched-pairs signed-ranks test.

[b]Comparison of 4-week changes between KBG-treatment period and control period by MANOVA test.

Data are expressed as mean ± S.E., $^*P < 0.05$, $^{**}P < 0.01$.

reported that polyphenols of Cinnamomi Cortex and Paeoniae Radix have endothelium-dependent relaxative effects [25, 26]. Cinnamaldehyde contained in Cinnamomi Cortex also has endothelium-dependent relaxation effect [27, 28]. These effects of KBG and composing herbal medicines are assumed to contribute to the efficacy of KBG on endothelial function.

It is reported that the elevated level of NEFA is related to metabolic syndrome and endothelial dysfunction, and that it is an independent predictive factor for cardiovascular events [29]. In our previous study, KBG decreased the plasma level of NEFA in cholesterol-fed rabbits [30], and in the present clinical trial, NEFA was also decreased. Therefore, KBG seems to have the actual potential to decrease NEFA, and this effect might contribute to the improvement of endothelial function.

In the present study, the levels of MDA decreased. Oxidative stress has been reported to impair endothelial function and accelerate the progression of atherosclerosis. A highly oxidized condition aggravates endothelial dysfunction and depresses nitric oxide production [31, 32], and drugs having antioxidant activity possess protective effects against endothelial dysfunction. Previous reports have indicated that each component of the diagnostic criteria of metabolic syndrome

Table 4: Effects of KBG on various parameters in group A ($n = 27$).

	Period	Week 0	Week 4	P-value[a]	P-value[b]
BMI (kg/m^2)	KBG	24.1 ± 0.6	24.2 ± 0.6	0.3362	0.6182
	Control	24.2 ± 0.7	24.2 ± 0.7	0.5916	
Waist circumference (cm)	KBG	85.6 ± 1.8	85.8 ± 2.0	0.6699	0.9871
	Control	87.2 ± 1.9	87.3 ± 1.9	0.8962	
SBP (mmHg)	KBG	126.3 ± 3.2	125.4 ± 3.2	0.6820	0.9476
	Control	122.6 ± 2.8	121.5 ± 2.8	0.5591	
DBP (mmHg)	KBG	76.3 ± 2.5	75.9 ± 2.0	0.3790	0.4292
	Control	71.2 ± 1.8	73.3 ± 1.7	0.3028	
L_RHI	KBG	0.58 ± 0.04	0.70 ± 0.05	0.0231*	0.0085**
	Control	0.65 ± 0.05	0.55 ± 0.04	0.1834	
TG (mg/dL)	KBG	129.2 ± 14.6	128.1 ± 12.7	0.7791	0.5937
	Control	123.4 ± 11.0	116.6 ± 8.9	0.5277	
HDL-cholesterol (mg/dL)	KBG	65.4 ± 3.8	66.0 ± 3.6	0.8341	0.6859
	Control	64.5 ± 2.7	64.5 ± 2.4	0.8432	
LDL-cholesterol (mg/dL)	KBG	138.5 ± 5.9	143.1 ± 6.8	0.2268	0.0715
	Control	130.5 ± 5.3	127.3 ± 5.5	0.1422	
NEFA (μEq/L)	KBG	527.8 ± 49.1	445.4 ± 33.9	0.0306*	0.0004**
	Control	440.0 ± 30.3	545.9 ± 42.4	0.0028**	
FPG (mg/dL)	KBG	103.7 ± 3.3	105.9 ± 4.6	0.3768	0.2550
	Control	108.9 ± 5.0	106.6 ± 5.2	0.7477	
IRI (μU/mL)	KBG	6.71 ± 0.58	6.05 ± 0.58	0.0593	0.1223
	Control	6.86 ± 0.78	7.18 ± 0.79	0.5777	
HOMA-IR	KBG	1.73 ± 0.17	1.63 ± 0.19	0.2001	0.3213
	Control	1.91 ± 0.26	2.00 ± 0.30	0.4815	
Creatinine (mg/dL)	KBG	0.70 ± 0.03	0.70 ± 0.03	0.5742	1.0000
	Control	0.71 ± 0.03	0.71 ± 0.04	0.9734	
hs-CRP (mg/dL)	KBG	0.10 ± 0.03	0.10 ± 0.02	0.1962	0.2681
	Control	0.07 ± 0.01	0.11 ± 0.02	0.0423*	
MDA (nmol/mL)	KBG	10.2 ± 1.5	8.7 ± 1.4	0.0007**	0.5068
	Control	12.8 ± 2.5	11.9 ± 2.1	0.3444	
sVCAM-1 (ng/mL)	KBG	769.5 ± 72.6	708.2 ± 66.3	0.0121*	0.2791
	Control	735.8 ± 51.3	711.6 ± 48.3	0.7389	

KBG: keishibukuryogan; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; L_RHI, natural logarithmic scaled reactive hyperemia; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein; NEFA: nonesterified fatty acid; FPG: fasting plasma glucose; IRI: immunoreactive insulin; HOMA-IR: homeostasis model assessment as an index of insulin resistance; hs-CRP: high-sensitive C-reactive protein; MDA: malondialdehyde; sVCAM-1: soluble vascular cell adhesion molecule 1.

[a]Comparison between week 0 and week 4 by Wilcoxon signed-rank test.

[b]Comparison of 4-week changes between KBG-treatment period and control period by MANOVA test.

Data are expressed as mean ± S.E., $^*P < 0.05$, $^{**}P < 0.01$.

is individually related to oxidative stress and endothelial dysfunction [33]. We reported the antioxidative effect of KBG, and this effect is beneficial for the prevention of endothelial dysfunction and arteriosclerosis [9–12].

As for sVCAM-1, in our previous study dealing with rheumatoid arthritis patients, KBG decreased the plasma levels of sVCAM-1 [34]. VCAM-1 is expressed on the impaired endothelium is regulated by various factors such as oxidative stress and cytokines [35, 36] and has been considered to be an important risk factor to the progression of atherosclerosis and cardiovascular events [37]. In the present study, the value of sVCAM-1 was decreased by KBG-treatment in group A. We speculated that the antioxidant effect of KBG might lead to the downregulation of VCAM-1.

Table 5: Effects of KBG on various parameters in group B ($n = 22$).

	Period	Week 0	Week 4	P-value[a]	P-value[b]
BMI (kg/m^2)	Control	22.8 ± 0.6	22.7 ± 0.6	0.4477	0.3443
	KBG	22.7 ± 0.6	22.7 ± 0.6	0.7660	
Waist circumference (cm)	Control	82.7 ± 1.7	82.8 ± 1.6	0.9129	0.8807
	KBG	84.1 ± 1.5	84.1 ± 1.3	0.8125	
SBP (mmHg)	Control	123.8 ± 2.6	122.5 ± 2.3	0.4676	0.0808
	KBG	123.4 ± 3.3	115.5 ± 2.2	0.0037**	
DBP (mmHg)	Control	75.7 ± 2.2	75.2 ± 1.6	0.5524	0.7949
	KBG	72.5 ± 1.8	71.5 ± 1.9	0.1567	
L_RHI	Control	0.62 ± 0.05	0.66 ± 0.05	0.2870	0.1751
	KBG	0.56 ± 0.05	0.70 ± 0.05	0.0074**	
TG (mg/dL)	Control	128.3 ± 14.7	122.2 ± 8.6	0.9750	0.7320
	KBG	118.4 ± 8.4	117.2 ± 7.8	0.9251	
HDL-cholesterol (mg/dL)	Control	60.4 ± 3.2	57.7 ± 3.1	0.0718	0.3085
	KBG	57.8 ± 3.2	57.2 ± 3.3	0.4357	
LDL-cholesterol (mg/dL)	Control	132.6 ± 8.0	128.2 ± 9.0	0.1619	0.7604
	KBG	125.5 ± 7.7	122.5 ± 6.7	0.5393	
NEFA (μEq/L)	Control	590.5 ± 56.6	509.5 ± 39.0	0.1289	0.9939
	KBG	539.1 ± 42.8	457.7 ± 41.0	0.0312*	
FPG (mg/dL)	Control	101.4 ± 2.6	98.8 ± 2.5	0.0881	0.6065
	KBG	97.6 ± 2.9	96.1 ± 2.7	0.1706	
IRI (μU/mL)	Control	5.51 ± 0.63	5.12 ± 0.59	0.1525	0.2374
	KBG	4.53 ± 0.50	4.76 ± 0.44	0.6260	
HOMA-IR	Control	1.42 ± 0.19	1.28 ± 0.17	0.1342	0.2206
	KBG	1.12 ± 0.14	1.15 ± 0.12	0.8140	
Creatinine (mg/dL)	Control	0.71 ± 0.03	0.70 ± 0.03	1.0000	0.4789
	KBG	0.74 ± 0.04	0.75 ± 0.04	0.7539	
hs-CRP (mg/dL)	Control	0.16 ± 0.07	0.09 ± 0.04	0.4895	0.4382
	KBG	0.17 ± 0.10	0.16 ± 0.07	0.8877	
MDA (nmol/mL)	Control	13.1 ± 2.8	12.8 ± 3.3	0.6747	0.0464*
	KBG	13.7 ± 1.7	10.5 ± 1.4	<0.0001**	
sVCAM-1 (ng/mL)	Control	710.1 ± 50.7	716.9 ± 56.4	0.4684	0.3898
	KBG	666.1 ± 36.3	640.3 ± 41.4	0.3021	

KBG: keishibukuryogan; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; L_RHI: natural logarithmic scaled reactive hyperemia; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein; NEFA, nonesterified fatty acid; FPG: fasting plasma glucose; IRI: immunoreactive insulin; HOMA-IR: homeostasis model assessment as an index of insulin resistance; hs-CRP: high-sensitive C-reactive protein; MDA: malondialdehyde; sVCAM-1: soluble vascular cell adhesion molecule 1.

[a]Comparison between week 0 and week 4 by Wilcoxon matched-pairs signed-ranks test.

[b]Comparison of 4-week changes between control period and KBG-treatment period by MANOVA test.

Data are expressed as mean ± S.E., $^*P < 0.05$, $^{**}P < 0.01$.

From the results of the present study, it is suggested that KBG has the potential to prevent the progression of endothelial dysfunction and arteriosclerosis by its antioxidative effect, and early detection of endothelial dysfunction with PAT and early treatment with KBG might contribute to the prevention of arteriosclerosis.

5. Conclusion

Our present controlled clinical trial with crossover design revealed that KBG improves endothelial function as evaluated by L_RHI in patients with metabolic syndrome-related factors, suggesting that KBG has beneficial effects against the progression of endothelial dysfunction and arteriosclerosis.

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Research Article

Metabolomics-Based Study of Clinical and Animal Plasma Samples in Coronary Heart Disease with Blood Stasis Syndrome

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The aim of this study is to explore a bridge connecting the mechanism basis and macro syndromes of coronary heart disease with experimental animal models. GC-MS technique was used to detect the metabolites of plasma samples in mini swine models with myocardial infarction (MI) and patients with unstable angina (UA). 30 metabolites were detected in the plasma samples of more than 50 percent of model group and control group in swine, while 37 metabolites were found in the plasma samples of UA patients and healthy control group. 21 metabolites in the plasma samples of swine model and 20 metabolites in patients with UA were found of significant value. Among which, 8 shared metabolites were found of low level expression in both swine model and UA patients. Independent Student's *t*-test, principal component analysis (PCA), and hierarchicalcluster analysis (HCA) were orderly applied to comprehend inner rules of variables in the data. The 8 shared metabolites could take place of the 21 or 20 metabolites in classification of swine model with MI and UA patients, which could be considered as a bridge connecting the mechanism basis and macrosyndromes of swine model with MI and UA patients.

1. Introduction

Coronary heart disease (CHD) causes more than one million Chinese to death each year [1]. Unstable angina (UA) is one of the most dangerous types of CHD that has a high mortality and morbidity in the world. Comparing the metabolites in swine model and patients with UA of blood stasis syndrome at the level of metabolomics to explore the underlying mechanism is a new way deserved trying.

Metabonomics, the study of metabolites and their roles in various disease states, is a novel methodology arising from the postgenomics era. In the last decade, "metabonomics" has demonstrated enormous potential in further understanding of many diseases, including work in cardiovascular research [2]. Metabonomics is now recognized as an independently and widely used technique for deriving new biochemical-based assays for disease diagnosis, understanding the relationships between gene function and metabolic control in health and disease, and identifying combination biomarkers for disease [3].

GC (gas chromatography) plays an important role in metabonomics research nowadays. Chromatography has been used mainly in biofluid analysis, especially for target component analysis but not for whole sample profiling combined with chemometrics [4]. The high separation power and the ability to achieve high sensitivity are strong incentives for the consideration of its use in biofluid fingerprinting as well. So chromatography would provide additional and complementary information that cannot be achieved with NMR [5].

In this study, blood samples of UA patients were collected under clinical epidemiology and chemically detected by metabolomics methods. Meanwhile, blood samples of swine model with MI were detected by metabolomics methods. As we had found in former research, feature selection-based data mining methods is more suitable for identifying biomarkers for UA [6]. Alternatively, we combined independent *t*-test and classification-based data mining methods. The novel method presented here provides a better insight into the pathology of a disease.

2. Materials and Methods

2.1. Animal Sample Preparation. Seventeen male miniswine weighed 25 ± 5 kg were randomly divided into two groups. The model group was composed of nine swine, while the control group was eight. The swine in model group ($n = 9$) was placed with Ameroid constrictor (Research Instrument SW, USA) at the left anterior descending coronary artery to gradually induce chronic myocardial infarction (MI). The swine in control group ($n = 8$) was chest-opened and coronary artery-isolated without placing any constrictor. Based on early results of dynamic observations, evaluations were performed four weeks after operation. Meanwhile, blood was obtained from former cava vena.

2.2. Evaluation of Swine Model with MI and Blood Stasis Syndrome. Evaluation of swine with MI and blood stasis syndrome was performed from five parts. Basic phenotype changes, ECG, echocardiography, coronary angiography, and hemorrheology were used to assess the animal model.

ECG. More than 0.1 mV ST segment depression and inversion T waves were observed in several leads of surface electrocardiogram of model animals. But no significant arrhythmia was found in experimental period.

Echocardiography. The structure and function of left ventricular were evaluated by echocardiography. Compared with sham-operated animals, there was great increase of end-systolic volume ($P < 0.05$), end-diastolic volume ($P < 0.05$), end-systolic diameter ($P < 0.01$), and end-diastolic diameter ($P < 0.01$) in model animals. Anterior wall thickness of both papillary muscle and apex level was decreased at end-diastolic and end-systolic in model animals ($P < 0.05$, $P < 0.01$). Meanwhile, the apex of the left ventricular anterior systolic wall thickening decreased ($P < 0.01$). In addition, septal thickness at the end-systolic decreased ($P < 0.05$). All the results above showed a segmental dysfunction of the left ventricular. There were no significant changes in stroke volume, ejection faction, fraction of shortening, and E/A, which suggested that cardiac function was still in compensatory period.

Coronary Angiography. Ex vivo angiography performed more than 90% occlusion or even completely block of the left anterior descending artery by the Ameroid constrictor in model animals. TIMI flow grade I or II was observed in most model animals. There was good filling in the left anterior descending artery with TIMI flow grade III in sham-operated animals.

Phenotype Information. Model animals appeared mental stress, irritation, fear, violent behaviors, strong self-defense, pilose disorderly, and lack of luster, while sham-operated animals' performances gradually returned to normal, with increased appetite, neat, and shiny pilose.

Hemorrheology. Compared with sham-operated animals, the blood viscosity of model animals increased significantly at a shear rate of high ($P < 0.05$), mid ($P < 0.01$), low ($P < 0.01$).

It was found that swine in MI group had significant changes when compared with the sham operation group from each aspect. Combined the five aspects above, the swine could be diagnosed as MI with blood stasis syndrome.

The results of echocardiography and hemorrheology of mini swine were given in Supplemental Table 1. (see Supplementary material available online at doi:10.1155/2012/638723)

2.3. Clinical Blood Sample Collection. Patients who suffered from UA and blood stasis at Dongzhimen Hospital affiliated to Beijing University of Chinese Medicine were included in the screening of metabolite biomarker cohort study. All patients aged from 55 to 75 were eligible for enrollment. Diagnosis standard of CHD refers to "Treatment Guide of Stable Angina" (ACC/AHA/ACP-ASIM, 1999) and "Diagnosis and Treatment Recommendations of Unstable Angina" (Chinese Society of Cardiology, 2000) [7, 8]. Diagnosis standard of blood stasis syndrome refers to "Guiding principles for the clinical study of Chinese Medicines" (2002) and Standard of syndrome differentiation of coronary heart disease (1990) [9, 10].

Patients were excluded from the case population in four conditions: those who suffered from acute myocardial infarction (AMI), infective cardiomyopathy, cardiac neurosis, or intercostal neuralgia; those who suffered from angina that was caused by polyarthritis rheumatica acuta, great pox, inborn coronary abnormity, hypertrophic cardiomyopathy or aortic valve stenosis; besides UA, those who also suffered from stroke, diabetes mellitus, pulmonary infection, nephritis, renal failure, urinary system infection, rheumatism, or osteoarthrosis; women in pregnant or in lactation.

The control group included healthy people that of no significantly different baseline compared with the case group. After these analyses, blood samples from a total of 27 UA patients with blood stasis syndrome and 15 healthy controls were enrolled for the further metabolomic analysis. The local ethics committee of Beijing University of Chinese Medicine approved the study protocol, and all patients provided written and informed consent.

The demographic details of included subjects were given in Supplemental Table 2.

2.4. Gas Chromatography: Mass Spectrometry (GC-MS) Analysis of Human and Animal Plasma. Add 250 μL acetonitrile and centrifuged to 100 μL plasma samples after ice-bathing for 10 minutes. Place 250 μL supernatant plasma extraction liquid in derivation reaction bottle and blew by N2. Add 50 μL methoxylamine pyridine solution with a concentration of 15 mg/mL to uniformly mixed, which was in oximation for one hour and then added by 50 μL derivation reagent (volume ratio of MSTFA : TMCS is 100 : 1) to uniformly mixed for one hour. Add 100 μL skellysolve C containing 0.1 mg/mL docosane to uniformly mixed and then centrifuged for 10 minutes. The resulting clear supernatant liquid was extracted

to small volume sample injection vessel for GC-MS analysis, whose conditions were given as following. Temperature of sample injection was 270°C, none-split stream sampling was used, and quantity of injection was 1 μL. Solvent was delayed for 7 minutes. Temperature was initialed at 80°C for 5 minutes and then gradually raised to 300°C with a speed of 10°C/min. It remained at 300°C for 5 minutes. Interface temperature was 280°C, and ion source temperature was 230°C. Ionizing voltage was 70 eV; quadrupole temperature was 150°C. Helium was used as carrier gas with a flow rate of mL/min. 45~550 m/z frequency was used to completely scan the samples.

2.5. Data Mining Methods. Independent Student's t-test, Principal component analysis (PCA), and hierarchical cluster analysis (HCA) were orderly applied to comprehend inner rules of variables in the data. These methods linearly investigated the data from one variable (t-test associated method) to multiple variables (PCA and so on). Moreover, the methods involved unsupervised methods (PCA and HCA). The systematical application of the statistical methods guaranteed the useful information of the disease mined by them.

3. Results

3.1. Detection of Significantly Different Metabolites by Student's t-Test Statistics. Student's t-test was initially employed to detect metabolites that are of significant difference between model and sham operation in swine as well as between UA patients and healthy people. 21 metabolites in the plasma samples of swine model, and 20 metabolites in patients with UA were found of significantly value. Among which, 8 shared metabolites between swine model and UA patients were lined in Table 1. The different metabolites were lined in Table 2. Supplemental Table 3 listed the identified compounds and their retention time.

3.2. The Results of PCA Analysis. PCA results showed that 21 metabolites classified miniswine model from sham operation group, 20 metabolites classified UA patients from healthy people, moreover, the 8 shared metabolites between swine model and UA patients can distinguish model group from sham operation group in swine and UA patients from healthy people as well. PCA results of miniswine with MI and blood stasis syndrome were showed in Figure 1. PCA results of UA patients with blood stasis syndrome and healthy people were showed in Figure 2.

3.3. The Results of Hierarchical Cluster Analysis (HCA). It showed that the 8 shared metabolites are of the same value with the 21 or 20 metabolites in classification of model group from sham operation group in swine and UA patients from healthy people (Figures 3 and 4).

Table 1: The shared metabolites in swine model and UA patients.

Number	Metabolite	P of swine model	Ascending (↑) or descending (↓)	P of UA patients	Ascending (↑) or descending (↓)
1	1,4-Benzenedicarboxylic acid	0	↓	0.019387	↓
2	1,5-Anhydroglucitol	0.044	↓	0.005023	↓
3	2-Keto-d-gluconic acid	0.003	↓	0.056989	↓
4	Azelaic acid	0	↓	0.056989	↓
5	Heptanedioic acid	0	↓	0.031348	↓
6	Pentanedioic acid	0.019	↓	0.049076	↓
7	Ribitol	0.03	↓	0.010673	↓
8	Serine	0.001	↓	0.021365	↓

Table 2: The different metabolites in swine model and UA patients.

Animal model		Clinical patients	
Metabolite	P	Metabolite	P
2-Keto-1-gluconic acid	0.002	Tetracosanoic acid	0.017749
4-Pyrimidinamine	0.011	11-Eicosanoic	0.012502
Aminomalonic acid	0	4(1H) Pyridinone	0.003801
Butanedioic acid	0.008	Docosanoic acid	0.023955
Butanoic acid	0.032	Glucopyranoside	0.005449
Decanoic acid	0.015	Myo-Inositol	0.010473
D-Fructose	0	Octadecanoic acid	6.22E-05
Glutamine	0.002	citrazinic acid	0.003344
L-Cysteine	0.032	D-gluconic acid	0.015724
L-threonine	0.001	Glucose oxime	0.005449
L-Valine	0	Hexadecanoic acid	0.00186
Thiazolidine-4-carboxylic acid	0.044	Tetracosanoic acid	0.017749
Urea	0.001		

4. Discussion

Low level expressions of the eight molecules, 1,4-benzenedicarboxylic acid, 1,5-anhydroglucitol, 2-keto-d-gluconic acid, azelaic acid, heptanedioic acid, pentanedioic acid, ribitol, and serine were detected in swine models as well as in UA patients, which could be considered as a bridge connecting the mechanism basis and macrosyndromes of swine model with MI and UA patients. But further researches need to be carried out, and more evidence needs to be investigated to confirm this hypothesis. Plasma concentration of 1,5-anhydroglucitol decreased in diabetic patients has been confirmed [11, 12]. In order to eliminate interferences,

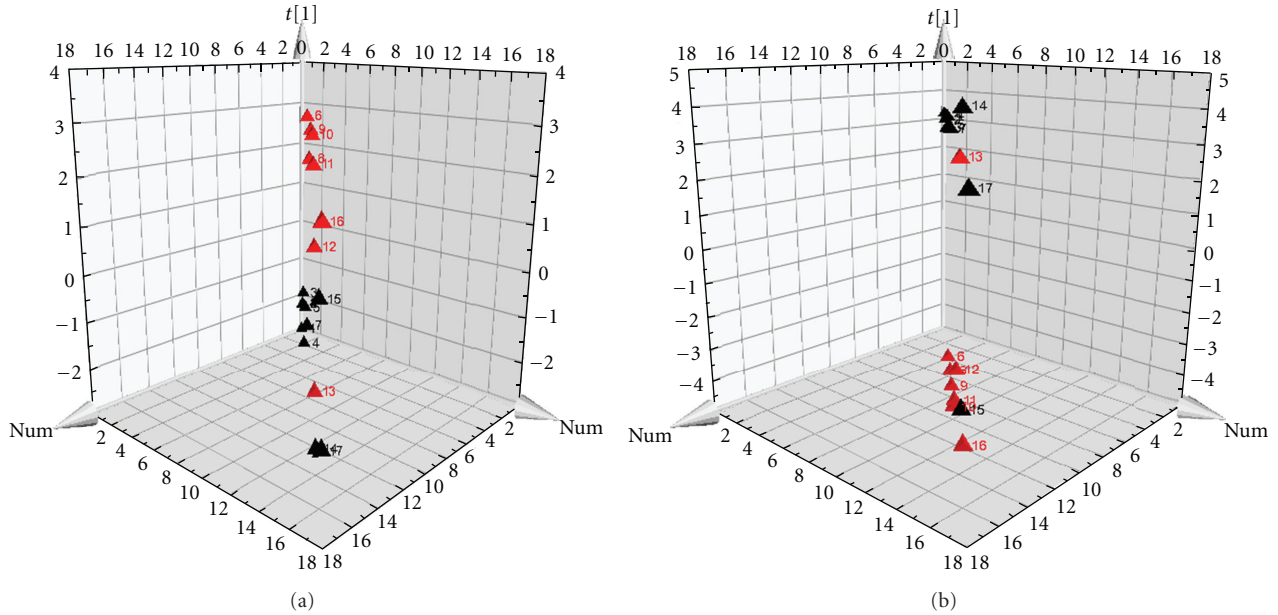

Figure 1: PCA scores' plots of miniswine. (a) is classified by the 21 metabolites in the plasma samples of swine model, and (b) is classified by the 8 same metabolites.

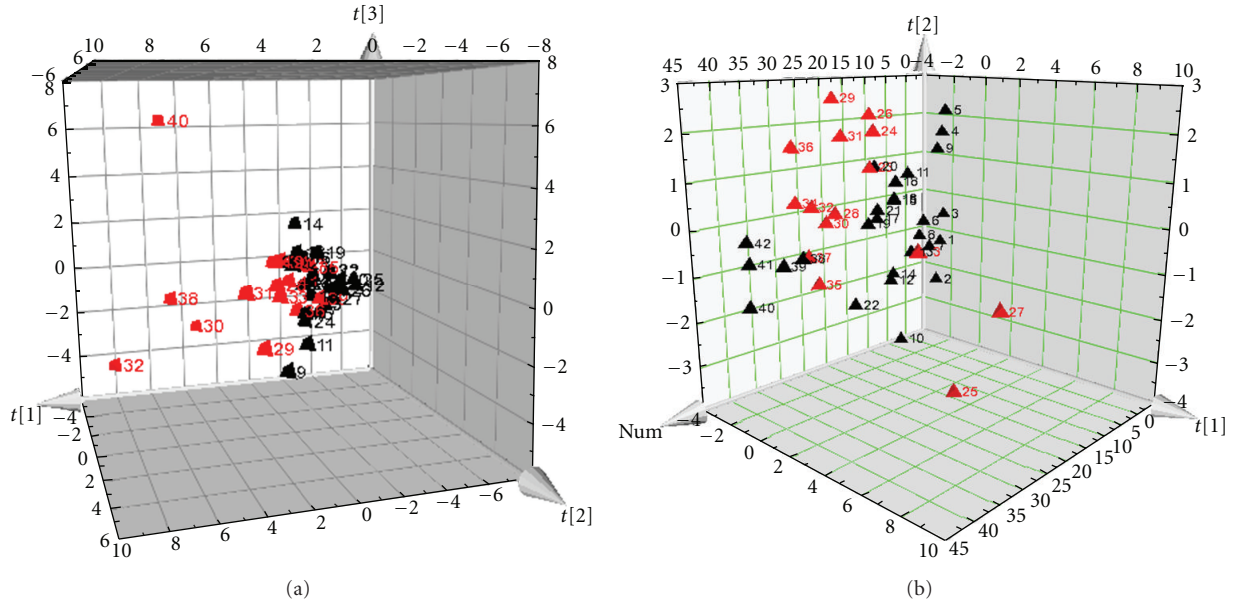

Figure 2: PCA scores plots of UA patients. (a) is classified by the 20 metabolites in the plasma samples of patients with UA, and (b) is classified by the 8 same metabolites. Notes: X-axis represents the first component of the metabolite data. Y-axis represents the second component, and Z-axis represents the third component.

diabetes patients were excluded in our research. Our study found that low expression of 1,5-anhydroglucitol existed in UA patients with blood stasis syndrome as well as in MI swine with blood stasis syndrome. Besides 1,5-anhydroglucitol, the other seven low expressed molecules are newly found. We also found that sugar and fatty acids decreased in UA patients [13]. It indicated that UA may correlate with energy metabolic obstacle and inflammatory reaction. Low level expression of the eight molecules observed in our study may provide an important target for future study.

GC-MS is mainly used in plant metabonomics [14]. In order to apply GC-MS analysis, samples need to undergo the derivatization process to increase its stability and volatility. Therefore, GC-MS analysis is limited to detect volatile substances and cannot be used to analyze thermal instable substances, such as lipid membrane, or metabolites of high

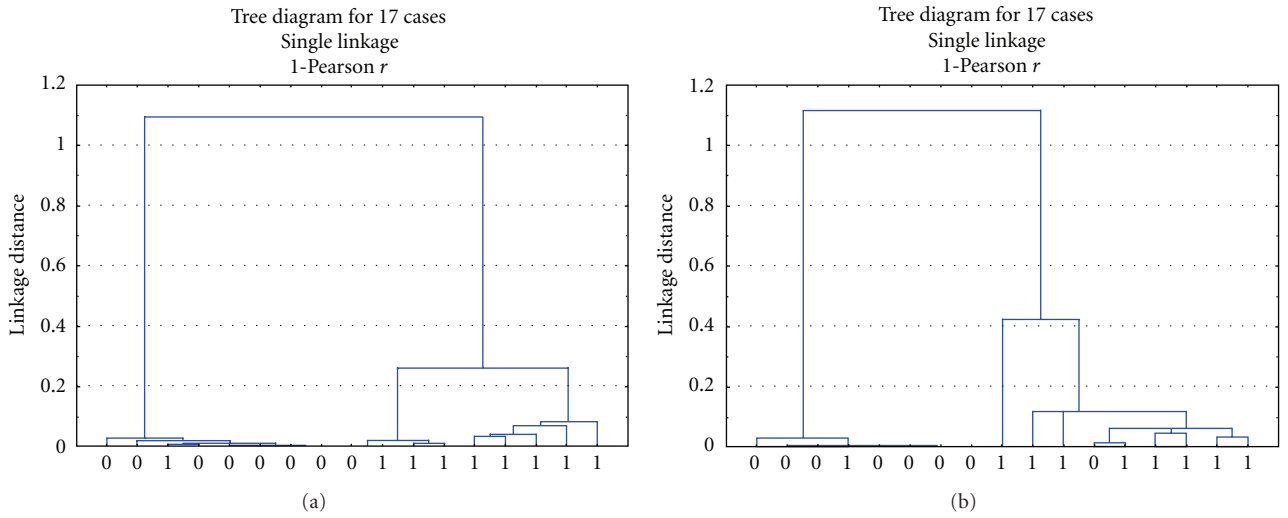

Figure 3: Hierarchical cluster diagrams of miniswine. (a) is classified by the 21 metabolites in the plasma samples of swine model and control, and (b) is classed by the 8 same metabolites. Notes: X-axis represents the sample numbers. The Y-axis represents the linkage distance (0 = control group; 1 = model group).

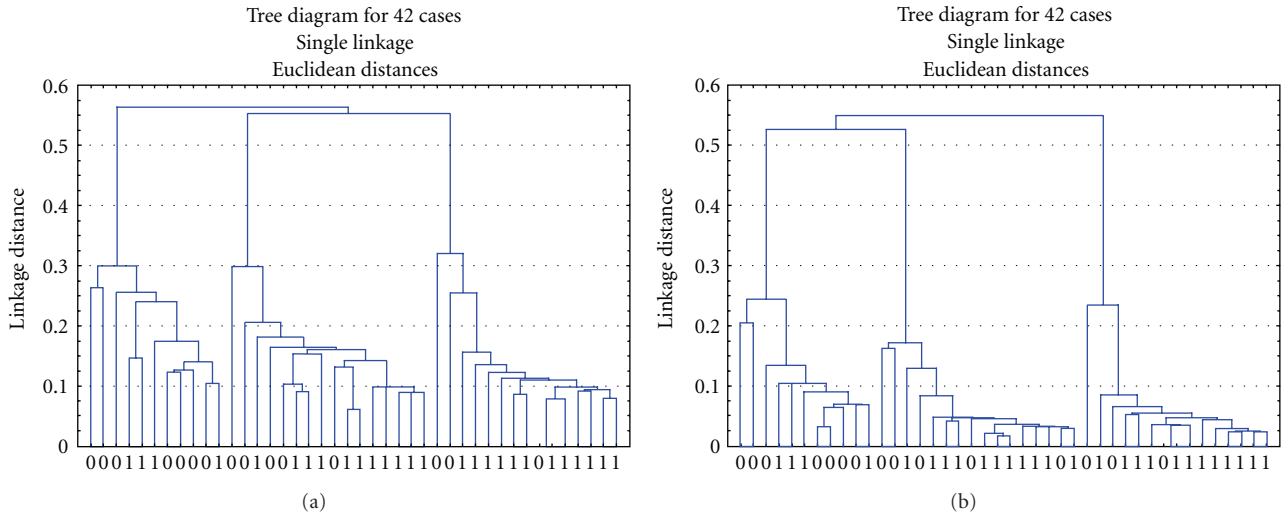

Figure 4: Hierarchical cluster diagrams of UA patients and healthy controls. (a) is classified by the 20 metabolites in the plasma samples of UA patients and healthy controls, and (b) is classified by the 8 same metabolites. Notes: X-axis represents the sample numbers. Y-axis represents the linkage distance (0 = healthy control group; 1 = UA patients group).

molecular weight. In this study, we used GC-MS to analyze metabolites difference of plasma in swine model and in UA patients. In future study, other metabonomics technology can be used in corporately to examine more metabolites and further explore the underlying mechanisms of UA. Another interesting research is to investigate whether similar metabolic changes also exist in other animal models of coronary heart disease.

5. Conclusion

In this study, the plasma samples of UA patients and MI swine model with blood stasis syndrome were used to select biomarkers in the level of metabolomics. Student's t-test was initially employed to detect metabolites that are of significant difference between model and sham operation group in swine as well as between UA patients and healthy control group. 21 metabolites in the plasma samples of swine model and 20 metabolites in patients with UA were found of significant value. Among which, 8 shared metabolites were found of low level expression in both swine model and UA patients. Then, independent student's t-test, principal component analysis (PCA), and hierarchical cluster analysis (HCA) were orderly applied to comprehend inner rules of variables in the data. The results indicated that the 8 shared metabolites can take place of the 21 or 20 metabolites in the classification of swine model with MI and patients with UA, which could be considered as a bridge connecting the mechanism basis and macrosyndromes of swine model with

MI and UA patients. This research is for the first time trying to explore a bridge connecting the mechanism basis and macrosyndromes of human disease with experimental animal models by comparing the metabolites differences in human and animal plasma samples. But further researches need to be carried out, and more evidences need to be investigated to confirm this hypothesis.

Authors' Contribution

Z. Huihui, C. Jianxin, S. Qi, and M. Xueling contributed equally to the work.

Acknowledgments

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Research Article

The Effects of Jiang-Zhi-Ning and Its Main Components on Cholesterol Metabolism

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To examine how Jiang-Zhi-Ning (JZN) regulates cholesterol metabolism and compare the role of its four main components. We established a beagle model of hyperlipidemia, fed with JZN extract and collected JZN-containing serum 0, 1, 2, 4, and 6 h later. Human liver cells Bel-7402 were stimulated with 10% JZN-containing serum as well as the four main components of JZN and Atorvastatin. The mRNA expression of LDL receptor (LDL-R), 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoAR), cytochrome P450 7A1 (CYP7A1), and acetyl-Coenzyme A acetyltransferase 2 (ACAT2) was measured by real-time PCR. LDL-R surface expression and LDL-binding and internalization were examined by flow cytometry. The results showed that JZN-containing serum significantly increased the mRNA expression of LDL-R, HMG-CoAR, and CYP7A1 in Bel-7402 cells. All the four components significantly increased the mRNA and protein expression of LDL-R and HMG-CoAR and decreased the mRNA and protein expression of ACAT2 in Bel-7402 cells. Hyperinand chrysophanol also markedly increased the mRNA expression of CYP7A1. Stimulation with stilbene glycosidesignificantly increased the surface expression of LDL-R and the binding and internalization of LDL. In conclusion, JZN and its four components have close relationship with the process of cholesterol metabolism, emphasizing their promising application as new drug candidates in the treatment of hyperlipidemia.

1. Introduction

Numerous studies have shown that cholesterol plays a key role in the development of atherosclerosis, which is the main pathological basis of cardiovascular diseases [1]. Increase in the level of serum total cholesterol, especially low-density lipoprotein (LDL) cholesterol, is of significant importance in developing diseases. Multiple clinical experiments have shown that lowering the level of serum total cholesterol, especially LDL cholesterol, can decrease lipid content in atherosclerotic plaques, lessen the shear force from blood on the cap of fibrous tissues, and reduce the secretion of proteases from foam cells that can hydrolyze extracellular matrix. This progress can stabilize plaques or even reduce their size, stop disease progression, and decrease cardiovascular morbidity and mortality [2].

Cholesterol metabolism is a complicated homeostasis involving multiple steps, including cholesterol absorption, synthesis, conversion, and modification. LDL receptor (LDL-R) plays a critical role in cholesterol absorption. In the serum, cholesterol mainly exists in the form of cholesterol ester and is carried and transported by lipoproteins, such as LDL, apolipoprotein B100 (Apo B100), and apolipoprotein E (Apo E). LDL-R binds to these lipoproteins and internalizes them into cells, providing lipids for cell proliferation and synthesis of steroid hormones and bile salt. These animals metabolized 7.1 pools of LDL-cholesterol (LDL-C) per day, and 79% of this degradation took place in the liver. Of this total turnover,

the LDLR accounted for 88% while the remaining 12% was receptor independent. 91% of the receptor-dependent transport identified in these animals was located in the liver while only 38% of the receptor-independent uptake was found in this organ [3]. Indeed, a functional deficit in LDL-R is one of the major causes of hypercholesterolemia and atherosclerosis [4, 5].

Cholesterol in the body can be obtained from food intake or from liver biosynthesis, the latter accounting for generating 70% to 80% of serum total cholesterol [6]Thus, inhibiting cholesterol biosynthesis is an effective way to reduce total cholesterol levels. Cholesterol synthesis includes 30 steps of enzymatic reaction, where 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoAR) is a rate-limiting enzyme. Currently available cholesterol-lowering drugs are mainly statins, whose main target is HMG-CoAR.

Another important step in cholesterol metabolism is the conversion of cholesterol into bile acid through cytochrome P450-meidiated oxidation. The rate-limiting enzyme for the dominant pathway of bile acid synthesis, the so-called classic pathway, is cytochrome P450 7A1 (CYP7A1). Human CYP7A1 gene defect can cause cholesterol accumulation in the liver, which has been associated with hypercholesterolemia [7]. In contrast, high-level expression of CYP7A1 increases the mRNA expression of LDL-R in liver cells and decreases the concentration of circulating LDL-cholesterol even in LDL-R-deficient mice [8]. In human, CYP7A1 expression is induced by its substrate cholesterol and inhibited by negative feedback from bile acid [9].

Another interesting participant in cholesterol metabolism is acetyl-CoA acetyltransferase 2 (ACAT2), which esterifies cholesterol in the small intestine and liver. Cholesterol esters synthesized by ACAT2 are packed into very-low-density lipoprotein and secreted into blood. It is observed in an atherosclerosis model of Apo E-deficient mice that the formation of atherosclerosis can be prevented by simultaneous knockout of ACAT2, suggesting that ACAT2-mediated cholesterol esterification is important for atherosclerosis [10].

Among all current lipid-regulating drugs, Atorvastatin shows the most significant clinical effects. Atorvastatin decreases the cholesterol level by inhibiting HMG-CoAR and promotes the transcription of LDL-R by activating the transcription factor steroid response element binding protein 2 (SREBP2). The defined targets and marked lipid-lowering effects of Atorvastatin have made it a major breakthrough in lipid-lowering drugs. Atorvastatin has now become a preferred first-line lipid-regulating drug. However, statins may cause serious side reactions, such as liver toxicity and statin myopathy, with symptoms including increases in alanine aminotransferase and aspartate aminotransferase, dermatomyositis and polymorphic myositis, and so forth. In severe cases, statins can lead to rhabdomyolysis. Therefore, it is imperative to develop novel, safe, and effective lipid-regulating drugs.

Traditional Chinese Medicine (TCM) has been used to prevent and cure atherosclerosis and lower lipid for thousands of years. Jiang-Zhi-Ning (JZN), a widely used ready-made Chinese medicine, is composed of stilbene glycoside (from ShouWu, fleeceflower root), hyperin (from ShanZha, fructus crataegi), nuciferine (from HeYe, folium nelumbinis), and chrysophanol (from JueMingZi, semen cassiae). JZN has been in clinical application for more than 1300 years and has been shown to significantly lower serum cholesterol levels. The four herbs in JZN, Fleeceflower Root, Fructus Crataegi, Folium Nelumbinis, and Semen Cassiae have been used in clinic on obesity for centuries as in "QianJinFang" (Prescriptions Worth Thousands Gold). Though at that time the disease is not called as hyperlipidemia, but recent research has confirmed that those herbs have significant effect on lowing serum cholesterol levels [11–15].

This study aims to examine the underlying mechanisms of the lipid-lowering role of JZN and compare how its four main components contribute to this function. Cholesterol metabolism after drug treatment was examined by analyzing the expression of LDL-R, HMG-CoAR, CYP7A1, and ACAT2.

2. Materials and Methods

2.1. Animals and Cell Lines. Adult laboratory beagles weighted between 10 ± 1 kg were provided by Beijing TongLi Laboratory Animal Culture Company (Beijing, China). Liver cell Bel-7402 was provided by Institute of Basic Medical Sciences of Chinese Academy of Medical Sciences (Beijing, China). After thaw, Bel-7402 cells were first cultured at 37°C in a CO_2 incubator in RPMI-1640 culture solution containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin.

2.2. Preparation of JZN Extracts and the Four Components. Fleeceflower root is the processed product of the dried root of polygonum multiflorum thunb. Folium nelumbinis is dried leaf of nelumbo nucifera gaertn. Fructus crataegi and semen cassiae are dried mature fruits of *Crataegus pinnatifida* Bge. var. major N. E. Br. and Cassia obtusifolia L., respectively. All were purchased from Beijing TongRenTang Inc. (Beijing, China) and authenticated by Professor Guijun Zhang from Beijing University of Chinese Medicine.

Fleeceflower root (25 g) and folium nelumbinis (75 g) were mixed and 25-fold of 50% ethanol was added. The mixture was heated and refluxed for 1.5 h. Then, ethanol extract was swilled and the residues were filtered and distilled twice. The three extracts were combined and concentrated into ointment, which was named as whole solid I. Fructus crataegi (500 g) and semen cassiae (25 g) were mixed and 7-fold of water was added. The mixture was heated and refluxed for 2 h. Then, the liquid extract was swilled and the residues were filtered and distilled. The two liquid extracts were combined and concentrated into ointment, which was named as whole solid II. The two whole solids were mixed and then dried using decompression drying method at 50°C.

Hyperin (from ShanZha) and stilbene glycoside (from ShouWu) were dissolved in sterilized water. Nuciferine (from HeYe) was dissolved in hydrochloric acid and then the pH was adjusted to 7.0 by sodium hydroxide. Chrysophanol and Atorvastatin were dissolved in DMSO.

2.3. Preparation of JNZ-Containing Serum. To establish a beagle model of hyperlipidemia, beagles were adaptively fed for one week and then fed with high-fat feed for two months. The fat feed was composed of 88% of usual feed, 10% of lard, and 2% of cholesterol. Beagles with hyperlipidemia were then fasted for 12 h and fed with 0.4 g/kg of JZN extract. 10 mL of vein blood was withdrawn from the back limb of beagles after 0, 1, 2, 4, and 6 h. The blood was centrifuged at 3000 r/min for 10 min to obtain serums, which were heat-inactivated for 30 min at 56°C and filtered through 0.22 μm micropore membrane. The concentration of serum LDL was measured using chemically selective inhibition method.

2.4. Isolation of LDL from Human Serum. The human serum was collected and centrifuged with gradient solution in a density of 1.0, 1.1, and 1.3 at 4500 r/min for 10 min. The yellow lipid in the center of the solution was extracted as LDL. After a 48-h dialysis in the dialysate, which contains 0.02 M Tris, 0.01% EDTA, and 0.9% NaCl, pH 7.4, the isolated LDL was kept at 4°C. The separation of LDL was confirmed by gelose gel electrophoresis with oil red O staining. LDL concentration was determined by the bicinchoninic acid method.

2.5. Stimulation of Bel-7402 Cells with JZN-Containing Serum. Bel-7402 cells were cultured in 6-well plates at $3 * 10^5$ cells per mL in 10% 1640 culture media. Alternatively, after cells became 80% confluent, the 10% 1640 media were replaced by serum-free 1640 media. After starvation for 24 h to synchronize cells to the G_0 phase, the serum-free media were replaced with 1 mL of 1640 culture media containing 10% JZN-containing serum from different time points. Isolated human LDL was added to the culture media to make the final LDL concentration in the media equal to the LDL concentrations in JZN-containing serum at different time points.

2.6. Stimulation of Bel-7402 Cells with Four Components of JZN. Bel-7402 cells in logarithmic growth were cultured in 35 mm or 100 mm flasks at $3 * 10^5$ cells per mL and starved in serum-free 1640 culture media for 24 h. After cells became 80% confluent, they were treated with the four components of JZN or Atorvastatin. The effects of each component were examined at three different concentrations: the low, medium, and high levels. The three concentrations of stilbene glycoside, nuciferine, and chrysophanol were 1, 10, and 100 μM. The three concentrations of hyperin were 0.1, 1, and 10 μM, respectively. The concentration of Atorvastatin was 10 μM. The drugs, after incubated in the CO_2 incubator with 10% BSA for 2 h, were added to cells for 24 h.

2.7. RT-PCR. Total RNA was extracted from drug-stimulated Bel-7402 cells using Trizol one-step method and then reverse-transcribed into cDNA using AMV Reverse Transcriptase. RT-PCR was conducted by the CYBR green method using following primers (Beijing Bioko Biotechnology Company, Beijing, China). β-Actin: F: 5′-GGCATCCTCACCCTGAAGTA-3′; R: 5′-GGGGTGTTGAAGGTCTCAAA-3′. LDL-R: F: 5′-GCTTGTCTGTCACCTGCAAA-3′; R: 5′-AACTGCCGAGAGATGCACTT-3′. HMG-CoAR: F: 5′-CTGGGAGCATAGGAGGCTAC-3′; R: 5′-CCACCCACCGTTCCTATCTC-3′. CYP7A1: F: 5′-GTCTTTCCAGCCCTGGTAGC-3′; R: 5′-GAGGACCACGAGGTGTGTCT-3′. ACAT2: F: 5′-CAAGGAGGTGAAGGACAAGC-3′; R: 5′-ATTGGACATGCTCTCCATCC-3′. PCR reactions were as follows: one cycle of 5 min at 95°C; 35 cycles of 1 min at 94°C, 45 s at 60°C, and 1 min at 72°C; final extension of 10 min at 72°C.

2.8. Western Blot. Equal amount of protein (50 μg) was loaded onto each lane for SDS-PAGE and transferred to membranes. Membranes were first stained with XXX primary antibodies, then with horseradish peroxidase-linked secondary antibodies, and developed with a ECL kit (Amersham, UK). Films were scanned and band intensity was quantified by ImageMaster Total Lab 1.0.

2.9. Flow Cytometry. Cells from the high-dosage group of stilbene glycoside treatment were washed twice with PBS and then once with serum-free medium. Cells were incubated for 1 h in serum-free medium containing 2% BSA and then washed with 0.5% BSA. For examining surface expression of LDL-R, cells were incubated with FITC-conjugated anti-LDL-R primary antibody and then analyzed by flow cytometry. For analyzing LDL binding and internalization, cells were incubated with 2 μL DIL-LDL for 1 h at 4°C and 37°C, respectively, and then washed and analyzed by flow cytometry.

3. Results

3.1. Effects of JZN-Containing Serum on Cholesterol Metabolism in Liver Cells. We first established a beagle model of hyperlipidemia, fed these beagles with JZN extract, and collected their serum after 0, 1, 2, 4, and 6 h. In Bel-7402 cells, 10% of JZN-containing serum from these beagles significantly increased the mRNA expression of LDL-R, HMG-CoAR, and CYP7A1 after X h stimulation ($P < 0.05$, Table 1). The maximum effect was induced by 1-h JZN-containing serum for LDL-R and HMG-CoAR and by 2-h JZN-containing serum for CYP7A1 (Table 1). In contrast, 1-h JZN-containing serum significantly decreased the mRNA expression of ACAT2 (Table 1).

3.2. Effects of Four Components of JZN on Cholesterol Metabolism in Liver Cells (mRNA Analysis). As a positive control, we first tested the effects of Atorvastatin on cholesterol metabolism. Atorvastatin treatment increased the mRNA expression of LDL-R, HMG-CoAR, and CYP7A1 and decreased the mRNA expression of ACAT2 ($P < 0.05$, Tables 2, 3, 4, and 5).

Stilbene glycoside and nuciferine showed a similar pattern of influence on cholesterol metabolism in liver cells. Compared with the no-drug control group, mRNA expression of LDL-R and HMG-CoAR in liver cells was significantly

Table 1: The effects of JZN-containing serum on cholesterol metabolism in liver cells.

Time point	LDL-R	HMG-CoAR	CYP7A1	ACAT2
0 h	1	1	1	1
1 h	3.66±0.07**	1.95 ± 0.04*	1.74±0.03**	0.73 ± 0.07
2 h	1.48 ± 0.03*	0.97 ± 0.11	1.98±0.06**	0.87 ± 0.21
4 h	1.11 ± 0.08	1.27 ± 0.06*	1.26 ± 0.08*	1.2 ± 0.04
6 h	0.87 ± 0.12	0.94 ± 0.09	0.73 ± 0.07	0.99 ± 0.09

Note: comparison with 0-h time point, $^*P < 0.05$, $^{**}P < 0.01$.

Table 2: The effects of stilbene glycoside treatment on cholesterol metabolism in liver cells (mRNA analysis).

Treatment	LDL-R	HMG-CoAR	CYP7A1	ACAT2
No stilbene glycoside	0.65 ± 0.60	0.70 ± 0.05	0.24 ± 0.05	0.98 ± 0.06
Stilbene glycoside: low dose	0.74 ± 0.04*	0.90 ± 0.08**	0.21 ± 0.07	1.07 ± 0.07
Stilbene glycoside: medium dose	0.99 ± 0.12**	0.87 ± 0.12**	0.19 ± 0.05	0.85 ± 0.22**
Stilbene glycoside: high dose	1.26 ± 0.36**	1.02 ± 0.19**	0.26 ± 0.09	0.87 ± 0.32**
Atorvastatin	1.15 ± 0.14**	1.03 ± 0.24**	0.53 ± 0.04	0.65 ± 0.08*

Note: comparison with no-drug control group, $^*P < 0.05$, $^{**}P < 0.01$.

Table 3: The effects of nuciferine treatment on cholesterol metabolism in liver cells (mRNA analysis).

Treatment	LDL-R	HMG-CoAR	CYP7A1	ACAT2
No nuciferine	1	1	1	1
Nuciferine: low dose	1.86 ± 0.84**	2.18 ± 0.26**	0.94 ± 0.17	0.97 ± 0.24
Nuciferine: medium dose	2.95 ± 0.25**	2.58 ± 0.14**	0.93 ± 0.26	0.95 ± 0.28
Nuciferine: high dose	3.57 ± 1.01**	2.96 ± 0.25**	1.15 ± 0.31	0.78 ± 0.19**
Atorvastatin	5.83 ± 0.19**	6.58 ± 0.28**	1.29 ± 0.12*	0.49 ± 0.33**

Note: comparison with no-drug control group, $^*P < 0.05$, $^{**}P < 0.01$.

Table 4: The effects of hyperin on cholesterol metabolism in liver cells (mRNA analysis).

Treatment	LDL-R	HMG-CoAR	CYP7A1	ACAT2
No hyperin	1	1	1	1
Hyperin: low dose	1.64 ± 0.27*	2.18 ± 0.19**	1.21 ± 0.18*	1.03 ± 0.21
Hyperin: medium dose	2.82 ± 0.36**	3.29 ± 0.31**	1.57 ± 0.22**	0.65 ± 0.36**
Hyperin: high dose	2.66 ± 0.24**	2.87 ± 0.25**	1.06 ± 0.19	0.61 ± 0.29**
Atorvastatin	5.82 ± 0.27**	6.48 ± 0.29**	1.29 ± 0.45**	0.49 ± 0.38**

Note: comparison with no-drug control group, $^*P < 0.05$, $^{**}P < 0.01$.

increased after stilbene glycoside or nuciferine stimulation ($P < 0.05$, Tables 2 and 3). Stimulation with stilbene glycoside (median and high doses) or nuciferine (high dose) significantly decreased mRNA expression of ACAT2 ($P < 0.01$, Tables 2 and 3). CYP7A1 mRNA expression was not affected by stilbene glycoside or nuciferine ($P > 0.05$, Tables 2 and 3).

Hyperin and chrysophanol showed similar influence on cholesterol metabolism in liver cells. Compared with the control group, stimulation with hyperin or chrysophanol also significantly increased the mRNA expression of LDL-R and HMG-CoAR ($P < 0.05$, Tables 4 and 5). Different from stilbene glycoside and nuciferine, hyperin (low and medium doses) and chrysophanol (medium and high doses) significantly increased Cyp7A1 mRNA levels ($P < 0.05$, Tables 4 and 5). Expression of ACAT2 mRNA was significantly reduced by hyperin (medium and high doses) or chrysophanol (all three doses) treatment ($P < 0.01$, Tables 4 and 5).

3.3. Effects of Four Components of JZN on Cholesterol Metabolism in Liver Cells (Protein Analysis). Atorvastatin and the four components of JZN showed similar affects. Protein expression of LDL-R and HMG-CoAR was significantly increased under all treatments ($P < 0.01$, Tables 6, 7, 8, and 9, Figures 1, 2, 3, and 4). In contrast, ACAT2 protein expression was significantly reduced under all treatments ($P < 0.01$ except for low dose of stilbene glycoside, Tables 6, 7, 8, and 9, Figures 1, 2, 3, and 4).

Table 5: The effects of chrysophanol treatment on cholesterol metabolism in liver cells (mRNA analysis).

Treatment	LDL-R	HMG-CoAR	CYP7A1	ACAT2
No chrysophanol	0.42 ± 0.07	0.41 ± 0.11	0.28 ± 0.07	0.64 ± 0.15
Chrysophanol: low dose	0.62 ± 0.04*	0.54 ± 0.19*	0.31 ± 0.12	0.42 ± 0.16**
Chrysophanol: medium dose	0.75 ± 0.03**	0.72 ± 0.14**	0.33 ± 0.09*	0.37 ± 0.18**
Chrysophanol: high dose	0.88 ± 0.09**	0.81 ± 0.22**	0.37 ± 0.14**	0.30 ± 0.07**
Atorvastatin	0.93 ± 0.07**	0.86 ± 0.18**	0.49 ± 0.13*	0.31 ± 0.13**

Note: comparison with no-drug control group, $^{*}P < 0.05$, $^{**}P < 0.01$.

Table 6: The effects of stilbene glycoside treatment on cholesterol metabolism in liver cells (protein analysis).

Treatment	LDL-R	HMG-CoAR	ACAT2
No stilbene glycoside	0.32 ± 0.02	0.38 ± 0.05	1.24 ± 0.04
Stilbene glycoside: low dose	0.41 ± 0.06**	0.44 ± 0.07	1.26 ± 0.23
Stilbene glycoside: medium dose	1.09 ± 0.05**	0.96 ± 0.21**	1.07 ± 0.25
Stilbene glycoside: high dose	1.27 ± 0.07**	1.20 ± 0.24**	1.02 ± 0.31
Atorvastatin	1.58 ± 0.03**	1.77 ± 0.08**	0.51 ± 0.06**

Note: comparison with no-drug control group, $^{*}P < 0.05$, $^{**}P < 0.01$.

Table 7: The effects of nuciferine treatment on cholesterol metabolism in liver cells (protein analysis).

Treatment	LDL-R	HMG-CoAR	ACAT2
No nuciferine	0.25 ± 0.02	0.26 ± 0.03	1.23 ± 0.02
Nuciferine: low dose	0.60 ± 0.11**	0.48 ± 0.16**	0.76 ± 0.16**
Nuciferine: medium dose	0.91 ± 0.14**	0.53 ± 0.14**	0.87 ± 0.25**
Nuciferine: high dose	0.98 ± 0.26**	0.51 ± 0.23*	0.71 ± 0.18**
Atorvastatin	1.68 ± 0.21**	0.68 ± 0.19**	0.58 ± 0.22**

Note: comparison with no-drug control group, $^{*}P < 0.05$, $^{**}P < 0.01$.

Table 8: The effects of hyperin on cholesterol metabolism in liver cells (protein analysis).

Treatment	LDL-R	HMG-CoAR	ACAT2
No hyperin	0.17 ± 0.05	0.73 ± 0.03	0.74 ± 0.06
Hyperin: low dose	0.33 ± 0.08**	1.41 ± 0.07**	0.53 ± 0.02**
Hyperin: medium dose	0.29 ± 0.11*	1.47 ± 0.16**	0.48 ± 0.15**
Hyperin: high dose	0.62 ± 0.09**	1.54 ± 0.07**	0.57 ± 0.16*
Atorvastatin	0.86 ± 0.13**	2.69 ± 0.25**	0.52 ± 0.18*

Note: comparison with no-drug control group, $^{*}P < 0.05$, $^{**}P < 0.01$.

Table 9: The effects of chrysophanol treatment on cholesterol metabolism in liver cells (protein analysis).

Treatment	LDL-R	HMG-CoAR	ACAT2
No chrysophanol	0.23 ± 0.08	0.25 ± 0.04	0.36 ± 0.12
Chrysophanol: low dose	0.38 ± 0.11*	0.27 ± 0.17	0.32 ± 0.06
Chrysophanol: medium dose	0.61 ± 0.15**	0.29 ± 0.12	0.21 ± 0.11*
Chrysophanol: high dose	0.95 ± 0.13**	0.51 ± 0.14**	0.19 ± 0.09*
Atorvastatin	1.61 ± 0.26**	0.97 ± 0.28**	0.22 ± 0.07*

Note: comparison with no-drug control group, $^{*}P < 0.05$, $^{**}P < 0.01$.

3.4. Effects of Stilbene Glycoside Treatment on Surface Level of LDL-R. Our flow cytometry analysis showed that similar to Atorvastatin, stilbene glycoside significantly increased surface expression of LDL-R, as well as binding and internalization of LDL ($P < 0.01$, Table 10 and Figure 5).

4. Discussion

Hyperlipidemia causes progressive atherosclerosis, which is the major cause of cardiovascular diseases. Clinically, 80% to 90% of acute coronary events are triggered by sudden collapse of atherosclerotic plaques [16]. The stability of plaques is related to lipid content: the higher the lipid content, the lower the stability. In addition, hyperlipidemia is also an important risk factor for hypertension, impaired glucose tolerance, and diabetes. Hyperlipidemia can also lead to hepatic adipose infiltration, cirrhosis, gallstones, pancreatitis, fundus hemorrhage, blindness, peripheral vascular disease, claudication, and hyperuricemia. Some primary and familial hyperlipidemia may also cause tendon-like, nodular, palm print, periorbital xanthomas, and arcus juvenilis. Therefore, hyperlipidemia treatment has become a hot topic in recent years.

Many different types of lipid-regulating drugs, including resins, statins, niacin, and unsaturated fatty acid lipid-regulating drugs, have been developed. However, most of these drugs only focus on one step of cholesterol metabolism. Few currently available drugs target cholesterol synthesis, absorption, modification, and conversion all together. In addition, most lipid-regulating drugs cause serious side effects, including gastrointestinal problems and liver and kidney dysfunction.

Numerous lipid-lowering formulae have been recorded in TCM. However, their application has been limited due to a lack of thorough understanding of both their effective ingredients and underlying mechanisms. There is also no standardized quality control in drug preparation. Therefore, it is imperative to examine active ingredients from known lipid-lowering TCM for their pharmacological effects. This has been proven to be an effective strategy to develop novel, more efficacious lipid-regulating drugs with fewer side effects. It has been reported that the activity of LDL-R can be enhanced by tea polyphenol as well as the water-soluble and ethanol-soluble extracts of turmeric [17, 18]. The expression of LDL-R in rat liver is increased by guanxinkang decoction that is composed of astragalus, trichosanthes, scalion white, and salvia miltiorrhiza [19, 20]. The expression of LDL-R and high-density lipoprotein receptor is also increased by tiaogandaozhuo decoction consisting of processed polygonum multiflorum, radix bupleuri, cassia, alisma, salvia miltiorrhiza, fructus leonuri, tumeric, cattail pollen, and so forth [21]. Up to now, studies in this area have mainly focused on regulation on LDL. The current study aims to extend research on lipid-lowering TCM to cholesterol absorption, synthesis, conversion, and other steps in cholesterol metabolism.

JZN shows significant cholesterol-lowering effects. Polygonum multiflorum thunb, hawthorn, lotus leaf, and cassia seed are all frequently used as lipid-lowering TCM. Total glycoside from polygonum multiflorum thunb shows significant lipid-lowering and antioxidant effects [22]. The total cholesterol and triglyceride values in hyperlipidemic mice are significantly reduced by hawthorn flavonoids of different purity [23]. Furthermore, different proportions of cassia seed extract and hawthorn extract have been shown to reduce the levels of total cholesterol, triglyceride, LDL-cholesterol, and Apo B in hyperlipidemic mice [24].

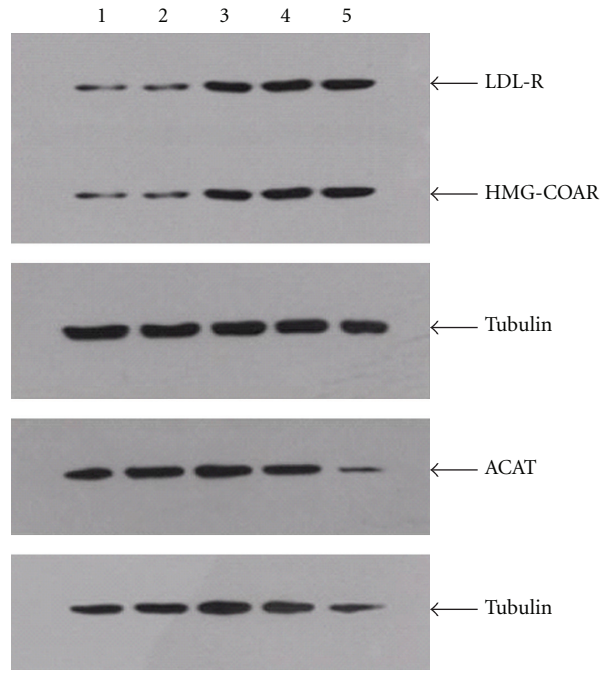

Figure 1: LDL-R, HMG-CoAR, and ACAT2 protein expression under stilbene glycoside treatment. Notes: Lane 1: no-drug control; 2: low dose of stilbene glycoside; 3: medium dose of stilbene glycoside; 4: high dose of stilbene glycoside; 5: Atorvastatin.

However, the current understanding of JZN or its four major components is still far from satisfactory. Stilbene glycoside has been shown to exert neuroprotectivity, antioxidation, and lipid-lowering effects [25, 26]. But its mechanisms remain unclear. Pharmacological studies of hyperoside mainly focus on analgesia and the protection of cardiovascular system whereas its effect on hyperlipidemia has not been reported [27]. The role of nuciferine has been studied in models of obese hyperlipidemic rats [28]. The function of chrysophanol is hardly known.

In the current study, we first examined the role of JZN in lipid metabolism in liver cells. It is worth noting that for a long time, a routine TCM pharmacological experiment is conducted as follows: ingredients of TCM are first extracted and separated and then added directly to an *in vitro* system (e.g., cell culture), and the effects of various ingredients are added to illustrate the effectiveness of a TCM recipe. Research on pharmacodynamic material basis and mechanism of Chinese herbal compound is still the difficult and hot spot in Chinese medicine research. Study on serum pharmacochemistry of herbal compound

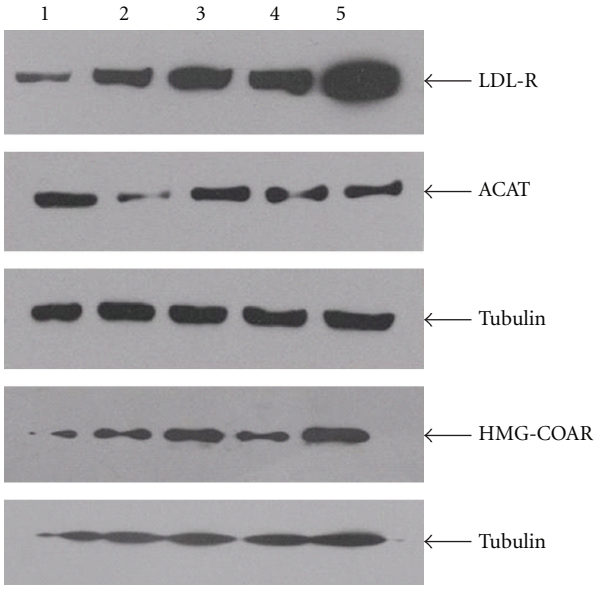

Figure 2: LDL-R, HMG-CoAR, and ACAT2 protein expression under nuciferine treatment. Notes: Lane 1: no-drug control; 2: low dose of nuciferine; 3: medium dose of nuciferine; 4: high dose of nuciferine; 5: Atorvastatin.

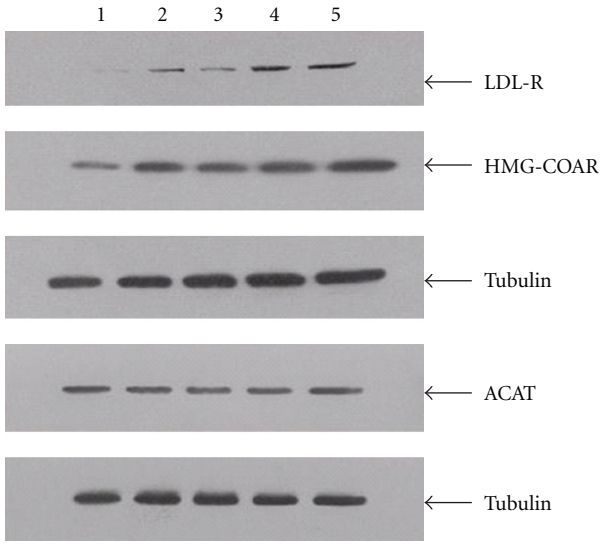

Figure 3: LDL-R, HMG-CoAR, and ACAT2 protein expression under hyperin treatment. Notes: Lane 1: no-drug control; 2: low dose of hyperin; 3: medium dose of hyperin; 4: high dose of hyperin; 5: Atorvastatin.

directly from the components that absorbed into the body directly narrows the scope of constituent research and provides a new way for herbal compound research. But it is still at the exploratory stage, there are many difficulties in practical research. In our experiment, we found that the serum medicine concentration is low in case of Chinese herbal compound taken by oral and due to the experimental conditions and technical limitations, which is still a challenge

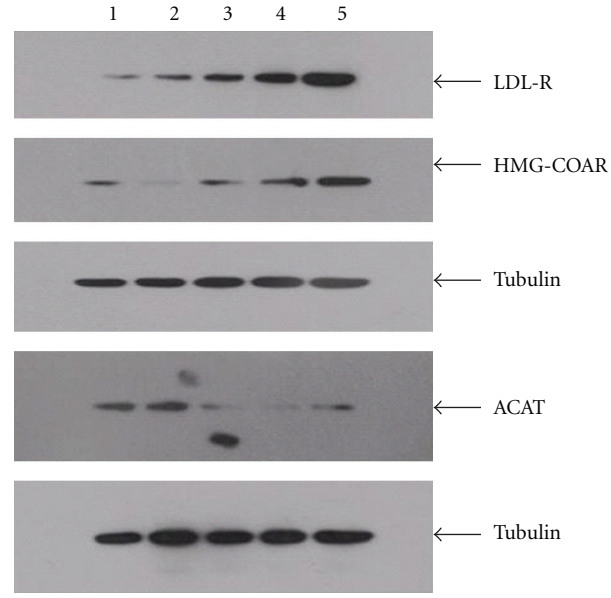

Figure 4: LDL-R, HMG-CoAR, and ACAT2 protein expression under chrysophanol treatment. Notes: Lane 1: No-drug control; 2: Low dose of chrysophanol; 3: Medium dose of chrysophanol; 4: High dose of chrysophanol; 5: Atorvastatin.

in drug-testing. However, this method neglects the following characteristics of TCM: (1) in most cases, TCM is taken orally and thus the ingredients entering blood after metabolism in the gastrointestinal tract and liver may differ significantly from the original ingredients; (2) the components of TCM may interact and cross-talk and therefore, the overall effect of TCM does not equal the sum of the effects of individual ingredients; (3) the physical and chemical properties of TCM components in extracts are not constant from study to study, making it difficult to reach consensus conclusions. Based on the above considerations, Shinichi Tashiro proposed using medicine-containing serum for *in vitro* pharmacological studies [29]. Chinese herbal compound exerts its pharmacodynamics effect after oral administration and gastrointestinal absorption into blood and body interactions. Applying serum pharmacology method, the use of the medicine-containing serum *in vitro* experiment is more closely related to the real process of exerting pharmacological effects *in vivo* environment. This method provides a new way for pharmacodynamic material basis research of Chinese herbal compound.

Medicine-containing serum is collected from animals fed with TCM after a certain period of time. This TCM serum method mimics the genuine route of TCM to take effects *in vivo* and thus provides more information about the pharmacological role of TCM. We adopted this method and demonstrated that in liver Bel-7402 cells, 10% JZN-containing serum significantly increases the mRNA expression of LDL-R, HMG-CoAR, and CYP7A1. Since it is crucial to observe the efficacy of different time points of JZN-containing serum to further determine which point in time to extract and separate the serum-containing material.

Table 10: The effects of stilbene glycoside treatment on the surface expression of LDL-R.

Treatment	LDL-R Expression	LDL Binding	LDL Internalization
No-drug	11.68 ± 3.72	18.47 ± 2.34	7.94 ± 2.95
Stilbene glycoside	24.42 ± 5.21**	34.06 ± 6.29**	81.01 ± 5.22**
Atorvastatin	51.07 ± 6.34**	109.88 ± 10.60**	135.23 ± 17.63**

Note: comparison with no-drug control group, $^{**}P < 0.01$.

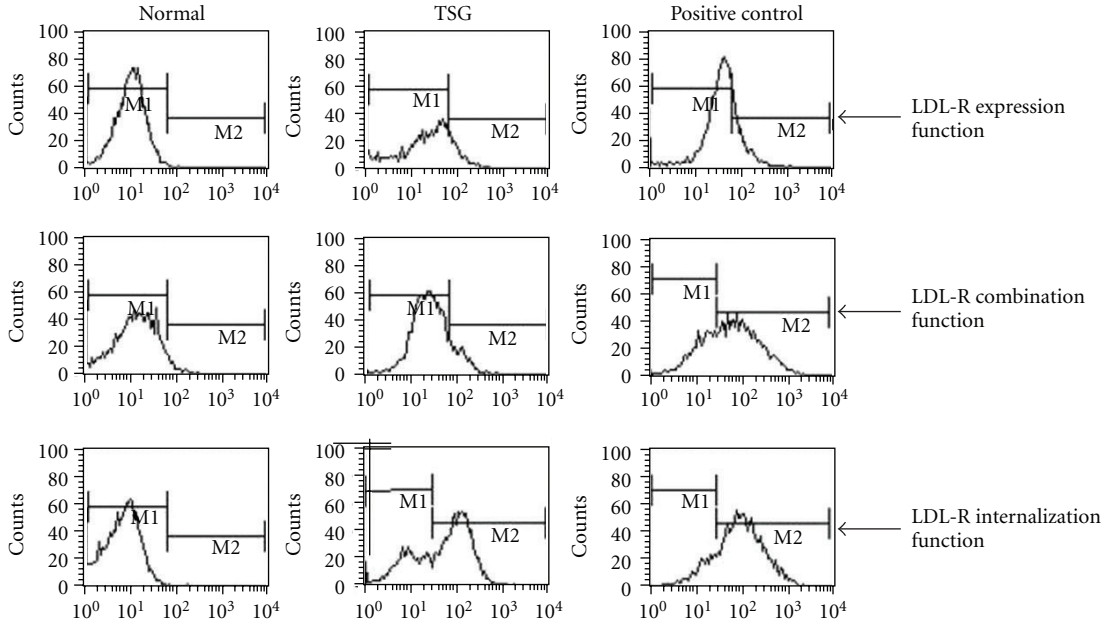

Figure 5: The effects of stilbene glycoside treatment on the surface expression of LDL-R. Treatment of stilbene glycoside or atorvastatin increased LDL-R surface expression as well as LDL binding and internalization.

According to the reported drug metabolism *in vivo*, about 80 percent reported that the process of drug metabolism is within two hours. So our experiments set 0, 1, 2, 4, 6 hours after administration as five time points. However, due to the complexity of endogenous components of the serum itself, if we use blank serum as a control, it will be another difficulty in determining the time points of blank serum. In this study, we use the LDL concentration in the medium calibration to ensure a consistent amount of stimulation.The maximum induction effect is achieved by 1-h JZN-containing serum for the former two molecules and by 2-h JZN-containing serum for the last one. JZN-containing serum collected after 1 h significantly reduces the mRNA expression of ACAT2. The different effects at different time points may reflect changes in effective ingredients of the plasma drug following drug uptake.

Cholesterol absorption and synthesis are negatively regulated by their end products. When cholesterol levels are low (e.g., when treated with Atorvastatin), the transcription factor SREBP is activated and triggers the transcription of LDL-R and HMG-CoAR [30]. The dominant regulator of CYP7A1 transcription is liver X receptor α (LXRα), a sterol-dependent nuclear receptor [31, 32]. The LXR response element DR4 has been found to interact with the promoter region of CYP7A1 in human and mice [33]. The current study suggested that JZN-containing serum increases the mRNA levels of LDL-R, HMG-CoAR, and CYP7A1 through the SREBP and LXR transcription pathways.

In this study, all four major components of JZN, stilbene glycoside, nuciferine, hyperin, and chrysophanol, significantly increase the mRNA and protein expression of LDL-R and HMG-CoAR and significantly reduce the mRNA and protein expression of ACAT2. Hyperin and chrysophanol also significantly increase the mRNA expression of CYP7A1. In addition, stilbene glycoside increases the surface expression of LDL-R as well as the surface binding and internalization of LDL. Our results showed that the components of JZN may exert lipid-lowering effects by increasing the surface expression and activity of LDL-R in liver cells, inhibiting intracellular cholesterol synthesis, increasing the conversion of cholesterol into bile acid, and reducing ACAT2-mediated cholesterol esterification. Our study suggested that these four components of JZN are promising candidates for developing new medications to treat hyperlipidemia and related diseases.

Authors' Contribution

Jianxin Chen, Huihui Zhao, and Xueling Ma contributed equally to this work.

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Research Article

Korean Red Ginseng Improves Blood Pressure Stability in Patients with Intradialytic Hypotension

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Introduction. Intradialytic hypotension (IDH) is a common complication during hemodialysis which may increase mortality risks. Low dose of Korean red ginseng (KRG) has been reported to increase blood pressure. Whether KRG can improve hemodynamic stability during hemodialysis has not been examined. *Methods*. The 8-week study consisted of two phases: observation phase and active treatment phase. According to prehemodialysis blood pressure (BP), 38 patients with IDH were divided into group A (BP $\geq$ 140/90 mmHg, $n = 18$) and group B (BP $<$ 140/90 mmHg, $n = 20$). Patients were instructed to chew 3.5 gm KRG slices at each hemodialysis session during the 4-week treatment phase. Blood pressure changes, number of sessions disturbed by symptomatic IDH, plasma levels of vasoconstrictors, blood biochemistry, and adverse effects were recorded. *Results*. KRG significantly reduced the degree of blood pressure drop during hemodialysis ($P < 0.05$) and the frequency of symptomatic IDH ($P < 0.05$). More activation of vasoconstrictors (endothelin-1 and angiotensin II) during hemodialysis was found. The postdialytic levels of endothelin-1 and angiotensin II increased significantly ($P < 0.01$). *Conclusion*. Chewing KRG renders IDH patients better resistance to acute BP reduction during hemodialysis via activation of vasoconstrictors. Our results suggest that KRG could be an adjuvant treatment for IDH.

1. Introduction

Intradialytic hypotension (IDH) is a common complication which occurs in 20–30% of all dialysis treatments [1]. It has a negative impact on quality of life due to associated symptoms such as nausea, vomiting, dizziness, and cramps. IDH may cause premature discontinuation of hemodialysis that may lead to chronic underdialysis and higher mortality risks [1, 2].

The mechanism responsible for IDH is an inappropriate response of cardiovascular and neurohormonal systems to the acute plasma volume removal during dialysis [3–5]. Hemodialysis prescriptions include ultrafiltration amount, dialysate composition, and solution temperature may affect the frequency of IDH [6–8]. Older age, ischemic heart disease, diabetes, and autonomic neuropathy increase the risk of developing IDH [9–11]. Common interventions to prevent IDH include adaptation of dialysis prescriptions, avoidance of food during dialysis, and administration of vasoconstrictor agents (e.g., midorine, adenosine antagonist) [1, 6, 12–14].

Panax ginseng, a naturally occurring compound that has been used for several thousand years in the orient [15], is one of the most popular herbs in the world due to its therapeutic

effects on modulating immune and cardiovascular functions [16]. Previous studies on *Panax ginseng* have confirmed its effect on regulating blood pressure [15]. Interestingly, *Panax ginseng* at low-doses can elevate blood pressure, while high-dose *Panax ginseng* has hypotensive effect in healthy subjects [17–19]. However, the potential therapeutic effect of *Panax ginseng* in patients with IDH has not been examined.

Korean red ginseng (KRG), a steamed form of *Panax ginseng* with preserved major constituents, has been shown to possess more biological activity than *Panax ginseng* [20–22]. Here we conducted a prospective study to evaluate the effects of KRG on the occurrence of symptomatic IDH during hemodialysis. We also examined the changes of endothelin-1 (ET-1), plasma renin activity (PRA), angiotensin II (AngII), and nitric oxide (NO) products during hemodialysis with and without oral KRG administration.

2. Material and Methods

2.1. Participants. The study was conducted at the hemodialysis center in Taoyuan Chang Gung Memorial Hospital (TCGMH), Taiwan. The study was approved by Chang Gung Memorial Hospital Ethical Review Committees. Each patient signed informed consent before enrollment. Patients aged from 20- to 80-year-old on thrice-weekly hemodialysis, with a treatment time of at least 180 minutes, and had at least three symptomatic episodes of IDH in the 30 days preceding enrollment, were enrolled in the study. IDH was defined as a decrease in systolic blood pressure ($\geq$20 mmHg) or a decrease in mean arterial pressure ($\geq$10 mmHg) accompanied by symptoms (dizziness, cramps, or fatigue, etc.), according to Kidney Disease Outcomes Quality Initiative Clinical Practice Guidelines for Cardiovascular Disease in Dialysis Patients [23]. The exclusion criteria included pregnancy or breast feeding, active infectious disease, current intake of antihypotensive medication or warfarin, and frequent changes of dry body weight ($> \pm 1\%$ during the screening phase) and severe medical conditions included liver cirrhosis, heart failure, autoimmune disease, and cancer. Of the 768 patients receiving chronic dialysis in our hospital, 74 patients were eligible for this study; 46 patients were enrolled, while 28 patients declined to participate (Figure 1).

2.2. Study Design. The prospective study was designed to evaluate the pre- and posttreatment differences, and each patient served as his/her own control. The 8-week study consisted of two phases: an observation phase (phase I) and an active treatment phase (phase II). Each phase was composed of twelve consecutive HD treatments (4 weeks). Patients were given standard treatment for IDH during phase I. These enrolled patients were divided into two groups according to their prehemodialysis blood pressure. Patients with average prehemodialysis blood pressure more than 140 mmHg systolic or 90 mmHg diastolic during phase I were clustered to group A, while patients with normal or low prehemodialysis blood pressure to group B.

2.3. Treatment. During the active treatment phase (phase II), patients were instructed to chew 3.5 gm KRG slices at each hemodialysis session. Each slice of KRG was put into the mouth until melted, then chewed and swallowed. Patients in group A (hypertensive at baseline) were given KRG slices 60 minutes after the onset of hemodialysis to prevent late onset of hypotension after ultrafiltration. In contrast, patients in group B (normotensive or hypotensive at baseline) were given KRG slices 30 minutes before hemodialysis to prevent early onset of hypotension [24]. Cheong-Kwan-Jang Korean red ginseng (Korea Ginseng Corporation; Korea) was used in this study. The active constituents of KRG are ginsenosides including Rb1 (1.96%), Rb2 (2.18%), Rc (1.47%), Rd (0.72%), Re (1.11%), Rf (0.24%), Rg1 (0.49%), Rg2 (0.13%), Rg3 (0.12%), Rh1 (0.12%), and Rh2 (0.003%) [25]. Routine dialysis prescriptions including dialyzer types, dialysate compositions, dialysate temperature, dialysis frequencies, treatment time, and antihypertensive medications were maintained constantly throughout two study phases.

2.4. Outcome Parameters. Arterial blood pressure was measured with an electronic digital sphygmomanometer every 60 minutes from the beginning to the end of each dialysis session. In the event of IDH, the blood pressure was checked every 10 minutes. Prehemodialysis (pre-HD), posthemodialysis (post-HD), and intradialytic lowest (nadir) blood pressure at each dialysis session were recorded. The difference of SBP, DBP, and MAP between prehemodialysis and nadir, and between prehemodialysis and posthemodialysis, were calculated. The number of sessions disturbed by symptomatic IDH was defined as hypotensive episodes that require medical intervention including transient reduction or premature stop of ultrafiltration, infusion of isotonic or hypertonic saline, or glucose solution. These parameters during each study phase were recorded by the nurses at the hemodialysis center. Any adverse effects that might be related to KRG were recorded.

2.5. Assays. In the last dialysis sessions of each study phase, plasma levels of ET-1, PRA, AngII, and NO products were checked before and after dialysis. Blood samples were collected with prechilled polypropylene tubes containing 1 mg/mL of K2-EDTA and 500 KIU/mL aprotinin (Sigma). Plasma was separated by centrifugation at 3000 rpm for 15 minutes at 4°C and stored at -70°C until assayed. Specific antibodies and radioimmunoassay kits were used to assay PRA (DiaSorin, MN, USA), AngII (Phoenix Pharmaceuticals Inc. USA), and ET-1 (Peninsula Laboratories, LLC, USA). The final products of NO metabolism were examined using an assay for detecting the plasma levels of the nitrate+nitrite (NT) (Cayman Chemical Company, Ann Arbor). Hematocrit, albumin, electrolytes, and alanine aminotransferase were monitored at the beginning and end of the study by autoanalyzer.

2.6. Statistical Analyses. All numerical values are expressed as mean$\pm$standard deviation (SD). Baseline variables are compared with the χ^2 test for dichotomous variables. The Mann-Whitney U test is applied to compare intergroup differences. The Wilcoxon signed-rank test is used for intragroup

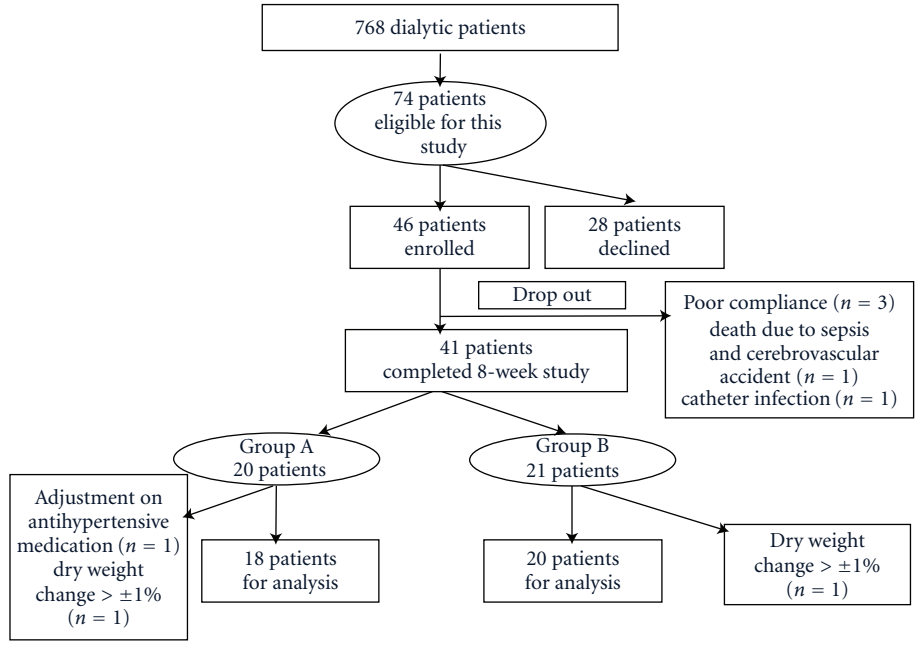

Figure 1: Flow chart of patient recruitment. Patients aged from 20 to 80 year old on thrice-weekly hemodialysis, with a treatment time of at least 180 minutes and had at least three symptomatic episodes of intradialytic hypotension (IDH) in the 30 days preceding enrollment, were enrolled in the study.

Table 1: Baseline characteristics of study patients.

	Total ($n = 38$)	Group A (HTN) ($n = 18$)	Group B (Non-HTN) ($n = 20$)	P value
Age (year)	52.6 ± 12.4	56.2 ± 12.7	49.3 ± 11.5	0.083
Sex (% Female)	25 (65.8%)	11 (61.1%)	14 (70%)	0.734
Dry BW (kg)	60.7 ± 16.6	62.5 ± 15.0	59.0 ± 18.1	0.515
BMI	23.6 ± 5.2	24.1 ± 4.2	23.1 ± 6	0.579
Time on dialysis (months)	93.0 ± 61.9	67.9 ± 48.7	115.5 ± 64.9	0.016*
Comorbidities				
DM	15 (39.5%)	13 (72.2%)	2 (10%)	<0.001**
HTN history	26 (68.4%)	18 (100%)	8 (40%)	<0.001**
Previous MI	1 (2.6%)	1 (5.6%)	0 (%)	0.474
Angina history	5 (13.2%)	3 (16.7%)	2 (10%)	0.653

Values are expressed as means ± SD or number (%); BMI, body mass index; BW, body weight; DM, diabetes mellitus; HTN, hypertension; MI, myocardial infarction; $^{*}P < 0.05$; $^{**}P < 0.01$.

comparison between phase I and phase II. A two-tailed $P < 0.05$ is considered statistically significant. Statistical analysis is performed with analytical Software SPSS 12.0 version.

3. Results

3.1. Patient Characteristics. Of the 46 enrolled patients, 21 patients were grouped into group A and 25 patients to group B according to prehemodialysis blood pressure in the observation phase (phase I) (Figure 1). In total, 41 patients completed the KRG treatment phase (phase II), and five patients dropped out of the study prematurely due to poor compliance ($n = 1$ in group A; $n = 2$ in group B), catheter infection ($n = 1$ in group B), and death due to sepsis and cerebrovascular accident ($n = 1$ in group A) which was not directly related to KRG use. For the statistical analyses, three patients were excluded due to adjustment of antihypertensive medications ($n = 1$ in group A) and changes of dry weight exceeding ±1% ($n = 2$ in group B). Finally, 18 patients in the group A and 20 patients in the group B were included for analysis.

Baseline patient characteristics of the 38 patients of the analysis population are listed in Table 1. The mean age was 53 ± 12 years; 25 (65.8%) were females and 13 (34.2%) were males. Compared to patients in group B (normal or low prehemodialysis BP), patients in group A (high prehemodialysis BP) had a significantly shorter average time on maintenance dialysis (68 ± 49 versus 116 ± 65 months, $P = 0.016$) and a higher percentage of diabetes mellitus (72.2% versus 10%, $P < 0.001$).

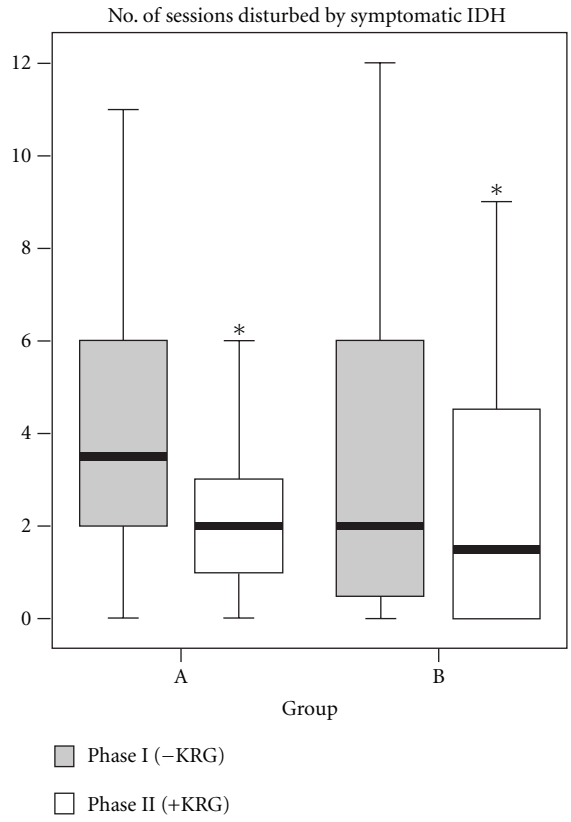

Figure 2: Comparison of the frequency of intradialytic hypotension (IDH) with and without Korean red ginseng (KRG) treatment. Phase I is the control phase (grey boxes). Phase II is the KRG treatment phase (white boxes). The number of sessions disturbed by symptomatic IDH reduced significantly after KRG treatment in both group A (hypertensive at baseline, $n = 18$, $P = 0.016$) and group B (normotensive or hypotensive at baseline, $n = 20$, $P = 0.035$). Data are shown as box and whisker plots. Horizontal lines represent median values. The boxes encompass the first and 3rd quartile of the included data. The bars give the 95% confidence interval of the values. $^*P < 0.05$.

3.2. Effect of KRG on Symptomatic IDH. We examined the clinical effects of KRG on symptomatic IDH. The number of sessions disturbed by symptomatic IDH reduced significantly from 3.9 ± 3.1 times (per 12 times) to 2.7 ± 3.1 times in group A ($P = 0.016$) and from 3.9 ± 3.9 times to 2.7 ± 3.5 times in group B ($P = 0.035$) (Figure 2). The average pre- and postdialysis body weight, dry weight, and the amount of actual ultrafiltration were not significantly different between two study phases (Table 2). Thus, we could exclude the effect of altered ultrafiltration amount that might have affected the occurrence of IDH.

3.3. Effect of KRG on Blood Pressure during Hemodialysis. We looked at the effects of KRG in regulating the blood pressure changes during hemodialysis (Table 3). We found that the nadir SBP in group B was significantly elevated from 76.3 ± 16.2 mmHg in phase I to 79.1 ± 15.8 mmHg in phase II ($P = 0.045$). A similar trend of increased nadir SBP was noted in group A (97.4 ± 16.0 in phase I versus 100.1 ± 18.6 in phase II; $P = 0.184$). However, the administration of KRG did not significantly change prehemodialysis or posthemodialysis blood pressure.

Furthermore, we calculated the differences of blood pressure measured at different time points (prehemodialysis to nadir and to posthemodialysis) (Table 4). Notably, we found that patients in group B experienced less blood pressure changes during phase II (−24.4 ± 10.1 mmHg of Nadir-Pre SBP in phase I to −20.4 ± 11.0 mmHg in phase II, $P = 0.045$; −13.2 ± 7.1 mmHg of nadir-pre-DBP in phase I to −10.4 ± 6.6 mmHg in phase II, $P = 0.011$; −16.9 ± 7.1 mmHg of nadir-pre-MAP in phase I to −13.8 ± 7.7 mmHg in phase II, $P = 0.015$; −11.0 ± 6.3 mmHg to −7.8 ± 6.3 mmHg in post-pre-DBP, $P = 0.025$). A similar trend was also noted in group A after KRG treatment (−50.7 ± 39.3 mmHg of nadir-pre-SBP in phase I to −42.0 ± 23.8 mmHg in phase II, $P = 0.053$; −40.3 ± 39.3 mmHg of nadir-pre-MAP in phase I to −31.8 ± 24.8 mmHg in phase II, $P = 0.02$). When all patients were included for analysis, the blood pressure drop reduced significantly in the KRG-treated period (−36.9 ± 30.7 mmHg of nadir-pre-SBP in phase I to −31.0 ± 21.3 mmHg in phase II, $P = 0.006$; −22.4 ± 14.5 mmHg of nadir-pre-MAP in phase I to −19.3 ± 12.7 mmHg in phase II, $P = 0.004$). These results indicate that KRG may help to keep hemodynamic stability in IDH patients.

3.4. Effect of KRG on PRA, ET-1, AngII, and NT. To investigate the action mechanism of KRG, we examined the plasma levels of PRA, ET-1, AngII, and NT (nitrite + nitrate). In the observation phase (phase I), PRA levels increased significantly after hemodialysis in group B ($P = 0.004$) but less evident in group A ($P = 0.071$). The level of ET-1 and AngII did not significantly increase despite an average ultrafiltration of 2.6 ± 1.0 kg with removal of fluid in both groups ($P > 0.05$).

After four weeks of KRG treatment (phase II), the posthemodialysis PRA levels increased significantly in both groups ($P = 0.005$ in group A, $P < 0.001$ in group B) (Figure 3). Similarly, the posthemodialysis ET-1 levels increased significantly by one- to threefold compared to the prehemodialysis levels ($P = 0.035$ in group A, $P = 0.011$ in group B), which is in contrast to the trend in phase I (Figure 3). The levels of AngII also significantly increased after dialysis in group A ($P = 0.033$), but not in group B ($P = 1.000$). A previous study has shown that NT can be removed by hemodialysis [4]. As expected, the levels of NT decreased significantly after dialysis in all groups. Nevertheless, the posthemodialysis NT levels which would cause vasodilation were significantly lower in the KRG treatment phase than those in the observatory phase ($P < 0.05$ in group A) (Figure 3). These results suggest that KRG treatment may improve the compensatory response mediated by various vasoconstrictors to acute volume change during hemodialysis.

Unexpectedly, we observed a two- to threefold declination of prehemodialysis ET-1 levels in both groups with KRG treatment (1.627 ± 1.460 to 0.497 ± 0.202 ng/mL in group A, $P = 0.014$; 2.042 ± 1.127 to 1.023 ± 1.190 ng/mL in group B, $P = 0.047$) as shown in Figure 3. The same holds true

Table 2: Comparison of body weights and ultrafiltration rates with and without Korean red ginseng treatment.

	Group A (HTN)		*P* value	Group B (non-HTN)		*P* value	All		*P* value
	Phase I (−KRG)	Phase II (+KRG)		Phase I (−KRG)	Phase II (+KRG)		Phase I (−KRG)	Phase II (+KRG)	
BW predialysis (Kg)	65.3 ± 15.5	65.4 ± 15.4	0.862	61.6 ± 18.8	61.7 ± 18.8	0.218	63.4 ± 17.2	63.4 ± 17.1	0.415
BW postdialysis (Kg)	62.7 ± 15.0	62.8 ± 15.0	0.296	59.0 ± 18.1	58.9 ± 18.1	0.888	60.7 ± 16.6	60.7 ± 16.6	0.994
Dry BW (Kg)	62.5 ± 15.0	62.6 ± 15.0	0.054	59.0 ± 18.1	59.0 ± 18.2	0.386	60.7 ± 16.6	60.7 ± 16.6	0.282
Target UF (L)	2.6 ± 1.0	2.6 ± 0.9	0.616	2.7 ± 1.0	2.8 ± 1.1	0.322	2.6 ± 1.0	2.7 ± 1.0	0.33
Actual UF (L)	2.8 ± 1.1	2.7 ± 1.0	0.850	2.7 ± 1.1	3.0 ± 1.6	0.198	2.7 ± 1.1	2.9 ± 1.4	0.338
% target UF	100.0 ± 21.5	106.4 ± 39.1	0.231	103.8 ± 10.4	103.0 ± 10.6	0.794	102.0 ± 16.5	104.6 ± 27.6	0.319

Values are expressed as means ± SD; BW, body weight; KRG, Korean red ginseng; UF, ultrafiltration; % target UF, percentage of actual UF/target UF. Phase I is the controlled phase without KRG treatment, expressed as −KRG; phase II is the KRG treatment phase, expressed as +KRG.

Table 3: Comparison of blood pressure measurements during hemodialysis with and without Korean red ginseng treatment.

	Group A (HTN)		*P* value	Group B (non-HTN)		*P* value
	Phase I (−KRG)	Phase II (+KRG)		Phase I (−KRG)	Phase II (+KRG)	
Pre- SBP (mmHg)	148.1 ± 39.3	142.9 ± 30.8	0.231	100.8 ± 16.9	99.5 ± 14.5	0.332
Pre-DBP (mmHg)	71.1 ± 10.4	71.3 ± 13.0	0.931	58.2 ± 9.7	56.8 ± 8.1	0.279
Pre-MAP (mmHg)	96.8 ± 18.7	95.2 ± 18.5	0.327	72.4 ± 11.9	71.0 ± 9.9	0.218
Nadir SBP (mmHg)	97.4 ± 16.0	100.1 ± 18.6	0.184	76.3 ± 16.2	79.1 ± 15.8	0.045[a]
Nadir DBP (mmHg)	53.9 ± 8.4	54.8 ± 9.4	0.338	45.0 ± 9.8	46.4 ± 10.3	0.341
Nadir MAP (mmHg)	68.4 ± 10.4	69.9 ± 11.9	0.231	55.5 ± 11.8	57.2 ± 12.02	0.296
Post-SBP (mmHg)	107.8 ± 17.2	111.1 ± 17.2	0.053	86.7 ± 30.9	84.8 ± 16.4	0.305
Post-DBP (mmHg)	58.4 ± 8.7	59.0 ± 8.8	0.396	47.2 ± 10.1	49.0 ± 10.9	0.135
Post-MAP (mmHg)	74.9 ± 10.7	76.4 ± 11.1	0.248	60.3 ± 16.3	61 ± 12.3	0.284

Values are expressed as means ± SD of the data; KRG: Korean red ginseng; pre-: prehemodialysis; post-: posthemodialysis; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; [a]$P < 0.05$.

Table 4: Changes of blood pressure during hemodialysis with and without Korean red ginseng treatment.

	Group A (HTN)		*P* value	Group B (non-HTN)		*P* value	All		*P* value
	Phase I (−KRG)	Phase II (+KRG)		Phase I (−KRG)	Phase II (+KRG)		Phase I (−KRG)	Phase II (+KRG)	
ΔNadir-pre-SBP (mmHg)	−50.7 ± 39.3	−42.9 ± 23.8	0.053	−24.4 ± 10.1	−20.4 ± 11.0	0.045*	−36.9 ± 30.7	−31.0 ± 21.3	0.006**
ΔNadir-pre-DBP (mmHg)	−17.3 ± 8.8	−16.6 ± 10.0	0.446	−13.2 ± 6.2	−10.4 ± 6.6	0.011*	−15.1 ± 7.7	−13.3 ± 8.8	0.020*
ΔNadir-pre-MAP (mmHg)	−28.4 ± 18.0	−25.4 ± 14.4	0.078	−16.9 ± 7.1	−13.8 ± 7.7	0.015*	−22.4 ± 14.5	−19.3 ± 12.7	0.004**
ΔPost-preSBP (mmHg)	−40.3 ± 39.3	−31.8 ± 24.8	0.020*	−14.1 ± 23.4	−14.7 ± 9.9	0.232	−26.5 ± 34.2	−22.8 ± 20.2	0.011*
ΔPost-preDBP (mmHg)	−12.7 ± 10.1	−12.3 ± 10.3	0.408	−11.0 ± 6.3	−7.8 ± 6.3	0.025*	−11.8 ± 8.2	−10.0 ± 8.6	0.027*
ΔPost-preMAP (mmHg)	−21.9 ± 18.6	−18.8 ± 14.9	0.048*	−12.0 ± 10.3	−10.1 ± 7.1	0.145	−16.7 ± 15.4	−14.2 ± 12.1	0.014*

Values are expressed as means ± SD of the data.
Pre-: prehemodialysis; post-: posthemodialysis; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure;
*$P < 0.05$; **$P < 0.01$; Δ: changes.

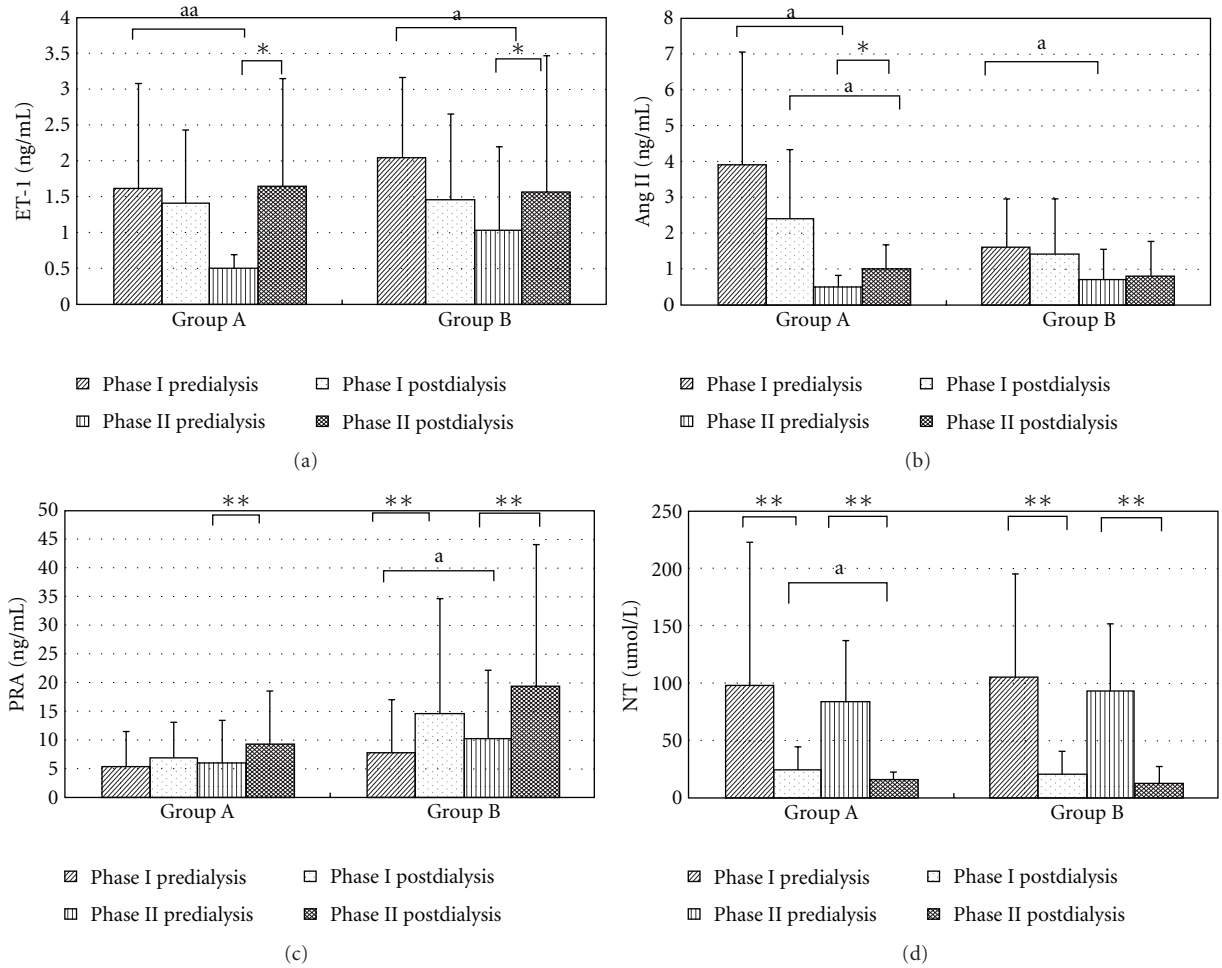

Figure 3: Changes of endotheline-1 (ET-1), plasma renin activity (PRA), angiotensin II (AngII), and NT (Nitrate+nitrite) plasma levels after KRG treatment in patients with intradialytic hypotension. Phase I is the control phase, and phase II is the KRG treatment phase. The ET-1 levels increased significantly after hemodialysis in phase II in both group A (hypertensive at baseline, $n = 18$, $P = 0.035$) and group B (normotensive or hypotensive at baseline, $n = 20$, $P = 0.011$). The posthemodialysis PRA levels increased significantly in phase II in both group A ($P = 0.005$) and group B ($P < 0.001$). In group A, the AngII levels significantly elevated after KRG treatment ($P = 0.033$). The levels of NT decreased significantly after dialysis in all groups in both phases ($P < 0.0001$). In group A, the posthemodialysis NT level was significantly lower in phase II than those in phase I ($P = 0.028$). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{a}P < 0.05$; $^{aa}P < 0.01$.

for the comparison of prehemodialysis AngII levels between two phases (3.902 ± 3.162 to 0.477 ± 0.353 ng/mL in group A, $P < 0.001$; 1.616 ± 1.387 to 0.694 ± 0.859 ng/mL in group B, $P < 0.001$). In contrast, the predialysis PRA levels remained unchanged in the group A but elevated in group B ($P = 0.012$) with KRG treatment. There were no significant changes in the prehemodialysis NT levels during the two study phases.

3.5. Side Effects. No significant adverse effects were observed during the study. Side effects reported by patients were palpitation ($n = 1$ in group A; $n = 1$ in group B) and thirsty ($n = 1$ in group B). Serum potassium levels increased slightly from 4.3 ± 0.5 to 4.6 ± 0.7 mEq/L ($P = 0.016$) in group B with KRG administration, but the difference were not significant when data from both groups was pooled (4.4 ± 0.8 versus 4.6 ± 0.7 mEq/L, $P = 0.189$) (Table 5). Serum phosphate levels were slightly elevated from 4.9 ± 1.4 to 5.3 ± 1.6 mg/dL ($P = 0.04$) with KRG treatment, but the levels were still within the normal range. No other significant changes in hematologic or biochemical parameters were observed (Table 5).

4. Discussion

The current treatments for IDH include stopping ultrafiltration, increasing dialysate sodium and glucose concentrations, and administration of hypertonic solutions and vasoconstrictor agents [1, 6, 12–14]. In the current study, we found that taking 3.5 g KRG slices at the start of hemodialysis can elevate the nadir blood pressure and reduce the frequency of symptomatic IDH by increasing the nadir blood pressure. KRG had no significant effects on the baseline blood pressure, suggesting that its beneficial effect

Table 5: Comparison of laboratory parameters with and without Korean red ginseng treatment.

	Group A (HTN)		P value	Group B (non-HTN)		P value	All		P value
	Phase I (−KRG)	Phase II (+KRG)		Phase I (−KRG)	Phase II (+KRG)		Phase I (−KRG)	Phase II (+KRG)	
Hct (%)	32.5 ± 3.6	33.2 ± 3.3	0.170	33.3 ± 3.2	32.9 ± 3.6	0.537	32.9 ± 3.4	33.0 ± 3.4	0.795
Hb (g/dL)	10.7 ± 1.1	10.8 ± 1.0	0.276	10.6 ± 1.1	10.5 ± 1.2	0.359	10.7 ± 1.1	10.6 ± 1.1	0.849
Ca (mg/dL)	9.7 ± 1.3	9.6 ± 0.8	0.938	10.1 ± 1.1	10.1 ± 1.2	0.618	9.9 ± 1.2	9.8 ± 1.0	0.543
P (mg/dL)	5.0 ± 1.7	5.6 ± 1.9	0.065	4.8 ± 1.0	5.1 ± 1.3	0.360	4.9 ± 1.4	5.3 ± 1.6	0.04*
K (mEq/L)	4.5 ± 1.0	4.5 ± 0.7	0.796	4.3 ± 0.5	4.6 ± 0.7	0.016*	4.4 ± 0.8	4.6 ± 0.7	0.189
BUN (mg/dL)	63.4 ± 22.1	64.3 ± 19.2	0.943	58.0 ± 11.0	62.2 ± 14.9	0.083	60.5 ± 17.2	63.2 ± 16.9	0.186
Cr (mg/dL)	10.1 ± 2.9	10.0 ± 2.7	0.523	11.3 ± 1.9	11.3 ± 2.2	0.985	10.7 ± 2.4	10.7 ± 2.5	0.768
ALT (U/L)	16.2 ± 10.0	14.7 ± 7.7	0.459	15.0 ± 6.2	13.8 ± 3.9	0.599	15.6 ± 8.1	14.2 ± 5.9	0.183

Values are expressed as means ± SD of the data.
Hct: hematocrit; Hb: hemoglobin; Ca: calcium; P: phosphate; K: potassium.
BUN: blood urea nitrogen; Cr: creatinine; ALT: alanine aminotransferase.
*$P < 0.05$; **$P < 0.01$.

is through restoring the vasoconstrictive response to acute plasma changes during hemodialysis. Furthermore, KRG has been used in different pathologic conditions [15], and its safety profile has been well studied in patients with normal renal function [16]. Our results confirmed the safety of using oral form KRG during hemodialysis. Although KRG has been reported to increase blood pressure in non-dialysis patients [17–19, 26, 27], we did not observe exacerbation of preexisting hypertension in our patients (group A). Our results suggest that KRG may be an alternative and adjuvant treatment for IDH.

The effects of *Panax ginseng* on regulating blood pressure were controversial due to its complexity of major components and different actions in various pathological conditions [18, 19, 22, 28, 29]. It has been reported that dammarenetriol glycosides in *Panax ginseng* have strong CNS excitatory actions that may cause hypertension, while dammarenediol glycosides in *Panax ginseng* have sedative and antihypertensive effects [26]. Three decades ago, Siegel proposed that low-dose *Panax ginseng* could cause hypertension [18]. However, high-dose *Panax ginseng* was shown to increase NO production in recent clinical trials and laboratory experiments [29], which may reduce blood pressure [28, 30]. Furthermore, *Panax ginseng* induced different responses in different blood vessels taken from rabbits, dogs and humans qualitatively and quantitatively [31]. Our data suggest that low-dose KRG (3.5 gm per dialysis session) could maintain the stability of blood pressure rather than exacerbating hypotension during hemodialysis.

The mechanism of IDH has been partly attributed to endothelial dysfunction in response to hemodynamic instability, with increased NO and decreased ET-1 during hemodialysis [4, 32–34]. ET-1 is the most potent vasoconstrictor that is locally produced from vascular endothelial cell [35, 36]. ET-1 levels decrease in IDH prone patients and increase in patients who have hypertension during hemodialysis, implying its importance in regulating hemodynamic stability [4]. Consistently, we did not found a significant increase of ET-1 levels during hemodialysis in these hypotension-prone patients during the observation period, suggesting a lack of adequate vasoconstrictive response. Indeed, we detected a significant increase in ET-1 levels during hemodialysis after KRG treatment. Similarly, we found that KRG treatment led to more activation of PRA and AngII during hemodialysis, indicating a gradually restoration of neurohormonal and cardiovascular responses to acute plasma volume change. Although NT concentrations is an indirect measurement of NO and can be removed by dialysis [4], we observed a significant decrease in the posthemodialysis NT levels after KRG treatment (group A). The result suggested that the beneficial effects of KRG treatment on IDH may be partially due to decreased NO production.

Previous studies have shown that hypotension-prone patients have higher baseline AngII levels compared to hypotension resistant dialysis patients [37]. This indicates that renin-angiotensin-aldosterone system may be abnormally activated in patients with recurrent IDH but still incapable to have adequate cardiovascular capacitance to the dialytic ultrafiltration. Elevation of AngII may lead to endothelial dysfunction and increase the risk of adverse cardiovascular events [38]. In the current study, we found that KRG treatment resulted in significantly reduced baseline AngII and ET-1. Our data suggest that KRG may have additional beneficial effects on endothelial dysfunction in patients with IDH by lowering the baseline of ET-1 and AngII levels [36, 39].

Despite the promising results obtained in this trial, several limitations should be acknowledged. First, the study had a relative small sample size, and further studies are

needed to confirm the results. Second, the study is a phase I pilot study, and we could only observe the differences within the same patient although this also help to eliminate the variation of blood pressure changes among different patients. Third, the long-term effects of KRG in hemodialysis patients were not studied, and further investigations are warranted.

5. Conclusions

Chewing low-dose KRG renders patients better resistance to acute BP reduction during hemodialysis. KRG treatment improves the compensatory response to acute volume change during hemodialysis via activation of vasoconstrictors (ET-1 and AngII). Our results suggest that KRG could be an adjuvant treatment for IDH.

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Research Article

The Protective Effect of Apamin on LPS/Fat-Induced Atherosclerotic Mice

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Apamin, a peptide component of bee venom (BV), has anti-inflammatory properties. However, the molecular mechanisms by which apamin prevents atherosclerosis are not fully understood. We examined the effect of apamin on atherosclerotic mice. Atherosclerotic mice received intraperitoneal (ip) injections of lipopolysaccharide (LPS, 2 mg/kg) to induce atherosclerotic change and were fed an atherogenic diet for 12 weeks. Apamin (0.05 mg/kg) was administered by ip injection. LPS-induced THP-1-derived macrophage inflammation treated with apamin reduced expression of tumor necrosis factor (TNF)-α, vascular cell adhesion molecule (VCAM)-1, and intracellular cell adhesion molecule (ICAM)-1, as well as the nuclear factor kappa B (NF-κB) signaling pathway. Apamin decreased the formation of atherosclerotic lesions as assessed by hematoxylin and elastic staining. Treatment with apamin reduced lipids, Ca^{2+} levels, and TNF-α in the serum from atherosclerotic mice. Further, apamin significantly attenuated expression of VCAM-1, ICAM-1, TGF-β1, and fibronectin in the descending aorta from atherosclerotic mice. These results indicate that apamin plays an important role in monocyte/macrophage inflammatory processing and may be of potential value for preventing atherosclerosis.

1. Introduction

Atherosclerosis is a progressive disease characterized by formation of a plaque, consisting mainly of cholesterol, other lipids, and debris from cellular death, in the inner lining of arteries [1]. It is increasingly regarded as a chronic inflammatory disease of the vessel wall [1]. Many macrophages can be observed in atherosclerotic lesions, and early lesions of atherosclerosis are characterized by the infiltration of monocytes/macrophages, proliferation of medial smooth muscle cells, and the presence of macrophage foam cells [2]. Therefore, the number of macrophages in lesions is an important measure of an atherosclerotic plaque.

Macrophages are multipotent inflammatory cells with the capacity to synthesize and secrete proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-8, and IL-6 [3]. These cytokines play a central role during development of atherosclerosis. Proinflammatory cytokines are regulated at the transcriptional levels by several transcription factors, including activator protein (AP)-1 and nuclear factor-kappa B (NF-κB) [4, 5]. NF-κB is a transcription factor that plays a key role in the regulation of host immune and inflammatory responses by increasing the expression of gene encoding cytokines, chemokines, growth factors, cell adhesion molecules, and several acute phase proteins [6]. NF-κB affects different steps in the atherosclerotic process, including initiation of atherosclerosis, foam cell formation, proliferation of smooth muscle cells, and fibrous cap formation [7]. Proinflammatory cytokines and adhesion molecules, such as vascular cell adhesion molecule (VCAM)-1 and intercellular cell adhesion molecule (ICAM)-1, induce

atherosclerosis by NF-κB activation [8, 9]. Cytoplasmic dissociation of NF-κB from inhibitor of κB (IκB) is regulated by activation of IκB inhibitor complex (IKK). Activated IKK phosphorylates IκBα, which frees NF-κB dimers to translocate to the nucleus. NF-κB then interacts with κB elements in the promoter region of several inflammatory genes to activate their transcription [10]. Potent inhibitors of IKKs that prevent NF-κB activity through blockage of IκB release can be useful for treatment of inflammatory diseases [11, 12]. Therefore, inhibiting transcription factors related to the activation of inflammation is a good biological target for anti-inflammatory activity.

Apamin is an integral part of bee venom (BV), comprising about 2-3% of its dry weight [13]. BV from the honeybee (*Apis mellifera*) has been traditionally used in China, Korea, and Japan for arthritis, bursitis, back pain, rheumatism, skin disease, and other chronic conditions. BV contains a variety of peptides including melittin, apamin, adolapin, and master cell degranulating peptide. It also contains enzymes, biological amines, and nonpeptide components [14]. It would be interesting to show that apamin in BV is responsible for altering transcription expression of proinflammatory cytokines, although the mechanisms behind that regulation remain unclear. Apamin has long been known as a highly selective blocker of Ca^{2+}-activated K^+ channels [15]. Several studies have confirmed that some calcium channel blockers can decrease areas of atherosclerotic lesions, production of oxidative stress, and expression of inflammatory cytokines without conspicuously effecting blood lipid levels [16]. However, the molecular mechanisms of the anti-atherosclerotic effects of apamin have not been elucidated. To gain a better insight into these mechanisms, the aim of this study is to evaluate the anti-atherosclerotic mechanisms of apamin in THP-1-derived macrophages and to investigate the anti-atherosclerotic effects of apamin in mouse models of atherosclerosis.

2. Materials and Methods

2.1. Cell Culture. The human monocytic cell line THP-1 was obtained from the American Type Culture Collection and cultured in with RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% antibiotics. Cells were cultured in humidified incubator at 37°C in a 5% CO_2 atmosphere. THP-1 cells (1×10^6 cells/mL) were differentiated into macrophages with the use of 50 nM phorbol-12-myristate-13-acetate for 48 h. For macrophage inflammation, cells incubated in serum-free culture medium prior to treatment with apamin (0.5, 1, 2 μg/mL, Sigma, MO, USA) for 6 h. After this incubation with apamin, THP-1-derived macrophages were activated with LPS (1 μg/mL, Sigma, MO, USA) for 24 h.

2.2. Enzyme-Linked Immunosorbent Assay (ELISA). Concentrations of TNF-α in culture supernatant and serum were measured with a solid-phase sandwich ELISA using a quantikine human or mouse TNF-α kit (R&D Systems, MN, USA). The absorbance was measured at 450 nm in an ELISA reader (BMG labtechnologies, Mornington, Germany).

2.3. Western Blot Analysis. Cells or tissues were homogenized in a lysis buffer (50 mM Tris pH 8.0, 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, 100 mM PMSF, 1 M DTT, 10 mg/mL leupeptin, and aprotinin; all from Sigma, MO, USA). For cytosolic fractions, cells were suspended in extraction buffer (10 mM HEPES pH 8.0, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, 300 mM sucrose, 0.1% NP-40, and 0.5 mM PMSF) for 15 min on ice and were centrifuged $6000 \times$ g for 15 min. The supernatant from this step is the cytosolic fraction, and the pellet is the nuclear fraction. The nuclear fractions were collected by different extraction buffer (20 mM HEPES pH 8.0, 20% glycerol, 100 mM KCl, 100 mM NaCl, 0.2 mM EDTA, 0.5 mM PMSF, and 0.5 mM DTT) for 15 min on ice. The nuclear fractions were centrifuged $12000 \times$ g for 10 min at 4°C to remove insoluble protein. Then, protein concentration was determined using the Bradford assay. Total protein was separated on 10% to 12% SDS-polyacrylamide gels and transferred to polyvinylidene fluoride membrane (Millipore, MA, USA). Membranes were blocked in 5% skim milk for 1 h at room temperature. Protein samples were incubated with primary antibodies for 3 h. Primary antibodies used in this study were the following: anti-VCAM-1, anti-ICAM-1, and anti-TGF-β1 were obtained from R&D Systems (MN, USA); anti-IKK, anti-phospho-IKK, anti-IκBα, anti-phospho-IκBα, anti-NF-κB p65 and anti-phospho-NF-κB p65 were purchased from Cell Signaling Technology (MA, USA); anti-fibronectin and anti-F4/80 were obtained from Abcam (MA, USA), anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and histone H2B were purchased from Santa Cruz (CA, USA). The membranes probed with a horseradish peroxidase (HRPO)-conjugated secondary antibodied were used for detection. Target proteins were detected using an enhanced chemiluminescence detection system (Amersham, NJ, USA) and film.

2.4. Electrophoretic Mobility Shift Assay (EMSA). A DIG Gel shift kit (Roche, Mannheim, Germany) was used for EMSA assays. The NF-κB oligonucleotide probe (5′-CTT GAA GGG ATT TCC CTG GCT TGA AGG GAT TTC CCT GG-3′; only sense strands are shown) was end-labeled with DIG-ddUTP. For binding reaction, 9 μg nuclear extract protein was mixed with binding buffer (0.5 μg poly dI-dC, 0.1 μg poly L-lysine, and 0.8 μg labeled oligonucleotide, final volume of 20 μL) and was incubated at 37°C for 30 min. The nuclear protein-DNA complex was separated by 6% nondenaturing polyacrylamide gel in TBE buffer (22.5 mM Tris, 22.5 mM boric acid, 0.5 mM EDTA, and pH 8.3) at 80 V for 1.5–2 h at 4°C. Samples were transferred to Hybond-XL membranes (Amersham Biosciences, Amersham, UK) for 30 min and crosslinked for 10 min under ultraviolet light. Membrane was incubated with anti-digoxigenin-AP Fab fragments (1 : 10,000) for 30 min, and the nuclear protein-DNA bands were developed with detection solution including 100 mM Tris-HCl, 100 mM NaCl, pH 9.5,

and 100 μg/mL disodium 3-[4-metoxyspiro {1,2-dioxetane-3,2′(5′-chloro)tricyclo(3.3.1.1^{3,7})decan}-4-yl] phenyl phosphate.

2.5. NF-κB Promoter Activity Assay. Reporter gene activity was evaluated by cell-based analysis methods for assaying NF-κB activity. NF-κB promoter-luciferase construct was transiently transfected by using transfection reagent lipofectamine 2000 (Invitrogen, CA, USA). After harvesting, cells were lysed in reporter lysis buffer (Promega, WI, USA), cell extract (50 μg/20 μL) was mixed with 100 μL of luciferase assay reagent, and the emitted light intensity was measured using luminometer FLUOstar OPIMA (BMG Labtech, Germany). The luciferase activity was represented as the fold induction compared with normal control cells.

2.6. Experimental Animals. Male C57BL/6 mice (8 weeks old, 20–25 g) were obtained from Samtako (Osan, Republic of Korea) and were allowed one week for stabilization. Mice were kept in a room maintained at 21–25°C under 12 h dark/light cycles. The animal experiments were performed in accordance with the NIH guidelines for the care and use of laboratory animals. Mice were randomly subdivided into four groups ($n = 10$/group) and were maintained under various conditions for 12 weeks. The normal control (NC) group was fed with chow diet (Samyang Feed, Daejeon, Republic of Korea). The apamin (Apa) group was fed with chow diet and ip injected with 0.05 mg/kg apamin (Sigma, MO, USA) twice a week. The LPS/fat group (atherosclerotic mice) was fed with an atherogenic diet (1.25% cholesterol, 15% fat, and 0.5% cholic acid) and ip injected with 2 mg/kg LPS (Sigma, MO, USA) three times a week. The LPS/fat+Apa group was atherosclerotic mice treated with 0.05 mg/kg apamin twice a week.

2.7. Biochemical Analysis. Blood was collected from inferior vena cava and immediately centrifuged at 8000 × g for 10 min at 4°C to separate serum. Serum total cholesterol (TC) and triglycerides (TG) were measured using a commercial kit (Asan, Hwaseong, Republic of Korea). Serum Ca^{2+} accumulation was measured using a commercial kit (BioAssay Systems, CA, USA). The concentration of Ca^{2+} accumulation was determined with reference to a standard curve constructed with each assay, and mean plus standard deviation was calculated.

2.8. Reverse-Transcription Polymerase Chain Reaction (RT-PCR). Total RNA was isolated from the aorta with TRIzol Reagent (Gibco, NY, USA) according to manufacturer's recommendations. RNA (0.5 μg) was reverse-transcribed using M-MLV reverse transcriptase (Promega, WI, USA). Single-stranded cDNA was amplified by PCR with primers (Bioneer, Daejeon, Republic of Korea) specific to mouse VCAM-1, ICAM-1, TGF-β1, fibronectin, and GAPDH used as a positive control. Primer sequences are the following: ICAM-1 sense: 5′-AGC ACC TCC CCA CCT ACT TT-3′; ICAM-1 antisense: 5′-AGC TTG CAC GAC CCT TCT AA-3′; VCAM-1 sense: 5′-TAC CAG CTC CCA AAA TCC TG-3′; VCAM-1 antisense: 5′-TCT GCT AAT TCC AGC CTC GT-3′; TGF-β1 sense: 5′-CCT GCT GCT TTC TCC CTC AAC C-3′; TGF-β1 antisense: 5′-CTG GCA CTG CTT CCC GAA TGT C-3′; fibronectin sense: 5′-TGT GAC AAC TGC CGT AGA CC-3′; fibronectin antisense: 5′-GAC CAA CTG TCA CCA TTG AGG-3′; GAPDH sense: 5′-GTG GAC ATT GTT GCC ATC AAC G-3′; GAPDH antisense: 5′-GAG GGA GTT GTC ATA TTT CTC G-3′. PCR products were visualized by 2% agarose gel. The band intensity was quantified by using Image Analysis (Las 3000, Fuji, Japan).

2.9. Histological Analysis. All aorta specimens were fixed overnight in 10% formalin solution, dehydrated, and embedded in paraffin. Thin sections were mounted on glass slides, dewaxed, rehydrated in distilled water, and stained with hematoxylin and eosin (H&E) and Verhoeff' elastic tissue staining solution (alcoholic hematoxylin, 10% ferric chloride and iodine solution). For immunohistochemistry, sections were incubated with anti-ICAM-1 (R&D Systems, MA, USA) and anti-F4/80 (Abcam, MA, USA) for 1 h at 37°C. Signals were visualized using an Envision system (DAKO, CA, USA) for 30 min at 37°C. DAB (3,3′-diaminobenzidine tetrahydrochloride) was used as the coloring reagent, and hematoxylin was used as counterstain. For immunofluorescence staining, aorta sections were incubated with anti-F4/80 (Abcam, MA, USA) and anti-mouse biotinylated secondary antibodies conjugated with FITC (Jackson ImmunoResearch Laboratories, PA, USA). Slides were mounted using VECTASHIELD Mounting Medium (VECTOR Laboratories, CA, USA). Specimens were examined and photographed using a fluorescence microscope. Sections were counterstained with Hoechst 33342 (Immunochemistry, MN, USA).

2.10. Statistical Analysis. Data were collected from three independent experiments and analyzed with SPSS 12.0 software (SPSS Inc., IL, USA). Results were expressed as mean ± SD, and P value < 0.05 was considered as statistical significance.

3. Results

3.1. Apamin Inhibits Expression of Proinflammatory Cytokine and Adhesion Molecules. To investigate the effect of apamin on inflammatory response, this study assessed the effect of apamin on LPS-induced cytokine secretion in THP-1-derived macrophages (Figure 1(a)). Expression levels of proinflammatory cytokine were validated by an ELISA kit. THP-1-derived macrophages expressed TNF-α after exposure to LPS. Upregulation of TNF-α in LPS-treated THP-1-derived macrophages was suppressed by apamin in a concentration-dependent manner. Expression levels of adhesion molecules, including VCAM-1 and ICAM-1, were determined by western blot (Figure 1(b)). Protein levels of VCAM-1 and ICAM-1 were higher in LPS-treated THP-1-derived macrophages than in normal control cells. Treatment with apamin resulted predominantly in the dose-dependent downregulation of VCAM-1 and ICAM-1 expression levels

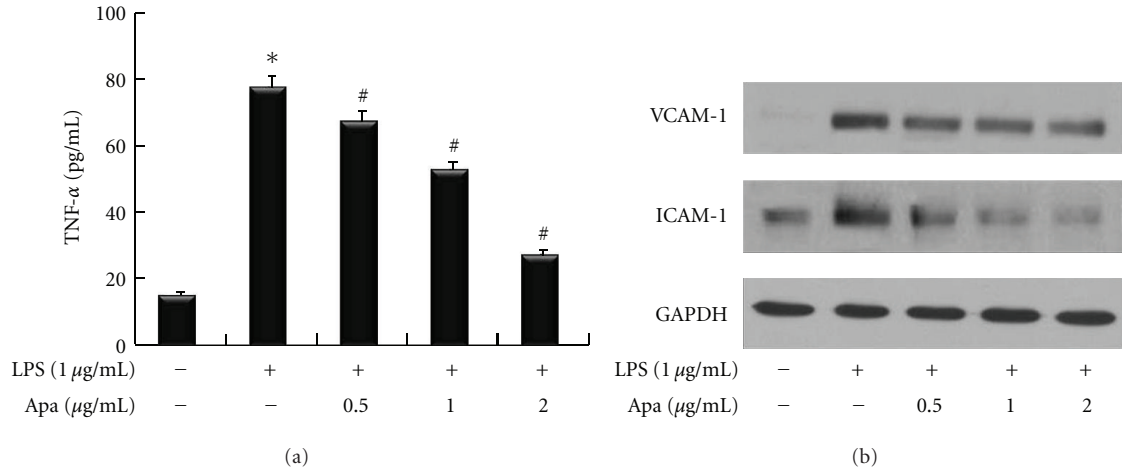

Figure 1: The effect of apamin on expression levels of proinflammatory cytokine and adhesion molecules in LPS-treated THP-1-derived macrophages. (a) Expression levels of TNF-α in culture supernatant, as determined by ELISA, significantly inhibited by apamin in a dose-dependent manner. (b) Expression levels of VCAM-1 and ICAM-1 were determined by western blot. They were decreased by apamin in a dose-dependent manner. As a loading control, GAPDH was used to confirm equal sample loading. $^{*}P < 0.05$ compared to control cells, $^{\#}P < 0.05$ compared to control cells treated with LPS alone.

in response to LPS. These results indicate that apamin efficiently discourages the activity of proinflammatory cytokine and adhesion molecules in THP-1-derived macrophages.

3.2. Apamin Inhibits NF-κB Activation and Nuclear Translocation of NF-κB. To determine the involvement of the IKK, IκB, and NF-κB signaling pathways in the anti-inflammatory property of apamin, activation of these three proteins was examined by western blots of their dually phosphorylated forms (Figure 2(a)). Upon LPS treatment, the total IKK protein did not change from the cytosolic fractions, whereas the phosphorylated IKK protein was particularly increased when compared to the normal control. Addition of apamin inhibited the LPS-induced phosphorylated IKK expression level. Expression of IκB phosphorylation tended to increase when treated with LPS. Addition of apamin reduced LPS-induced IκB phosphorylation. In the nuclear fraction, phosphorylated NF-κB was also inhibited by apamin in a dose-dependent manner. To determine whether the inhibitory effect of apamin on LPS-induced NF-κB activation was due to inhibition of IκB phosphorylation and NF-κB translocation, nuclear NF-κB p65 subunit levels were measured following treatment with LPS in the presence and absence of apamin. While LPS treatment increased nuclear NF-κB p65, apamin cotreatment suppressed this translocation. These results supported the explanation that apamin inhibits NF-κB activation by suppression of IκB phosphorylation at the transcription level in LPS-treated THP-1-derived macrophages. After demonstrating that apamin decreased NF-κB protein expression as measured by western blot, the next experiment attempted to further examine the effect of apamin on transcriptional activity of NF-κB by EMSA. The DNA-binding activity of NF-κB nuclear protein was markedly higher in LPS-treated THP-1-derived macrophages. LPS-induced NF-κB nuclear protein-DNA binding activity was noticeably inhibited by apamin in a dose-dependent manner (Figure 2(b)). To investigate the transcriptional activity of NF-κB, expression of reporter genes in cells transfected with plasmid NF-κB was analyzed. As shown in Figure 2(c), treatment of THP-1-derived macrophages with LPS resulted in increased NF-κB activity that was suppressed by apamin in a dose-dependent manner. The results were consistent view that apamin inhibits the expression of NF-κB, probably at the transcriptional level.

3.3. Apamin Reduces Lipid Levels, Ca^{2+} Accumulation, and TNF-α Expression of Serum. The effects of apamin on serum TC and TG levels were determined. After 12 weeks of feeding a high-fat diet and LPS treatment, mice developed severe atherosclerosis with significant elevation of serum TC and TG levels, compared to the NC and Apa groups. TC and TG levels were significantly decreased by apamin (Figures 3(a) and 3(b)). To confirm that apamin affected Ca^{2+} accumulation in atherosclerotic mice, Ca^{2+} accumulation of serum was measured. As shown in Figure 3(c), Ca^{2+} accumulation was markedly enhanced in the LPS/fat group compared to the NC and Apa groups. Ca^{2+} accumulation was predominantly reduced in serum from apamin-treated atherosclerotic mice compared to the LPS/fat group. The expression level of TNF-α was measured by an ELISA kit. The NC and Apa groups did not display a significant difference in expression level of TNF-α (Figure 3(d)). Expression level of TNF-α was elevated in the LPS/Fat group. In atherosclerotic mice treated with apamin, expression level of TNF-α was reduced compared to the LPS/Fat group.

3.4. Apamin Attenuates Formation of Atherosclerotic Lesions. Pathological evaluations of aortic lesions were carried out. Consecutive cross-sections of cuffed descending aortas stained with H&E revealed induction of atherosclerotic

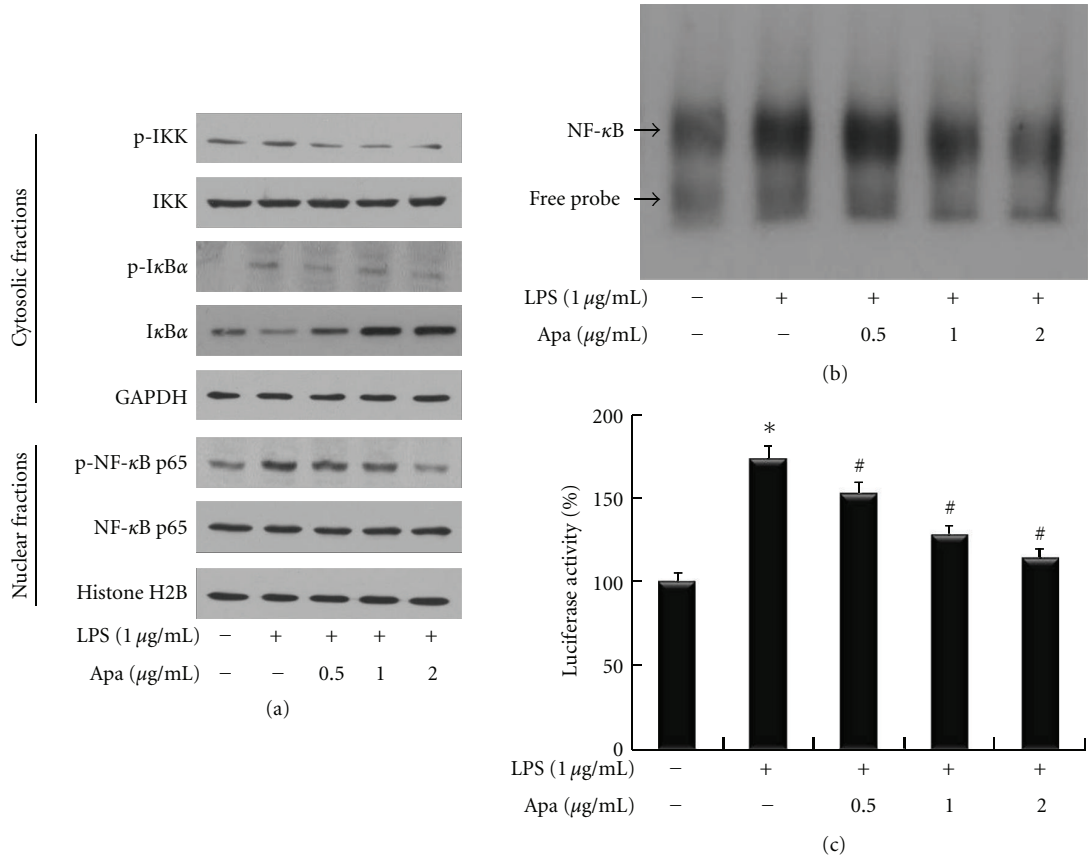

Figure 2: The effect of apamin on NF-κB signaling pathway in LPS-treated THP-1-derived macrophages. (a) Expression levels of IKK and IκBα in the cytosolic fraction and NF-κB in the nuclear fraction were determined by western blot. GAPDH and histone H2B were used as the internal controls for cytosolic and nuclear fraction loading control, respectively. (b) Nuclear NF-κB activity was examined by EMSA. The arrow indicates the specific NF-κB band. (c) Luciferase activity was measured with a luminometer. $^{*}P < 0.05$ compared to control cells, $^{\#}P < 0.05$ compared to control cells treated with LPS alone.

lesion formation. The LPS/fat group showed a larger number of atherosclerotic lesions in the aorta compared to the NC and Apa groups (Figure 4(a)). In the LPS/fat group, nearly all animals developed fatty streaks in the aortic arch, with accumulation of lipids localized mainly in areas subjacent to the endothelium. Compared to the LPS/fat group, treatment with apamin changed the size of atherosclerotic lesions in aortas suggesting that apamin exerted apparent protective atherogenic actions. These inhibitory effects of apamin on atherosclerotic mice were markedly displayed among the four groups when entire aortas were stained by elastic stain (Figure 4(b)). In atherosclerotic mice treated with apamin, atherosclerotic lesions were significantly decreased compared to the LPS/fat group. This result was consistent with H&E stain analysis.

3.5. Apamin Attenuates Expression Levels of Adhesion Molecules and Fibrotic Factors. Protein and mRNA levels of adhesion molecules (VCAM-1 and ICAM-1) and fibrotic factors (TGF-β1 and fibronectin) after apamin treatment in atherosclerotic mice were detected by western blot and RT-PCR, respectively. As shown in Figure 5(a), aortas from the NC, and Apa groups showed little expression of VCAM-1 and ICAM-1. However, a more significant upregulation of these expression levels was observed in the LPS/Fat group, while treatment with apamin led to evident downregulation of VCAM-1 and ICAM-1 expression levels. Similarly, expression levels of TGF-β1 and fibronectin were decreased in the aorta of the LPS/fat+Apa group compared to LPS/fat group. Moreover, expression levels of ICAM-1 in atherosclerotic lesions were determined by immunohistochemical staining. As shown in Figure 5(b), ICAM-1 expression levels were barely detected in aortic sections from the NC and Apa groups. Suppressed expression levels in surface area lesions revealed that apamin inhibited ICAM-1 expression levels in atherosclerotic lesions, which agreed with the results from current *in vitro* experiment. These results indicated that apamin suppresses the expression of VCAM-1, ICAM-1, TGF-β1, and fibronectin in atherosclerotic mice.

3.6. Apamin Reduces Infiltration of Macrophages. To investigate whether apamin could influence atherosclerotic lesions, aortas of experimental mice were collected for immunohistochemistry. Greater macrophage infiltrations in atherosclerotic mice were demonstrated using F4/80, a specific marker of macrophages (Figure 6(a)). Atherosclerotic lesions in

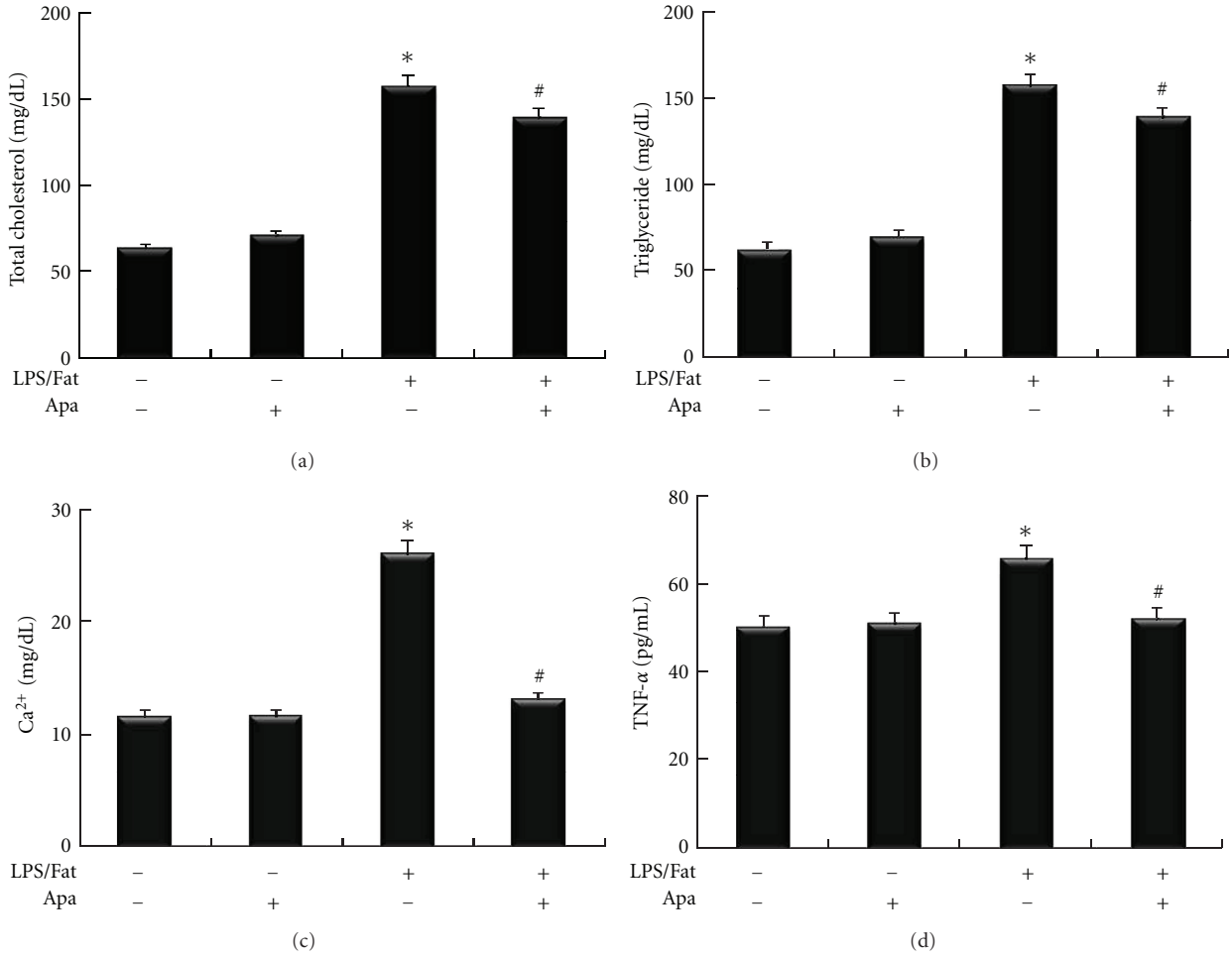

Figure 3: The effect of apamin on biochemical parameters in atherosclerotic mice. Total cholesterol (a), triglycerides (b), and Ca^{2+} accumulation (c) were measured using a commercial kit. Expression level of TNF-α (d) was determined by ELISA. $^{*}P < 0.05$ compared to normal control, $^{\#}P < 0.05$ compared to atherosclerotic mice.

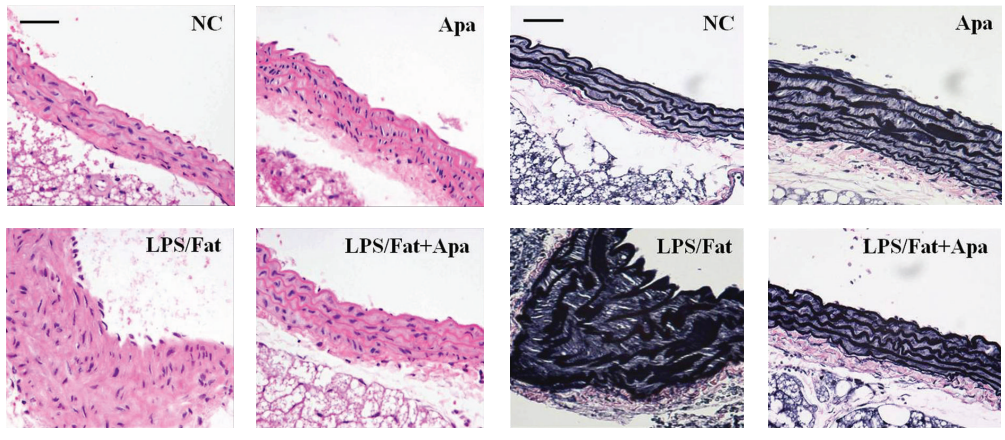

Figure 4: Histological cross-sections of descending aorta in atherosclerotic mice. (a) H&E staining and (b) elastic staining showed that atherosclerotic changes were attenuated in the LPS/Fat+Apa group by apamin. NC, normal control; Apa, normal control treated with apamin; LPS/Fat, LPS injection and high fat dieted mice (atherosclerotic mice); LPS/Fat+Apa, atherosclerotic mice treated with apamin. Scale bars, 50 μm.

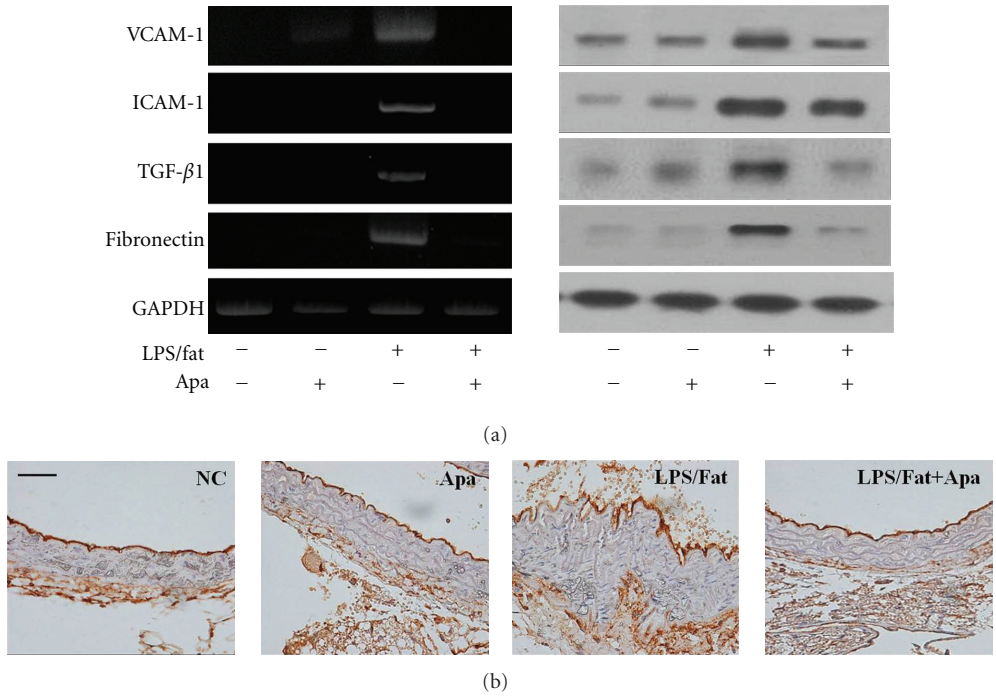

Figure 5: The effect of apamin on expression levels of VCAM-1, ICAM-1, TGF-β1, and fibronectin in atherosclerotic mice. (a) Western blot (left panel) and RT-PCR (right panel) suppressed expression levels of those protein by apamin. As a loading control, GAPDH was used to confirm equal sample loading. (b) Immunohistochemistry of ICAM-1 predominantly attenuated in the LPS/fat+Apa group. NC, normal control; Apa, normal control treated with apamin; LPS/fat, LPS injection and high-fat-dieted mice (atherosclerotic mice); LPS/fat+Apa, atherosclerotic mice treated with apamin. Scale bars, 50 μm.

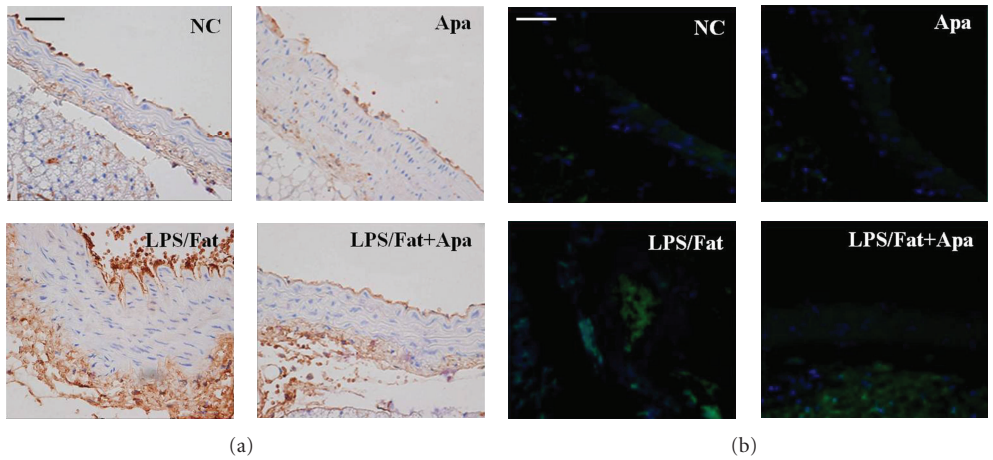

Figure 6: The effect of apamin on macrophage infiltrations in atherosclerotic mice. (a) Immunohistochemistry and (b) immunofluorescence examinations showed that apamin markedly inhibited macrophages infiltration in atherosclerotic mice. Micrographs display macrophages (green), nuclei (blue), and merged images. NC, normal control; Apa, normal control treated with apamin; LPS/Fat, LPS injection and high-fat-dieted mice (atherosclerotic mice); LPS/Fat+Apa, atherosclerotic mice treated with apamin. Scale bars, 50 μm.

atherosclerotic mice treated with apamin showed decreased infiltration of macrophages into the arterial wall. F4/80 positive areas in the mice treated with apamin were significantly reduced to the total cross-sectional area of aortas compared to the LPS/fat group. Similarly, macrophages infiltration of atherosclerotic lesions was markedly attenuated by apamin, as determined by immunofluorescence staining (Figure 6(b)). Taken together, these results indicate that apamin treatment substantially attenuates atherosclerotic lesions.

4. Discussion

Atherosclerosis is increasingly recognized as a chronic inflammatory disease. It is a multifactorial and progressive

disease in which inflammatory reaction and inflammation-related mediators play pivotal roles at all stages [17]. Various research studies of the anti-inflammatory effect of BV have recently been conducted [18–20]. Studies of the general pharmacological profiles of BV and its components have also been conducted [13, 21]. Apamin comprises 2-3% of the dry weight of apamin [22]. BV has been reported as having proinflammatory [23] and anti-inflammatory effects [18]. A number of studies have reported on a variety of mechanisms for the anti-inflammatory effect of BV and its constituents [13, 19, 22]. An anti-inflammatory effect of apamin accompanied by a reduction of seromucoid and haptoglobin levels has been reported [22].

In this study, apamin suppressed LPS-induced THP-1-derived macrophage inflammation via the NF-κB signal pathway. Furthermore, in atherosclerotic mice, apamin attenuated the regulation of various atherosclerotic factors by inhibiting inflammation.

Inflammation is pivotal to atherosclerosis, and monocytes/macrophages are critical participants. Monocytes/macrophages secrete IL-6, IL-1β, and TNF-α, and the serum concentrations of several markers of inflammation are associated with future cardiovascular risk [24]. This study used an *in vitro* cell-based system using THP-1-derived macrophages with LPS stimulation to test the anti-inflammatory effect of apamin. Apamin treatment significantly decreased TNF-α expression level in LPS-induced THP-1-derived macrophages. Adhesion molecules also play important roles in cellular interactions during inflammatory responses. Expression of VCAM-1 and ICAM-1 may influence the organization of cells that promote the production of cytokines in inflammatory cells [3]. Our data proves that LPS stimulation promotes the expression of these adhesion molecules in THP-1-derived macrophages. The expression levels of VCAM-1 and ICAM-1 were predominantly reduced in a dose-dependent manner after treatment with apamin in mice.

A role for activation of the NF-κB in regulation of inflammatory responses was reported [25]. NF-κB activation by TNF-α is required for transcription of the gene encoding cell adhesion molecules [26]. Following exposure of macrophages to LPS, NF-κB is activated by phosphorylation and degradation of IκB. The activated NF-κB was then translocated into the nucleus, leading to transcriptional expression of genes associated with inflammatory responses [27]. In the current study, apamin suppressed LPS-inducible IκB phosphorylation and nuclear NF-κB p65 activation in a concentration-dependent manner in THP-1-derived macrophages. These results demonstrate that apamin mediated an anti-inflammatory effect via the NF-κB signaling system. More data is required to determine whether the potential differential effect of apamin occurs upstream of NF-κB and particularly upstream of IKK based on current results that apamin inhibited phosphorylation of IKK in a dose-dependent manner in THP-1-derived macrophages.

Based on these *in vitro* results, the effect of apamin in an animal model of atherosclerosis was investigated. Atherosclerosis can be induced in mice given LPS injection and fed a high-fat diet [20, 28]. Accumulation of cholesterol and lipids resulting in foam cell formation is regarded as a critical process in the development of atherosclerosis. Progressive lipid accumulation leads to increases in expression of proinflammatory cytokines and infiltration of inflammatory cells [29]. Proinflammatory cytokines have been reported to promote efflux of cholesterol [30]. Stimulation of the mechanism involved in decrease of lipids and cholesterol in macrophages may thus be an effective way to prevent atherosclerosis. Our results from treatment with apamin showed that serum TC and TG levels were significantly decreased in the atherosclerotic mice. Studies have demonstrated that modification of lipid can induce Ca^{2+} influx, that Ca^{2+} is closely associated with production of TNF-α involved in the inflammatory response, and that inhibition of the influx of Ca^{2+} might attenuate these responses [31, 32]. In this study, apamin significantly reduced Ca^{2+} accumulation. In addition, TNF-α level decreased in the serum of atherosclerotic mice following apamin treatment. These data suggest that the anti-atheroscleortic effects of apamin may occur via inhibition of Ca^{2+} accumulation and proinflammatory cytokines.

Expression levels of VCAM-1 and ICAM-1 were largely studied in a mouse model of atherosclerosis [33], and these molecules were upregulated by a high-cholesterol diet in an animal model [34]. Presently, protein and mRNA levels of these adhesion molecule expressions were predominantly decreased by apamin in atherosclerotic mice. Further, atherosclerotic lesion development was accompanied by upregulation of ICAM-1. This is consistent with other studies that reported that a high-fat diet induces both lesion development and expression of adhesion molecules in general [33, 34]. Treatment with apamin attenuated both lesion development and adhesion molecule expressions in atherosclerotic mice.

TGF-β1 expression is upregulated in plaque development and appears to be involved in atherosclerotic lesions under a variety of circumstances which is further related to extracellular matrix remodeling [35]. The extracellular matrix protein fibronectin is focally deposited in atherosclerosis where it contributes to inflammatory signaling [36]. Several studies have reported that monocyte binding to fibronectin causes the induction of potent proinflammatory cytokines, together with the ability of fibronectin to promote NF-κB activation [36, 37]. In the present study, protein and mRNA levels of TGF-β1 and fibronectin expression were inhibited by apamin in atherosclerotic mice.

Infiltration and activation of monocytes into the arterial walls are critical steps in atherogenesis [38]. Macrophages accumulate cholesterol and lipids, resulting in foam cell formation, which is considered a critical process in development of atherosclerosis [39]. We were able to witness the macrophage infiltration via western blot (data not shown), immunohistochemistry, and immunofluoresence staining. However, macrophage infiltration was significantly reduced by apamin in the aorta from atherosclerotic mice.

5. Conclusion

Treatment with apamin in THP-1-derived macrophages suppresses inflammatory responses by a decrease of the NF-κB signal pathway. Furthermore, atherosclerotic mice treated with apamin predominantly attenuate lipids, Ca^{2+} levels, proinflammatory cytokines, adhesion molecules, fibrotic factors, and macrophage infiltration. These mechanisms could be involved in the possible role of apamin in protection against atherosclerosis. Therefore, apamin may be a therapeutically useful agent for use in atherosclerosis prevention.

Acknowledgments

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Review Article

Lipid-Regulating Effect of Traditional Chinese Medicine: Mechanisms of Actions

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Traditional Chinese medicine (TCM) has been increasingly used for the treatment of dyslipidemia and cardiovascular disease. Recently, much progress has been made in studies on the mechanisms of action of the lipid-regulating effect of TCM in animal experiments. Current researches showed that the lipid-regulating effect of TCM may be related to the following actions: (1) inhibiting intestinal absorption of lipids; (2) reducing the biosynthesis of endogenous lipids; (3) increasing the catabolism of lipid, sterol substances in live system; (4) increasing the secretion of sterol substances in live system; (5) regulating transcription factors related to lipid metabolism. This paper provides an overview of the recent advances and discusses their implications in future development of lipid-lowering drugs from TCM.

1. Introduction

Dyslipidemia refers to a disruption of lipid metabolism with exceeding serum levels of cholesterol (TC), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), and/or lower level of high-density lipoprotein-cholesterol (HDL-C). Serum levels of lipids and lipoprotein lipids are among the most potent and best substantiated risk factors for atherosclerotic diseases, particularly coronary heart disease (CHD) [1].

In human, lipids homeostasis is regulated by well-balanced mechanisms of intestinal uptake, endogenous synthesis and metabolism, transport in lipoprotein particles, and biliary excretion. The metabolism of cholesterol and fatty acids and their associated lipid transport particles (the lipoproteins) occurs in the gut, liver, and the peripheral tissues. Drugs that alter lipids concentration act mainly by altering the kinetics of one or more parts of the metabolic cycle.

Traditional Chinese medicine (TCM) has been increasingly used for the treatment of dyslipidemia and cardiovascular disease. In a previous paper, we have reviewed the efficacy of TCM for the treatment of dislipidemia, which has been confirmed by numerous clinical studies as well as laboratory researches [2].

In this paper, we will focus on the underlying mechanisms of actions of the lipid-regulation effect of TCM.

2. Inhibiting the Intestinal Absorption of Cholesterol

The absorption of dietary lipids (cholesterol, fatty acids, phospholipids, etc.) in the intestine is an important source of serum lipids. Cholesterol absorption is a key regulatory target in human lipid metabolism because it determines the amount of endogenous biliary as well as dietary cholesterol that is retained, thereby influencing cholesterol balance. Cholesterol in the intestinal tract is derived from the diet and bile. Whereas dietary intake ranges from <50 mg/day (pure vegetarians) to 750 mg/day, biliary cholesterol input is 3 to 10 times higher and ranges from 500 to 2,400 mg/day.

Plant sterols (e.g., stigmasterol and β-sitosterol) reduce serum LDL cholesterol level by competitively inhibiting

intestinal cholesterol absorption. Recent findings also suggest that plant stanols/sterols actively influence cellular cholesterol metabolism within intestinal enterocytes, and, in response to the reduced supply of exogenous cholesterol, receptor-mediated lipoprotein cholesterol uptake is probably enhanced, as shown by increased LDL receptor expression [3].

Plant sterols are rich in a variety of traditional Chinese herbal medicine, such as fleece-flower root, cassia seed, eucommia, rhubarb, polygonum, and turmeric. In addition, cellulose, pectin, and agar, which are also rich in many Chinese herbal medicines, can reduce the absorption of cholesterol by forming a complex with cholate to impede the formation of cholesterol microparticles in the intestine [3, 4].

An *in vivo* experiment showed that the addition of 0.5% Fr2-3 (a tea leaf fraction containing 72% saponins with high *in vitro* antihypercholesterolemic activity) to a high-cholesterol diet suppressed the increase in serum cholesterol levels in rats. Fr2-3 induced a decrease in the liver cholesterol and triglyceride levels and an increase in the fecal excretion of cholesterol [4].

Anthraquinones in fleece-flower root inhibit absorption of cholesterol by increasing peristalsis in the intestine, enhance the converting of cholesterol into the bile acid, and therefore increase the cholesterol excretion via the bile [5]. Lecithin in fleece-flower root can also prevent the cholesterol deposition in the liver, increase the transportation and converting of cholesterol, thus reduce the concentration of cholesterol in serum and liver, and prevent its infiltrating into artery intima [5]. Furthermore, lecithin increases the activity of the cholesteryl esterase in vessel wall and inhibits the activity of Acyl coenzyme A-cholesterol acyltransferase (ACAT) [5]. Components in cassia proteins were reported to combine with bile acid and inhibit the absorption and aggradations of cholesterol in the body, leading to the decrease of serum level of TC, TG, and LDL in the hyperlipidemic rats [6].

Recent insights in the role of ATP-binding cassette (ABC) transporters ABCG5 and ABCG8, as well as the identification of Niemann-Pick C1 Like 1 (NPC1L1) protein as sterol transporter in the gut, focused attention on sterol transport processes in the small intestine and the liver [7–9]. NPC1L1 is the target of cholesterol absorption inhibitor Ezetimibe, which prevents NPC1L1 entering cells by avoiding NPC1L1 incorporated into vesicles, reducing the uptake of cholesterol by cells, impending the cholesterol absorption in intestine, and thus reducing the serum cholesterol concentrations significantly [10]. To date, no research has been reported on the effects of TCM on NPC1L1. We believe that screening TCM with suppressing activities on NPC1L1 will lead to the discovery of new components and new drugs for regulating cholesterol absorption in intestine and the new drugs of lipid lowering.

ACAT plays an important role in the absorption, transport, and storage of cholesterol by catalyzing the cholesterol and long-chain fatty acid to form cholesterol ester *in vivo*. Inhibition of ACAT may reduce the levels of plasma TC and LDL cholesterol, reduce the accumulation of cholesterol ester on the arterial wall, and prevent the formation of atherosclerosis. Hawthorn triterpene acids were reported to reduce blood TC by inhibiting the activation of ACAT in the hamster intestinal. Oleanolic acid (OA) and ursolic acid (UA) are responsible for the cholesterol-lowering effect of hawthorn by inhibiting intestinal ACAT activity. In addition, hawthorn and particularly its bioactive compounds (OA and UA) enhanced the cholesterol-lowering effect of plant sterols [11].

The microsomal triglyceride transfer protein (MTP) plays a pivotal role in the assembly and secretion of apolipoprotein B. Fresh garlic extract (FGE) at 3~6 g/L reduced MTP mRNA levels in both the human hepatoma HepG2 and intestinal carcinoma Caco-2 cells in dose-dependent fashion; maximal reductions reached to 72% and 59%, respectively. Rats fed FGE had significantly (46% of the control) lower intestinal MTP mRNA levels compared with the control rats. Long-term dietary supplementation of fresh garlic may exert a lipid-lowering effect partly through reducing intestinal MTP gene expression, thus suppressing the assembly and secretion of chylomicrons from intestine to the blood circulation [12].

3. Inhibiting the Endogenous Lipid Biosynthesis

Cells *in vivo* synthesize cholesterol via the mevalonate or HMG-CoA reductase (HMGCR) pathway. HMGCR and 7-dehydrocholesterol reductase (DHCR7) are the key rate-limiting enzymes of cholesterol synthesis and play vital roles in maintaining the cholesterol homeostasis. High-energy, high-fat, and high-saturated fatty acid diet, which can promote the synthesis of cholesterol, is the most common risk factor of hyperlipidemia, especially hypercholesterolemia.

Dozens of TCM herbs, including coptis (berberine), salvia, hawthorn, hawthorn-flavone, green tea and its active ingredient-catechol, triterpenoid in alisma, and gypenosides, have been reported to inhibit the endogenous synthesis of cholesterol [5].

Chang reported that berberine might inhibit the synthesis of cholesterol in the liver by upregulating the expression of hepatic Insig-2 gene at low dose, while at high dose, berberine reduces the expression of Insig-2 mRNA and protein [13].

Astragalus and *Angelica* were also reported to reduce the serum level of TC and LDL-C via inhibiting HMGCR activity in hyperlipidemic rat. Angelica and ferulic acid competed with 5-methyl acid pyrophosphate and inhibit the activity of methyl valerate-5-pyrophosphate decarboxylase of the rat liver cell and thereby inhibit the cholesterol biosynthesis [14].

High-dose aqueous extracts from artichoke leaves were found to inhibit cholesterol biosynthesis from 14C acetate in primary cultured rat hepatocytes in a concentration-dependent biphasic manner with moderate inhibition (approximately 20%). Cynaroside and particularly its aglycone luteolin in the artichoke extract were mainly responsible for inhibition of hepatic cholesterol biosynthesis in an indirect downmodulation of HMGCR, which may contribute to the recently confirmed hypolipidemic effect in human [15].

Fatty acid synthase (FAS) catalyzes the last step of fatty acid biosynthesis. Radix Notoginseng can inhibit FAS and thus lower serum TG level. Zhang reported that *Panax notoginseng* saponins [16] and green tea polyphenols [17, 18] have been reported to inhibit the mRNA and protein expression of Iκ-Bα and FAS in abdominal aortic tissue or in adipocyte, so as to lower lipid in high-fat hyperlipidemic rat.

4. Regulating Lipoprotein Lipase Activity

Lipoprotein lipase (LPL) and hepatic lipase (HL) play vital roles in the metabolism of chylomicrons and very low-density lipoprotein. Lacking of these two enzymes or their dynamic abnormalities might lead to dyslipidemia, metabolic syndrome, atherosclerosis, diabetes, preeclampsia, and other diseases [19, 20].

Increase in mRNA and protein levels of LPL might increase the activity of LPL in the adipose tissue and plasma, promote the clearance of VLDL and postprandial plasma lipid, reduce plasma triglycerides, increase HDL-C levels, and therefore prevent hypercholesterolemia induced by high fat diet and development of atherosclerosis [21, 22].

Berberine, hawthorn, turmeric, red yeast, rhubarb, and purslane might increase the expression and activity of the LPL [5, 23–26]. Upregulating the transcription of LPL mRNA and LDL-R-mRNA in liver may also be one of the molecular mechanisms of blood lipids regulation of Xuezhikang, the red yeast extract [27]. Red ginseng acidic polysaccharide (RGAP), isolated from Korean red ginseng, was also reported to significantly enhance the serum activity of LPL [28].

Hepatic lipase (HL) is another important glycoprotein that catalyzes the hydrolysis of lipoprotein triacylglycerols and phospholipids. The majority of HL is synthesized and secreted by the liver and bound to heparin sulfate proteoglycans on the surface of sinusoidal endothelial cells and external surfaces of microvilli of parenchymal cells in the space of Disse, promoting the uptake of HDL and apolipoprotein-B-containing remnant particles [21, 29, 30]. Its catalytic activity contributes to the remodeling of LDL and high-density HDL to smaller, denser particles. HL also participates, with surface proteoglycans, the scavenger receptor B1 (SR-B1) and the LDL receptor-like protein, as a ligand in promoting hepatic uptake of lipoproteins. Recent *in vivo* and *in vitro* studies suggest alternative pathways, both through its catalytic activity and, independently, by which HL may modulate the development of cardiovascular and cerebrovascular disease [29, 30].

Saponins from *Tribulus terrestris* increase the activity and expression of HL in liver and the activity of LPL in skeletal muscle. These might be, at least in part, the underlying mechanisms for its effect in reducing serum levels of TG and TC [31].

Studies in our laboratory showed that Fufang Zhenzhu Tiaozhi Fang (FTZ) could improve serum lipid profile (TC, TG, apoB, LDL-C and HDL-C, apoA). Experimental studies in rat demonstrated that FTZ upregulates LPL and HL expression and increases the activity of both LPL and HL [32].

Pleurotus eryngii water extract (PEE) showed significant inhibitory activity against pancreatic lipase by preventing interactions between lipid emulsions and pancreatic lipase *in vitro*. The hypolipidemic effect of PEE in fat-loaded mice may be due to low absorption of fat caused by the inhibition of pancreatic lipase [33]. The ethyl acetate fraction of the rhizome of *Alpinia officinarum* (AO) exhibited potent inhibition of pancreatic lipase. 3-Methylethergalangin was isolated from the fraction as an inhibitor of pancreatic lipase with an IC50 value of 1.3 mg/mL (triolein as a substrate). AO and its ethyl acetate fraction significantly lowered the serum TG level in corn oil feeding-induced triglyceridemic mice, and serum TG and cholesterol in Triton WR-1339-induced hyperlipidemic mice. The hypolipidemic activity of AO and 3-methylethergalangin was due to the inhibition of pancreatic lipase [34].

The ethanolic extract of *Ananas comosus* L. leaves (AC) (0.40 g/kg) significantly reduced the increased serum triglycerides by 40% in fructose-fed mice. AC also significantly inhibited serum TC in Triton WR-1339 and alloxan plus high-fat diets-induced hyperlipidemic mice. AC (0.01–100 μg/mL) selectively activated LPL activity by 200%–400% and significantly inhibited HMGCR activity by 20%–49% *in vitro*. Furthermore, AC (0.40 g/kg) did no increase mice liver weights as fenofibrate (0.20 g/kg) administration. Xie et al. recognized that AC will be a new potential natural product for the treatment of hyperlipidemia through the mechanisms of inhibiting HMGCR and activating LPL activities, which was different from those with fibrates but may be partly similar to those with statins. It is hopeful that AC may serve as the adjuvant for fibrates [35].

5. Regulation of Cholesterol Transport

Inhibiting plasma cholesterol ester transfer protein (CETP) might increase the content of HDL-C and ApoA1, which is helpful to reduce TC, TG, and LDL-C. Lin et al. used a software to select CEPT inhibitor fictitiously. Dihydrotanshinone I, chosen as the target molecule with the CEPT-inhibiting active ingredient from salvia, was found to increase HDL and decrease LDL levels and could reduce TC and TG levels in serum and liver in the experimental hypercholesterolemic rat [39].

Berberine, an alkaloid isolated from the Chinese herb *Coptis chinensis*, has been recently identified as a new cholesterol-lowering drug and reduced serum TC and LDL-C levels in hyperlipidemia rats in a dose-dependent manner. The LDL cholesterol-lowering effect of berberine was attributed to its activity on hepatic LDLR expression via a new mechanism distinct from that of statins [36–38]. In a human hepatoma cell line (HepG2) as well as in hyperlipidemic hamsters, Kong et al. showed that berberine upregulated the expression of LDLR through stabilization of its mRNA involving an extracellular-regulated-kinase-(ERK-)dependent mechanism [36].

Vijayakumar reported that hypolipidemic effect of a novel thermostable extract of Fenugreek seeds (*Trigonella*

foenum-graecum, TEFS) was due to inhibition of fat accumulation and upregulation of LDLR. Under sterol-enriched condition, TEFS upregulated low-density lipoprotein receptor (LDLR) expression resulting in enhanced LDL uptake. TEFS administration for 15 days decreased the elevated serum TG, LDL-cholesterol, and body weight in a dose- and time-dependent manner in fat-supplement-fed C57BL6/J mice [42].

6. Promotion of Cholesterol Converting into Bile Acid and Excreting

Cholesterol is transformed into bile acids and excreted from the digestive tract under the catalysis of liver cholesterol 7α-hydroxylase (CYP7A1). About 2/5 synthesized cholesterol is converted into bile acids, under the catalysis of CYP7A1. Promoting CYP7A1 activity might enhance cholesterol to convert into bile acid, thereby to remove the cholesterol from the body, which is the primary mechanism for maintaining in cholesterol homeostasis [53, 54].

Xue et al. reported that radix *Salviae miltiorrhizea* extract containing danshensu is absorbed into blood after oral administration. Sodium danshensu (25~200 μg/mL) increased the percentage of cell viability in experiment of amphotericin B cell model and upregulated the expression of CYP7A mRNA in BRL cells, indicating that Danshensu can inhibit the synthesis of endogenous cholesterol and increase the expression of CYP7A1 mRNA to promote cholesterol transformation into bile acid in hamster liver cells [40, 41].

Zhang et al. reported that hawthorn fruit aqueous ethanolic extract decreased serum TC and TG by 10% and 13%, respectively, in hamsters which were fed with semisynthetic diet containing 0.1% cholesterol. Compared with the control, hawthorn fruit led to greater excretion of both neutral and acidic sterols. Further enzymatic assays showed that hawthorn fruit might promote the excretion of bile acids by upregulation of hepatic CYP7A1 activity and inhibition of cholesterol absorption mediated by downregulation of intestinal acyl CoA cholesterol acyltransferase (ACAT) activity [24].

Garcia-Diez reported that the addition of pectin to the diet resulted in lower serum and liver cholesterol concentrations (−27% and −17%, resp.) in male Wistar rats fed a fiber-free or a pectin-supplemented (7 g/100 g) diet for 4 wk. Fecal bile acid excretion (+168%) and the hepatic activity of CYP7A1 (+70%) were significantly higher in pectin-fed animals. HMGCR activity was also significantly greater (+11%) in the presence of dietary pectin. Pectin may increase hepatic synthesis of bile acids and liver depletion of cholesterol in rats, resulting in a higher rate of cholesterol synthesis and reduced serum cholesterol concentrations [46].

Vergara-Jimenez reported that plasma LDL cholesterol, TG, apolipoprotein B, and hepatic cholesteryl ester were lower in guinea pigs fed pectin (PE) and psyllium (PSY) compared to the control group. In addition, a 45% higher number of hepatic apoB/E receptors were observed by PE and PSY intake. Hepatic ACAT, HMGCR, and CYP7A1 activities were higher in the high-fat (HF) compared to the low-fat (LF) groups. PSY intake with HF resulted in upregulation of CYP7A1 and HMGCR activities. ApoB secretion was reduced by pectin and psyllium intake, while LDL fractional catabolic rates were 100% faster in guinea pigs fed PE or PSY in the HF groups [47].

Psyllium, the husks from *Plantago ovata* (PO), is recognized as a potent agent in lowering plasma cholesterol. Plasma triglycerides and LDL cholesterol were 34% and 23%, respectively, lower in the PO groups compared with the control male Hartley guinea pigs. Lecithin cholesterol acyltransferase (LCAT) and cholesterol ester transfer protein (CETP) activities were significantly affected by the PO diets. The control group had 100% and 36% higher LCAT and CETP activities, respectively, compared with the PO groups. Hepatic cholesteryl ester concentrations were 50% lower in the PO groups compared with the control. The activity of HMGCR was upregulated in the PO groups by 37%. Similarly, the activity of CYP7A1 was 33% higher in the PO groups. Fecal bile acids were 3 times higher in the PO groups than in the control group. PO exerts its hypolipidemic effect by affecting bile acid absorption and altering hepatic cholesterol metabolism [48].

FTZ significantly decreased the levels of serum TC, TG, and LDL-C whilst elevated the serum HDL-C and decreased serum atherogenic index (A.I.) values in high-lipid-diet-induced hyperlipidemic rats. Furthermore, FTZ showed significant antihyperlipidemic effect by at least three pathways in the high-lipid diet-induced-hyperlipidemic rats: (1) upregulating the gene expression and activity of CYP7A1 which promotes the conversion of cholesterol into bile acid; (2) downregulating the gene expression and activity of HMGCR to reduce *de novo* synthesis of cholesterol; (3) increasing the cholesterol excretion from feces. In these three pathways, HMGCR and CYP7A1 are two pivotal enzymes in lipid cholesterol metabolism and are expressed mainly in hepatic cells, which support the new TCM treatment strategy: modulating liver to treat hyperlipemia [49].

7. Effects of TCM on Regulation of Transcription Factors Related to Lipid Metabolism

Metabolic enzymes and receptors involved in the lipid metabolism are subject to positive and negative regulation of dozens of transcription factors. At present, some of intensively investigated genes involved in the lipid metabolism are peroxisome proliferator-activated receptor (PPARs) [55, 56], sterol regulatory element binding protein (SREBPs) [57, 58, 60, 61], and liver receptor (the liver X receptors, LXR) [59–61] gene. In recent years, there have been increasing studies to explore the regulating effects of TCM on transcription factors related to lipid metabolism.

7.1. PPARs Regulation. Studies have showed that several active ingredients from TCM herbs are PPARs agonists, and their active ingredient can activate PPARs.

Coptis and its active ingredient berberine could activate the PPARs, including PPARα [50] and PPAR-γ [23]. Hawthorn flavonoids might activate PPAR-α and increase the

Table 1: The regulation of some vital targets related to lipid metabolism by TCM.

Herb/ingredients	Ch absorption	Ch transport (LDLR, ...)	Lipid catabolism (HL, LPL)	Ch synthesis (HMG-CoA R)	BA synthesis (CYP7A1)	Others	Reference
Ananas comosus leaves			LPL(+)	(−)			[35]
Astragalus, Angelica/ferulic acid				(−)			[5, 14]
Alpinia officinarum/3-Methylethergalangin			Pancreatic L(−)				[34]
Artichoke/cynaroside, luteolin				(−)			[15]
Cassia protein	(−)						[6]
Coptis/berberine		(+)	LPL(+)	(−)			[32, 36–38]
Danshen/dihydrotanshinone 1, salvianolic acid, danshensu	(+)	LDLR(+), CEPT(−)		(−)	(+)	ABCA4/11(+)	[39–41]
FTZ		(+)	HL(+), LPL(+)	(−)	(+)		[32]
Fenugreek (TEFS)		(+)					[42]
Fleece-flower root/anthraquinones, Lecithin,	(−)	(+)			(+)	ACAT(−)	[5]
Garlic						MTP(−)	[12]
Ginseng			LPL(+)				[28]
Green tea/catechol, saponin	(−)					FAS(−)	[17, 18]
Gynostemma pentaphyllum/gypenoside				(−)			[43]
Hawthorn/hawthorn-flavone		ACAT(−)	LPL(+)	(−)	(+)	ACAT(−)	[24]
Resin of the guggul tree/guggulsterone				(−)	(+)		[44]
Hawthorn, ligustrum lucidum/oleanolic acid				(−)		ACAT(−)	[11, 51]
Notoginseng/saponin						FAS(−)	[16]
Pectin				(+)	(+)		[46]
Pleurotus eryngii	(−)		Pancreatic L(−)				[33]
Plantago ovata		CEPT(−)		(+)	(+)	LACT(−)	[48]
Psyllium		CEPT(−)		(+)	(+)	ApoB/LCAT(−)	[47]
Red yeast/xuezhikang		(+)	LPL(+)	(−)			[27]
Tribulus terrestris/saponins			HL(+), LPL(+)				[31]
Turmeric/curcumin			LPL(+)				[25, 26]

Table 2: The regulation of transcription factors related to lipid metabolism by TCM.

Herb/ingredients	PPAR-$\alpha/\gamma/\delta$	LXR-α	SREBPs	FXR	Others	Reference
Berberine	$\alpha(+),\gamma(+)$		SRE1		Insig-i(+), CPTIA	[23, 36–38, 50]
Curcumin	$\gamma(+)$					[25, 26]
Danshen/dihydrotanshinone 1, salvianolic acid, and Danshensu rosmarinic acid	$\alpha(+)$	(+)	−1c(−)	(+)	ABCA1, ABCB4/11, SHP(+)	[40, 41]
Ginseng	$\alpha(+)$					[45]
Gynostemma pentaphyllum/gypenoside	$\alpha(+)$					[43]
Resin of the guggul tree/guggulsterone				(−)	AR/GR/MR(−); PR/ERα(+)	[44]
Ligustrum lucidum/oleanolic acid	$\alpha(+),\gamma(+)$				protein kinase B (+)	[51]
Hawthorn, hawthorn-flavone	$\alpha(+)$					[11, 24]
Morroniside	$\alpha(+)$		−1(−); −2(−)		NF-kβ, iNOS, (−)	[52]
FTZ	$\alpha(+)$		(−)			[49]
Fenugreek (TEFS)	$\gamma(-)$		−1(−)		cEBP-α(−)	[42]

Notes for tables:
(+): positive/upregulation; (−) negative/downregulation

LPL activity of the blood and muscle tissue of hyperlipidemic rats or mice [24]. Curcumin could activate PPAR-γ and thus increase LPL activity in tissues and blood [25, 26].

Gynostemma pentaphyllum has showed antihyperlipidemic and hypoglycemic effects in the obese Zucker fatty diabetic rat model. Its active ingredient gypenoside XLIX was proved as a naturally occurring PPARα activator [43].

Gao et al. found that oleanolic acid extracted from *Ligustrum lucidum* could lower TC, TG, and LDL-C by activating the gene expression of cell PPAR-α, γ and protein kinase B [51]. Ginseng can also lower serum lipid by activating PPAR-α in cells [45].

7.2. SREBP Regulation. Lipid homeostasis is subject to the regulation of SREBPs, a class of membrane-bound transcription factor [57, 58, 60, 61].

Berberine promotes the translation of LDL-R gene by directly stabilizing the structure of SRE1 of LDL-R gene 5′ flanking region [36].

TEFS inhibited accumulation of fat in differentiating and differentiated 3T3-L1 cells via decreased expression of adipogenic factors such as PPAR-γ, SREBP-1, and CAAT element-binding proteins-alpha (c/EBP-alpha). TEFS treatment also significantly decreased cellular TG and cholesterol concentrations in HepG2 cells via reduced expression of SREBP-1, at mRNA as well as protein level [42].

Morroniside significantly decreased the elevated serum TG and alanine aminotransferase levels as well as hepatic glucose and lipids contents in a dose-dependent manner in type 2 diabetes model mice (C57BLKS/J db/db mice). The administration of morroniside also increased the antioxidative effects in the liver of db/db mice with hyperglycemia and dyslipidemia. The elevated expressions of nuclear factor-kappa Bp65, cyclooxygenase-2, inducible nitric oxide synthase, SREBP-1, and SREBP-2 were downregulated in the liver of db/db mice, but significantly increased PPARa expression by morroniside [52].

7.3. LXRs Regulation by TCM. LXRs are the receptors of nuclear-oxidized steroid. [59–61]. LXRs were activated by inducing SREBP-1c and finally upregulated the genes involved in fatty acid and triglyceride synthetic genes and fatty acid synthase, such as FAS, SCD1. At the same time, LXRs were also involved in regulating insulin-induced SREBP-1c gene expression [62, 63].

The plant sterol guggulsterone [4, 17 (20)-pregnadiene-3,16-dione] (GS) is the active substance in guggulipid, the resin of the guggul tree (*Commiphora mukul*) used to treat a variety of disorders in humans, including dyslipidemia, obesity, and inflammation. GS is a highly efficacious antagonist of the farnesoid X receptor (FXR), a nuclear hormone receptor that is activated by bile acids. GS treatment decreases hepatic cholesterol in wild-type mice fed a high-cholesterol diet but is not effective in FXR-null mice. Inhibition of FXR activation is proposed as the basis for its cholesterol-lowering activity [44, 63].

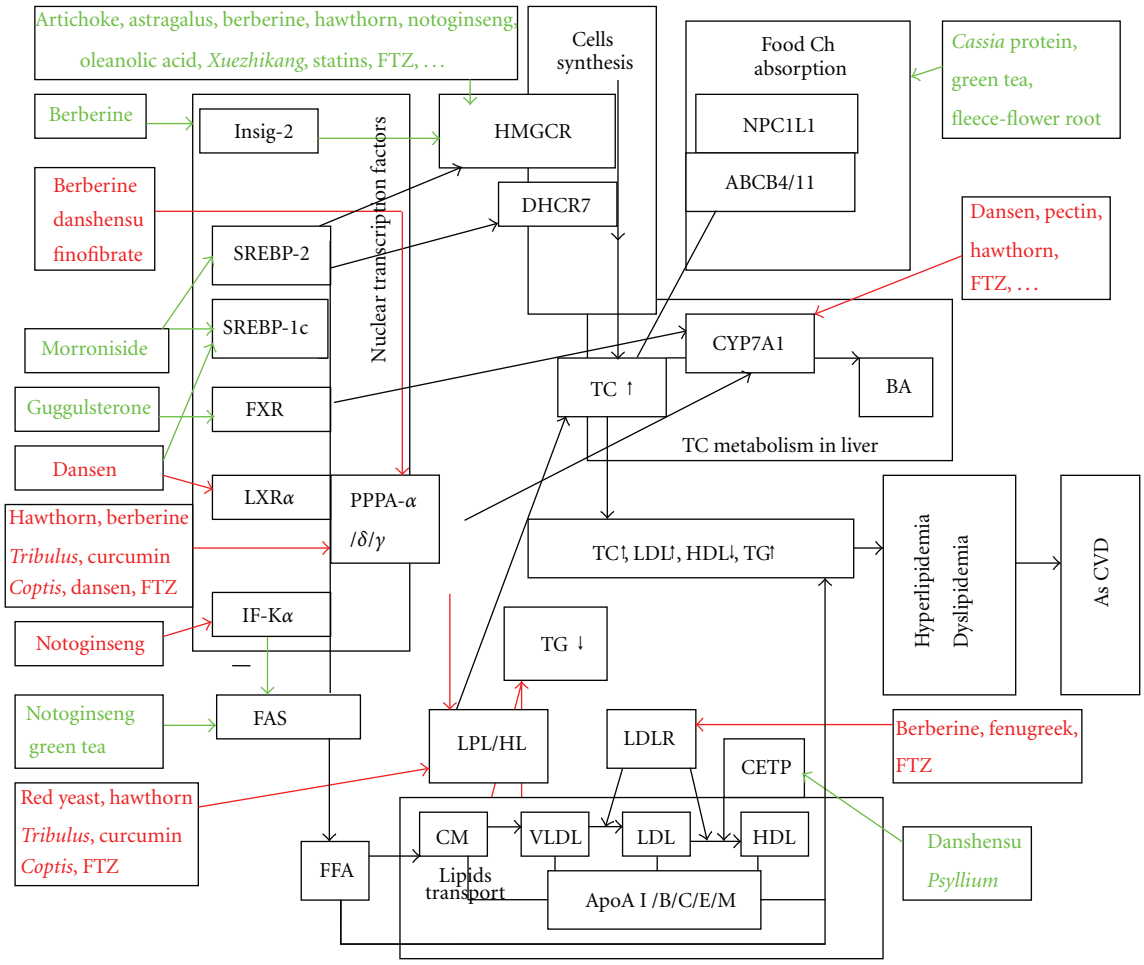

Figure 1: The regulation of some vital targets related to lipid metabolism by TCM. TCM might regulate all processes of lipid metabolism such as the synthesis, absorption, transport and metabolism of cholesterol and TG. Targets related to the regulation of lipid metabolism by TCM herbs are mainly including HMGCR FAS, LDL-R, CEPT, LPL, HL, CYP7A1, PPPA-α, SREBP, and LXRa. Some Chinese herb active components including berberine, danshensu, curcumin and hawthorn flavonoids, and ginseng saponins may regulate the different processes of lipid metabolism, and some vital targets relate to lipid metabolism as the figure arrow showed. Red arrows present positive or upregulation. Green arrows demonstrate negative or downregulation.

Extraction of salvia (PSME, containing Danshensu, salvianolic acid A/B, and rosmarinic acid) might also regulate the expression of FXR/LXRα and then induce the expression of ATP-binding cassette transporter protein family (ABCB11) and mouse Mdr2 P-glycoprotein (also known as ABCB4), which is responsible for bile cholesterol solubility and bile secretion in bile salts and bile phospholipids. The transcriptional activation experiments showed that PSME is a coagonist for FXR and LXR α. PSME might improve lipid spectrum of male high-fat high-cholesterol diet-induced hyperlipidemic SD rats by activating FXR/LXR α, and PSME decreases liver and plasma TG through an FXR-SHP-SREBP-1c pathway [41].

8. Conclusion

In summary, TCM might regulate all processes of lipid metabolism (Figure 1). Targets related to the regulation of lipid metabolism by TCM herbs are mainly HMG-CoA reductase, FAS, LDL-R, CEPT, LPL, HL, CYP7A1, PPPA-α, SREBP, LXRa, and other targets (Tables 1, 2, Figure 1). The Chinese herb active components include alkaloids (berberine), phenols (Danshensu, Tea Polyphenols), flavonoids (curcumin and hawthorn flavonoids), triterpenoid saponins (ginseng saponins, glycosides from Tribulus terrestris), and statins (*Monascus* prime, red yeast), which demonstrates multitarget, multicomponent features of traditional Chinese herb medicine for the regulation of lipid metabolism (Tables 1 and 2, Figure 1).

However, presently most of the studies on the mechanisms of TCM focus only on the efficacy of lipid lowering (serum lipid profile in TC, TG, LDL-C, and HDL-C) of a composite herb formula or its active ingredient and usually aim at one or two targets of lipid regulation. The interaction between the components in the composite acting on the same target is rarely involved, which failed to reflect the characteristics of the mechanism of Chinese herbal composite and the full-scale picture of Chinese medicine. It is

needed to unveil the mystery of TCM and mine underground advantage of the TCM composites in dealing with the complex dyslipidemia by further study on the profound mechanism of TCM composite involving interaction among the multitargets and multicomponents.

Abbreviations

ABC: ATP-binding cassette
ABCA1: ATP-binding cassette transporter A1
ABCB4: Mdr2 P-glycoprotein
ABCB11: ATP-binding cassette transporter protein family
ABCG5/8: ATP-binding cassette transporters ABCG5/ABCG8
AC: The ethanolic extract of *Ananas comosus* L. leaves
ACAT: Acyl coenzyme A cholesterol acyltransferase
ApoA/B: Apolipoprotein A/B
AR: Androgen receptors
BA: Bile acid
CETP: Cholesterol ester transfer protein
Ch: Cholesterol
c/EBP-alpha: CAAT element-binding proteins-alpha
ERα: Estrogen receptor alpha
CYP7A1: Cholesterol 7α-hydroxylase
FAS: Fatty acid synthase
FTZ: Fufang Zhenzhu Tiao Zhi
FXR: Farnesoid X receptor
GR: Glucocorticoid receptors
HDL-C: High-density lipoprotein cholesterol
HL: Hepatic lipase
HMGCR: HMG-CoA reductase
iNOS: Inducible nitrogen oxide synthase
Insig-2: Insulin-induced gene-2
LACT: lecithin cholesterol acyltransferase
LDL-C: Low-density lipoprotein cholesterol
LDLR: LDL receptor
LPL: Lipoprotein lipase
LXRα: Liver X receptor α
MR: Mineralocorticoid receptors
MTP: Microsomal triglyceride transfer protein
NF-kβ: Nuclear factor-kappa beta
NPC1L1: Niemann-Pick C1 Like 1
PPAR$\alpha/\gamma/\delta$: Peroxisome proliferators activated-receptor -alpha/gamma/delta
PR: Progesterone receptors
PSME: Salvia extract
SHP: Short heterodimer partner
SRE-1: Sterol regulatory element 1
SREBP-1/2: Sterol regulatory element-binding protein-1/2
SR-B1: Scavenger receptor B1
TC: Cholesterol
TCM: Traditional Chinese medicine
TG: Triglyceride
TEFS: Thermostable extract of fenugreek (*Trigonella foenum-graecum*) seeds
VLDL: Very low-density lipoprotein.

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Review Article

Chinese Medicine Shenfu Injection for Heart Failure: A Systematic Review and Meta-Analysis

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Objective. Heart failure (HF) is a global public health problem. Early literature studies manifested that Shenfu injection (SFI) is one of the most commonly used traditional Chinese patent medicine for HF in China. This article intended to systematically evaluate the efficacy and safety of SFI for HF. *Methods*. An extensive search was performed within 6 English and Chinese electronic database up to November 2011. Ninety-nine randomized controlled trails (RCTs) were collected, irrespective of languages. Two authors extracted data and assessed the trial quality independently. RevMan 5.0.2 was used for data analysis. *Results*. Compared with routine treatment and/or device support, SFI combined with routine treatment and/or device support showed better effect on clinical effect rate, mortality, heart rate, NT-proBNP and 6-minute walk distance. Results in ultrasonic cardiography also showed that SFI combined with routine treatment improved heart function of HF patients. There were no significant difference in blood pressure between SFI and routine treatment groups. Adverse events were reported in thirteen trails with thirteen specific symptoms, while no serious adverse effect was reported. *Conclusion*. SFI appear to be effective for treating HF. However, further rigorously designed RCTs are warranted because of insufficient methodological rigor in the majority of included trials.

1. Introduction

Heart failure (HF) is a leading cause of death, hospitalization, and rehospitalization worldwide. Despite advances in the treatment of HF, including use of drugs, devices, and heart transplantation, the condition remains associated with substantial morbidity and mortality [1].

International cooperation research program on cardiovascular disease in Asia showed that, on a total of 15,518 Chinese adults (35–74 years old) survey, the prevalence of HF was 0.9%, 0.7% for the males, and 1.0% for the females [2]. In the United States, HF incidence approaches 10 per 1,000 of the population over 65 years of age [3]. A report from the European Society of Cardiology (ESC) indicated at least 10 million patients with HF in these representing countries with a population of over 900 million. Half of the HF patients will die within 4 years, and more than half of those with severe HF will die within 1 year [4].

At present, the conventional therapeutic approaches in HF management include angiotensin-converting enzyme (ACE) inhibitors, β-blockers, and diuretics. Although several of them have led to an important effectiveness, HF remains the leading cardiovascular disease with an increasing hospitalization burden and an ongoing drain on health care expenditure [5]. Therefore, it remains necessary to search alternative and complementary treatment, in which Traditional Chinese Medicine takes a good proportion [6].

In TCM theory, pathogenesis of HF is related to deficiency of heart *yang* and heart *qi* and stasis of *blood* and excessive *water (fluid)*, as well as interaction within these pathological factors. Under physiological conditions, *yang* can promote *water* metabolism, while *qi* can accelerate *blood* circulation, so *yang* and *qi* are the vital elements for human body to maintain life activity. TCM theory holds that patients suffered from HF are in deficiency of heart *yang* and *qi* for a long course, which directly leads to excessive *fluid* retention and *blood* stasis (Figure 1).

Two Chinese herbal medicines, namely, Radix *Ginseng* (ginseng) and Radix *Aconiti Lateralis Preparata* (prepared aconite root), are used in treating HF over 2000 years.

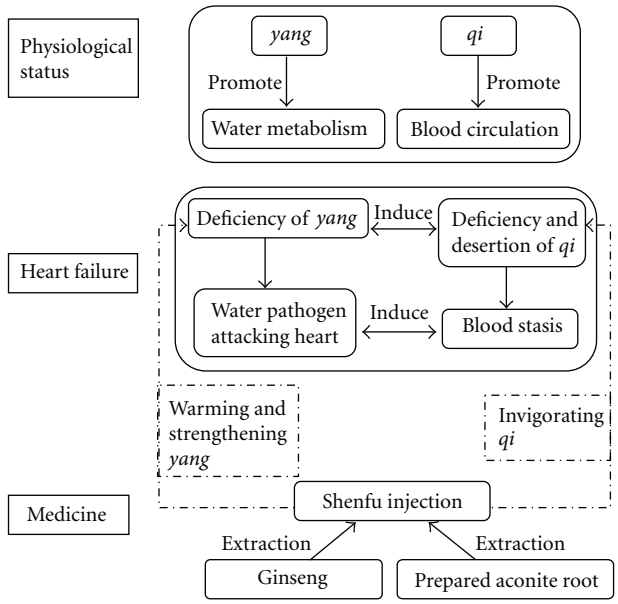

Figure 1: TCM theory on heart failure and Shenfu injection.

Ginseng invigorates *qi*, while prepared aconite root can warm and strengthen yang and lead to diuresis. Long-term clinical practice has proved that compatibility of ginseng and prepared aconite root can effectively ameliorate patients' symptom of HF and improve quality of life (Figure 1).

Shenfu injection (SFI) has been used in treating cardiac diseases for a long time in China [7]. The main active components of SFI are extraction of traditional Chinese herbs, namely, ginsenosides and higenamine. Modern pharmacological research shows that ginsenosides can improve ischemic myocardium metabolism, scavenge free radicals, protect myocardial ultrastructure, and reduce Ca^{2+} overload, and higenamine can enhance heart contractility, improve coronary circulation, and decrease the effect of acute myocardial ischemia [8].

Currently, SFI used alone or integrated with routine treatments has been widely accepted as an effective method for the treatment of HF in China. Many clinical studies reported the effectiveness ranging from case reports and case series to controlled observational studies and randomized clinical trials, but the evidence for its effect is not clear. This paper aims to evaluate the beneficial and harmful effects of SFI for treatment of HF in randomized controlled trials.

2. Methods

2.1. Database and Search Strategies. A systematic search was conducted in 5 databases including PubMed (1980–2011), China National Knowledge Infrastructure (1994–2010), VIP Database for Chinese Technical Periodicals (1979–2010), Chinese Biomedical Literature Database (1995–2011), and Cochrane Library (Issue 10, 2011), with the following terms: (Shenfu injection or Shenfu or Shen-fu) AND (heart failure or cardiac dysfunction or cardiac inadequacy or cardiac failure or congestive heart failure). All of those searches ended before November 2011. And the bibliographies of included trials were searched for thorough references, irrespective of languages.

2.2. Inclusion Criteria. All the randomized controlled trails (RCTs) of SFI compared with routine or conventional treatment (control group) in adult patients with HF were included. RCTs combined SFI with conventional treatment and/or invasive respiratory support (SFI group) compared with conventional treatments and/or invasive respiratory support (control group) were included. Both acute heart failure and chronic heart failure were included. Outcome measures include clinical effect rate, death and adverse events, ultrasonic cardiography, heart rate and blood pressure, and quality of life.

2.3. Data Extraction and Quality Assessment. Two authors (S. Wen-Ting and C. Fa-Feng) extracted the data from the included trials independently, based on the inclusion criteria outlined above. Nonrandomized evaluations, pharmacokinetic studies, animal/laboratory studies, and general reviews were excluded, and duplicated publications reporting the same groups of patients were also excluded (Figure 2).

Extracted data was entered into an electronic database by two authors, S. Wen-Ting and C. Fa-Feng independently. The methodological quality of RCTs was assessed by using criteria from the Cochrane Handbook for Systematic Reviews of Interventions, Version 5.0.1. The quality of trials was categorized into low risk of bias, unclear risk of bias, or high risk of bias according to the risk for each important outcome within included trials, including adequacy of generation of the allocation sequence, allocation concealment, blinding, whether there were incomplete outcome data or selective outcome, or other sources of bias.

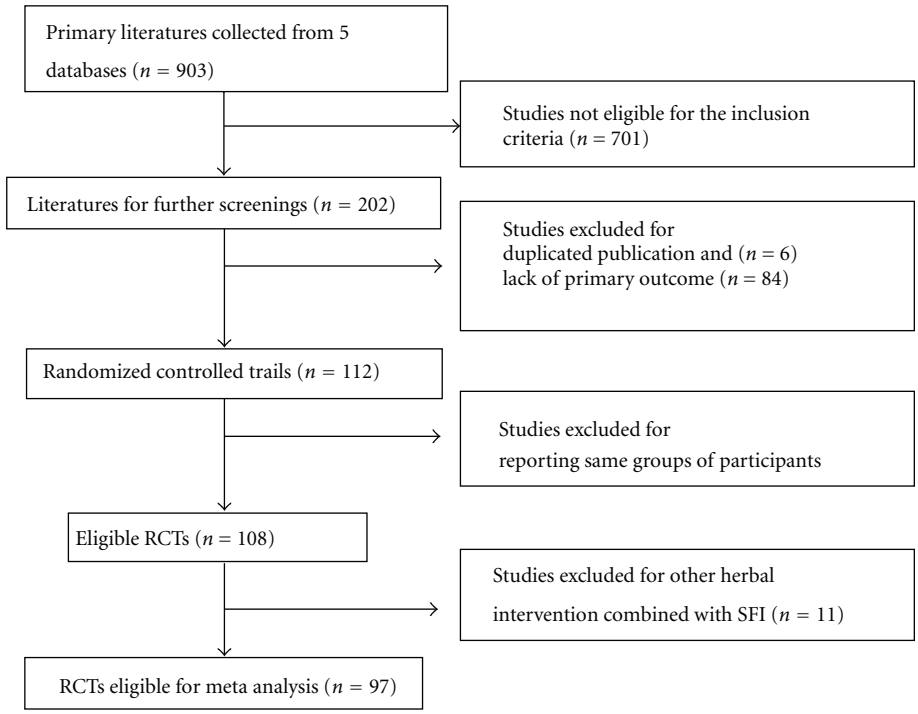

Figure 2: Diagram of the study selection flow.

2.4. Data Synthesis. The statistical package (RevMan 5.0.2), which is provided by The Cochrane Collaboration, was used to analyze collected data. Dichotomous data was presented as risk ratio (RR), with 95% confidence intervals (CIs). Continuous outcomes were presented as mean difference (MD), with 95% CI. Analyses were performed by intention-to-treat where possible. Heterogeneity between trials results was tested, and heterogeneity was presented as significant when I^2 is over 50% or $P < 0.1$. Random effect model was used for the meta-analysis if there was significant heterogeneity, and fixed effect model was used when the heterogeneity was not significant [21]. Publication bias was explored via a funnel-plot analysis.

3. Result

3.1. Search Flow. According to the search strategy, we screened out 903 potentially relevant studies for further identification (Figure 2). By reading titles and abstracts, we excluded 701 studies that were obviously ineligible, including review articles, case reports, animal/experimental studies, and non-randomized trials. 202 studies with full text papers were retrieved. After the full text reading, 6 studies were excluded because of duplicated publication. 84 studies were excluded due to lack of clinical effect rate which is the primary outcome evaluated in present study. 4 studies were excluded because the reported groups of participants were same as previous trials. In 108 RCTs, 11 studies were excluded due to other herbal intervention which was combined with SFI as treatment arm. Thus, 97 RCTs [9–20, 22–108] were included for systematic review.

3.2. Description of Included Trials. Ninety seven RCTs involved a total of 8,202 patients with HF, including 92 trails (7854 patients) of chronic HF and 5 trials (348 patients) of acute HF. The sample size varied from 24 to 248 participants, with an average of 42 patients per group. Since RCTs of HF on children were excluded, patients are adults (ranged from 28 to 89 years old). More males were included than females (52% males and 48% females). Disease duration was reported in 31 trials, ranging from 3 months to 26 years. 49 trials were observed in inpatients, 5 outpatients [22–26], 5 both inpatients and outpatients [27–31], and 39 unclear. All studies were published in Chinese.

Mortality was reported in eleven studies, while the rest of the eighty eight trials did not mention death. Effect rate was assessed in all the trials, based on the improvement of heart function. Ninety one trials used New York Heart Association (NYHA) Classification of Clinical Status, and six trials used Killip's Rating Standards [22, 25, 26, 33–35] for diagnosing HF and rating the patients. Patients in fifty one trails ranged from II to IV, seven trials II to III, twenty one trials III to IV, and five trials IV according to NYHA Classification; patients in five trials ranged from II to IV and one trial IV according to Killip's Standard.

Results of ultrasonic cardiography were reported in 61 trails (5135 patients) with left ventricular ejection fraction (LVEF) as main parameter. Other parameters such as left ventricular diastolic diameter (LVDd), cardiac output (CO), cardiac index (CI), stroke volume (SV), and A peak E-wave velocity ratio (E/A) were reported in 16, 17, 20, 18, and 11 trials, respectively. N-terminal pro-B-type nature tripeptide (NT-proBNP) level in blood was reported in 12 studies of 887 patients, and 6-minute walk distance (6-MWD) was reported

in 8 trials of 630 patients. Heart rate, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were reported in 27, 15, and 13 trials, respectively (Table 1).

3.3. Methodological Quality of Included Trials. According to our predefined quality assessment criteria, all of 97 included trials were evaluated as having unclear risk of bias (Table 2, Figure 3). None of the 97 trials reported sample size calculation. Eleven trials described randomization procedures, nine trials [9–11, 20, 30, 38–41] used a random number table, one drew lots [19], and one trial separated patients by odd and even number of patient ID as a quasirandomization [42]. Only one trial [43] blinded both patients and outcome assessors, and three trials [44–46] blinded patients. None of the trials reported adequate allocation concealment. Five out of ninety seven trials mentioned that followup ranged from 3 months to 12 months after treatment. One trial [47] followed all the patients for 12 months, one trail [38] for 6 month, and the rest [9, 11, 12] for 3 months. However, neither of them used intention to treat method.

3.4. Effect of the Interventions. The primary outcomes were effect rate and mortality. Secondary outcome measures included LVEF, LVDd, SV, CO, CI, HR, systolic blood pressure (SBP), diastolic blood pressure (DBP), NT-proBNP, and 6-MWD.

3.4.1. Primary Outcomes

Effect Rate. All the trials reported clinical effect rate to evaluate the outcome, which was based on NYHA Classification of Clinical Status and Killip's Rating Standards. Killip's Rating Standards were used by six trials with patients of myocardial infarction-induced HF, while other trials used NYHA Classification. Most of trails used three categories to evaluate treatment effect including markedly effective (an improvement of two classes on the classification), effective (an improvement of one class), and ineffective (no improvement, deterioration or death), and others only reported total effect. Total effect rate is the combination of markedly effect rate and effect rate. Trials of myocardial infarction-induced HF and nonmyocardial infarction-induced HF were separated into two subgroups. The meta-analysis showed a total significant difference between SFI and control groups on total effect rate (RR: 1.19, 95% CI [1.17, 1.21]; $P < 0.01$). And significant difference appeared in both subgroups separately, with RR ratio 1.19 in subgroup of myocardial infarction-induced HF (95% CI [1.16, 1.21]; $P < 0.01$), and 1.46 in the other subgroup (95% CI [1.25, 1.70]; $P < 0.01$) (Figure 4).

Death. Eleven studies reported mortality data, and total death number was 142 out of 978. Two trials [12, 38] assessed the mortality with 3- and 6-month followup, respectively, and other trials reported death at the end of treatment course. Trials were also separated into two subgroups depending on whether HF was induced by myocardial infarction. The result of meta-analysis indicated that SFI can significantly reduce mortality of patients of myocardial infarction-induced HF (RR: 0.52, 95% CI [0.37, 0.74]; $P < 0.01$). In the other subgroup, there was no significant difference between mortalities of SFI group and control group (RR: 0.68, 95% CI [0.36, 1.26]; $P = 0.22$). However, total result of both subgroups showed significant difference (RR: 0.56, 95% CI [0.41, 0.75]; $P < 0.01$) (Figure 5).

3.4.2. Secondary Outcomes

NT-proBNP. NT-proBNP level is used for screening and diagnosis of acute HF and may be useful to establish prognosis in HF, as it is typically higher in patients with worse outcome [109]. It was reported in 12 studies [20, 22, 38, 45, 49, 52, 54–59] on 887 patients. Consistent with effect rate and other outcomes, NT-proBNP levels of SFI group were significantly lower than control group (WMD: −201.26; 95% CI [−255.27, −147.25], $P < 0.01$) (Figure 6).

6-MWD. Eight trials [47–54] assessed 6-MWD of patients who received SFI or routine treatment. At the end of treatment, eight trails all showed significant increase in walking distance in SFI group, and meta-analysis result was WMD: 14.22; 95% CI [10.31, 18.13], $P < 0.01$ (Figure 7).

Heart Rate and Blood Pressure. Heart rate and blood pressure were reported in 27 and 15 trials, respectively. Meta-analysis showed that there was statistical significance between SFI group and control group (WMD: 6.31; 95% CI [5.18, 7.44], $P < 0.01$) (see Supplementary Figure 1 in Supplementary Material available online at doi: 10.1155/2012/713149). However, there was no significant difference between both SBP and DBP in two groups (WMD: −0.07; 95% CI [−0.42, 0.27], $P = 0.68$) (WMD: −0.37; 95% CI [−0.97, 0.23], $P = 0.22$) (Supplementary Figures 2 and 3).

Results of Ultrasonic Cardiography. LVEF is the ratio of the stroke volume and the left ventricular end-diastolic volume [107]. It is usually used for the assessment of HF and drug efficacy. Sixty-one studies reported the outcomes for LVEF. Meta-analysis showed that SFI group was better than control group in increasing LVEF (WMD: 6.31; 95% CI [5.18, 7.44], $P < 0.01$) (Supplementary Figure 4).

SV is the volume per stroke by left ventricle, and CO is the volume of blood being pumped by the heart in the time interval of one minute [107]. CI is a vasodynamic parameter that is relating CO to body surface area [107]. All the three parameters indicate left ventricular systolic function, as LVEF does. This paper made meta-analysis of these outcomes, respectively; results showed that SFI group was better than control group in these three parameters: SV (WMD: 7.25; 95% CI [4.60, 9.90], $P < 0.01$); CO (WMD: 0.67; 95% CI [0.47, 0.87], $P < 0.01$); CI (WMD: 0.36; 95% CI [0.23, 0.48], $P < 0.01$) (Supplementary Figures 5–7).

E/A ratio is widely accepted as a clinical marker of diastolic HF, and E/A ratio is reduced in diastolic dysfunction [108]. The result of meta-analysis of E/A ratio was WMD: 0.15; 95% CI [0.08, 0.22], $P < 0.01$, which indicated that SFI better improved diastolic function of heart on HF patients

Table 1: Characters of including trials.

Author Name	Inpatient (Y/N)	Course	Experiment group	Control group	NYHA classification	Disease duration	Followup (month)
Bao and Yu [61]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Chen [55]	Unclear	14 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	Unclear	No
Chen and Liu [14]	Y	60 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt plus metoprolol 6.25 mg, bid, po	Conventional medicine treatment plus metoprolol 6.25 mg, bid,po	II-III	1–15 y	No
Chen and Li [51]	Y	14 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt plus sodium nitroprusside 50 mg, iv.gtt	Conventional medicine treatment plus sodium nitroprusside 50 mg, iv.gtt	IV	Unclear	No
Chen et al. [52]	Y	15 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	4.5 y on average	No
Chen et al. [56]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	1.5 month–8 y	No
Cui [86]	Unclear	10 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Digoxigenin 0.25 mg	II-III	2–7 y	No
Deng and Tang [15]	Y	14 d	Conventional medicine treatment plus SFI 20–40 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Di [67]	Unclear	Unclear	Conventional medicine treatment plus SFI 40 mL, bid, iv.gtt	Conventional medicine treatment	II–IV	3–17 y	No
Dou [97]	Unclear	10 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	12 ± 1.5 y	No

Table 1: Continued.

Author Name	Inpatient (Y/N)	Course	Experiment group	Control group	NYHA classification	Disease duration	Followup (month)
Fan [60]	Y	21 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Metoprolol 12.5 mg, bid, po, +captopril 12.5 mg, tid, po	II–IV	Unclear	No
Fan et al. [101]	Unclear	14 d	Conventional medicine treatment plus SFI 40 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Geng et al. [27]	Both	12 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	0.5–9 y	No
Gu et al. [69]	Y	14 d	Conventional medicine treatment plus SFI 100 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	1.5–12 y	No
Guo et al. [49]	Unclear	14 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	Unclear	No
Guo et al. [23]	N	7 d	Conventional medicine treatment plus SFI 20 mL, iv + 50 mL, qd, iv.gtt plus non invasive positive pressure ventilation	Conventional medicine treatment plus non invasive positive pressure ventilation	Unclear	Unclear	No
Guo et al. [102]	Y	7 d	Conventional medicine treatment plus SFI 40–60 mL, qd, iv.gtt plus invasive respiratory support	Conventional medicine treatment plus invasive respiratory support	IV	Unclear	No
Han and Li [36]	Y	15 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	4.54 ± 2.1 y	No
He [70]	Unclear	14 d	Conventional medicine treatment plus SFI 40 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	1–14 y	No

Table 1: Continued.

Author Name	Inpatient (Y/N)	Course	Experiment group	Control group	NYHA classification	Disease duration	Followup (month)
He [98]	Unclear	7–20 d/ 10–30 d	Conventional medicine treatment plus SFI 20–40 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	3–16 y	No
Hong [44]	Unclear	14 d	Conventional medicine treatment plus SFI 80 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	Unclear	No
Hou and Hong [17]	Unclear	7 d	Conventional medicine treatment plus SFI 60–100 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Huang [13]	Unclear	7 d	Conventional medicine treatment plus SFI 20 mL iv + 40 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	Unclear	No
Huang [53]	Y	14 d	Conventional medicine treatment plus SFI 40 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Huang et al. [24]	N	Unclear	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt plus sodium nitroprusside 50 mg, iv.gtt	Conventional medicine treatment plus sodium nitroprusside 50 mg, iv.gtt	Unclear	Unclear	No
Jia and Yang [71]	Y	20 d	Conventional medicine treatment plus SFI 40 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Jian and Chen [88]	Unclear	14 d	Conventional medicine treatment plus SFI 60–80 mL, bid, iv.gtt	Conventional medicine treatment	III-IV	6.5 y on average	No
Jiang [62]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Jin and Guo [95]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No

Table 1: Continued.

Author Name	Inpatient (Y/N)	Course	Experiment group	Control group	NYHA classification	Disease duration	Followup (month)
Ju [37]	Unclear	14 d	Conventional medicine treatment plus SFI 30 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Lei and Li [92]	Y	7–10 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Lei et al. [12]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	1–18 y	3
Li et al. [9]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	1–20 y	3
Li et al. [72]	Y	15 d	Conventional medicine treatment plus SFI 30 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	5–26 y	No
Li [96]	Unclear	15 d	Conventional medicine treatment plus SFI 40 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	Unclear	No
Li et al. [73]	Unclear	15 d	Conventional medicine treatment plus SFI 100 mL, qd, iv.gtt plus sodium nitroprusside 50 mg	Conventional medicine treatment plus sodium nitroprusside 50 mg	IV	l–25 y	No
Li [93]	Unclear	10 d	Conventional medicine treatment plus SFI 1 mL/kg body weight, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Liu [75]	Y	21 d	Conventional medicine treatment plus SFI 40 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No

Table 1: Continued.

Author Name	Inpatient (Y/N)	Course	Experiment group	Control group	NYHA classification	Disease duration	Followup (month)
Liu and Sun [18]	Unclear	7 d	Conventional medicine treatment plus SFI 100 mL, qd, iv.gtt	Conventional medicine treatment	Unclear	Unclear	No
Liu and Chan [50]	Unclear	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Liu et al. [20]	Y	28 d	Conventional medicine treatment plus SFI 40 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	9 month–14 y	No
Liu [74]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Liu et al. [94]	Unclear	14 d	Conventional medicine treatment plus SFI 40 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Lv [57]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	Unclear	No
Luo et al. [76]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment plus sodium nitroprusside	III-IV	Unclear	No
Luo et al. [38]	Unclear	10 d/m 6 months	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	>3 months	6
Ma et al. [48]	Unclear	20 d	Conventional medicine treatment plus SFI 30–40 mL, qd, iv.gtt	Conventional medicine treatment	II-III	Unclear	No
Ma and Huang [99]	Y	14 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No

Table 1: Continued.

Author Name	Inpatient (Y/N)	Course	Experiment group	Control group	NYHA classification	Disease duration	Followup (month)
Ma [77]	Unclear	15 d	Conventional medicine treatment plus SFI 20 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Pan et al. [89]	Y	14 d	Conventional medicine treatment plus SFI 100 mL, qd, iv.gtt	Conventional medicine treatment plus dobutamine hydrochloride 40 ng, qd, iv.gtt	II–IV	2.5 month–11 y	No
Qiu [103]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Ru [46]	Unclear	10 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	Unclear	No
Shang [78]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Song [106]	Y	15 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II-III	Unclear	No
Song et al. [10]	Y	15 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Su [90]	Y	14 d	Conventional medicine treatment plus SFI 100 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Tan et al. [58]	Unclear	14 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Tian and Gong [16]	Y	14 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	2–20 y	No

Table 1: Continued.

Author Name	Inpatient (Y/N)	Course	Experiment group	Control group	NYHA classification	Disease duration	Followup (month)
Tian [80]	Y	15 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	>7 months	No
Tu and Yang [63]	Y	14 d	Conventional medicine treatment plus SFI 80 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Tu et al. [32]	Unclear	14 d	Conventional medicine treatment plus SFI 100 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
G. L. Wang and J. Wang [104]	Y	15 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	2.5–16 y	No
Wang [100]	Y	14 d	Conventional medicine treatment plus SFI 40 mL, qd, iv.gtt	Conventional medicine treatment	Unclear	22.3 ± 4.8 y	No
Wang [39]	Unclear	15 d	Conventional medicine treatment plus SFI 40 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Wang [28]	Both	14 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	0.6–7 y	No
Wang and Ye [87]	Y	10 d	Conventional medicine treatment plus SFI 40–100 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	14.2 y mean	No
Wang et al. [81]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	IV	3–10 y	No
Wu and Duan [45]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II-III	Unclear	No
Wu and Wang [64]	Y	14 d	Conventional medicine treatment plus SFI 100 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No

Table 1: Continued.

Author Name	Inpatient (Y/N)	Course	Experiment group	Control group	NYHA classification	Disease duration	Followup (month)
Wu et al. [40]	Unclear	10 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Yang and Wu [82]	Y	14 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	Unclear	No
Yang et al. [54]	Y	15 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	5.1 y	No
Yao and Lu [65]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Yin [83]	Y	14 d	Conventional medicine treatment plus SFI 40 mL, qd, iv	Conventional medicine treatment	II–IV	0.5–12 y	No
Yu et al. [84]	Unclear	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Yu [66]	Unclear	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Zhan and Yang [47]	Unclear	20 d	Conventional medicine treatment plus SFI 40 mL, qd, iv.gtt plus metoprolol. 25 mg–75 mg, bid po	Conventional medicine treatment plus metoprolol. 25 mg–75 mg, bid, po	II-III	Unclear	12
Zhang [79]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	3–15 y	No
Zhang et al. [85]	Y	20 d	Conventional medicine treatment plus SFI 30 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No

Table 1: Continued.

Author Name	Inpatient (Y/N)	Course	Experiment group	Control group	NYHA classification	Disease duration	Followup (month)
Zhang [42]	Y	21 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	II-III	Unclear	No
Zhang and Pan [30]	Both	14 d	Conventional medicine treatment plus SFI 40–60 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	2–16 y	No
Zhang [29]	Both	14 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	Unclear	No
Zhang [43]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Zhao et al. [11]	Y	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment plus isoket 10 mg,qd,iv.gtt	II–IV	1–20 y	3
Zhao [91]	Y	14 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	Unclear	Unclear	No
Zhou [59]	Y	14 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	IV	3–15 y	No
Zhou [19]	Y	10 d	Conventional medicine treatment plus SFI 80 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Zhou et al. [31]	Both	14 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Zhu and Ma [105]	Y	15 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	III-IV	Unclear	No

Table 1: Continued.

Author Name	Inpatient (Y/N)	Course	Experiment group	Control group	NYHA classification	Disease duration	Followup (month)
Zi and Li [41]	Y	14 d	Conventional medicine treatment plus SFI 40–100 mL, qd, iv or iv.gtt	Conventional medicine treatment plus dobutamine hydrochloride 50–100 mg	II–IV	Unclear	No
Guo et al. [22]	N	14 d	Conventional medicine treatment plus SFI 60–100 mL, bid, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No
Mo and Zhao [25]	N	7 d	Conventional medicine treatment plus SFI 60–100 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	1–7 d	No
Song and Zhang [33]	Y	10 d	Conventional medicine treatment plus SFI 40–60 mL, qd, iv.gtt	Conventional medicine treatment plus dobutamine hydrochloride 40 mg	II–IV	Unclear	No
Zeng et al. [34]	Y	7 d	Conventional medicine treatment plus SFI 50 mL, qd, iv.gtt	Conventional medicine treatment	IV	Unclear	No
Zeng [26]	N	10 d	Conventional medicine treatment plus SFI 60–100 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	1–72 h	No
Zhang [35]	Y	14 d	Conventional medicine treatment plus SFI 60 mL, qd, iv.gtt	Conventional medicine treatment	II–IV	Unclear	No

Conventional medicine treatment includes sitting up position, supplemental oxygen, vasodilator such as nitroglycerine, diuretics such as furosemide, and cardiotonic agents such as lanatoside C, ACE inhibitors, and β-blockers.

Table 2: Bias of including trails.

Author Name	Sequence generation	Allocation concealment	Blinding	Incomplete outcome data	Selective outcome reporting	Other source of bias	Risk of bias
Bao and Yu [61]	Unclear	Unclear	N	N	N	Unclear	Unclear
Chen [55]	Unclear	Unclear	N	N	N	Unclear	Unclear
Chen and Liu [14]	Unclear	Unclear	N	N	N	Unclear	Unclear
Chen and Li [51]	Unclear	Unclear	N	N	N	Unclear	Unclear
Chen et al. [52]	Unclear	Unclear	N	N	N	Unclear	Unclear
Chen et al. [56]	Unclear	Unclear	N	Y	N	Unclear	Unclear
Cui [86]	Unclear	Unclear	N	N	N	Unclear	Unclear
Deng and Tang [15]	Unclear	Unclear	N	N	N	Unclear	Unclear
Di [67]	Unclear	Unclear	N	N	N	Unclear	Unclear
Dou [97]	Unclear	Unclear	N	N	N	Unclear	Unclear
Fan [60]	Unclear	Unclear	N	N	N	Unclear	Unclear
Fan et al. [101]	Unclear	Unclear	N	N	N	Unclear	Unclear
Gao et al. [68]	Unclear	Unclear	N	N	N	Unclear	Unclear
Geng et al. [27]	Unclear	Unclear	N	N	N	Unclear	Unclear
Gu et al. [69]	Unclear	Unclear	N	N	N	Unclear	Unclear
Guo et al. [49]	Unclear	Unclear	N	N	N	Unclear	Unclear
Guo et al. [23]	Unclear	Unclear	N	N	N	Unclear	Unclear
Guo et al. [102]	Unclear	Unclear	N	Y	N	Unclear	Unclear
Han and Li [36]	Unclear	Unclear	N	N	N	Unclear	Unclear
He [70]	Unclear	Unclear	N	N	N	Unclear	Unclear
He [98]	Unclear	Unclear	N	N	N	Unclear	Unclear
Hong [44]	Unclear	Unclear	Single-blind	N	N	Unclear	Unclear
Hou and Hong [17]	Unclear	Unclear	N	N	N	Unclear	Unclear
Huang [13]	Unclear	Unclear	N	N	N	Unclear	Unclear
Huang [53]	Unclear	Unclear	N	N	N	Unclear	Unclear
Huang et al. [24]	Unclear	Unclear	N	N	N	Unclear	Unclear
Jia and Yang [71]	Unclear	Unclear	N	N	N	Unclear	Unclear
Jian and Chen [88]	Unclear	Unclear	N	N	N	Unclear	Unclear
Jiang [62]	Unclear	Unclear	N	N	N	Unclear	Unclear
Jin and Guo [95]	Unclear	Unclear	N	N	N	Unclear	Unclear
Ju [37]	Unclear	Unclear	N	N	N	Unclear	Unclear
Lei and Li [92]	Unclear	Unclear	N	N	N	Unclear	Unclear
Lei et al. [12]	Unclear	Unclear	N	Y	N	Unclear	Unclear
Li et al. [9]	Random number table	Unclear	N	N	N	Unclear	Unclear
Li et al. [72]	Unclear	Unclear	N	N	N	Unclear	Unclear
Li [96]	Unclear	Unclear	N	N	N	Unclear	Unclear

Table 2: Continued.

Author Name	Sequence generation	Allocation concealment	Blinding	Incomplete outcome data	Selective outcome reporting	Other source of bias	Risk of bias
Li et al. [73]	Unclear	Unclear	N	N	N	Unclear	Unclear
Li [93]	Unclear	Unclear	N	N	N	Unclear	Unclear
Liu [75]	Unclear	Unclear	N	N	N	Unclear	Unclear
Liu and Sun [18]	Unclear	Unclear	N	N	N	Unclear	Unclear
Liu and Chan [50]	Unclear	Unclear	N	N	N	Unclear	Unclear
Liu et al. [20]	Random number table	Unclear	N	Y	N	Unclear	Unclear
Liu [74]	Unclear	Unclear	N	Y	N	Unclear	Unclear
Liu et al. [94]	Unclear	Unclear	N	N	N	Unclear	Unclear
Lv [57]	Unclear	Unclear	N	N	N	Unclear	Unclear
Luo et al. [76]	Unclear	Unclear	N	N	N	Unclear	Unclear
Luo et al. [38]	Random number table	Unclear	N	Y	N	Unclear	Unclear
Ma et al. [48]	Unclear	Unclear	N	N	N	Unclear	Unclear
Ma and Huang [99]	Unclear	Unclear	N	N	N	Unclear	Unclear
Ma [77]	Unclear	Unclear	N	N	N	Unclear	Unclear
Pan et al. [89]	Unclear	Unclear	N	N	N	Unclear	Unclear
Qiu [103]	Unclear	Unclear	N	N	N	Unclear	Unclear
Ru [46]	Unclear	Unclear	Single-blind	N	N	Unclear	Unclear
Shang [78]	Unclear	Unclear	N	N	N	Unclear	Unclear
Song [106]	Unclear	Unclear	N	N	N	Unclear	Unclear
Song et al. [10]	Random number table	Unclear	N	N	N	Unclear	Unclear
Su [90]	Unclear	Unclear	N	N	N	Unclear	Unclear
Tan et al. [58]	Unclear	Unclear	N	N	N	Unclear	Unclear
Tian and Gong [16]	Unclear	Unclear	N	N	N	Unclear	Unclear
Tian [80]	Unclear	Unclear	N	N	N	Unclear	Unclear
Tu and Yang [63]	Unclear	Unclear	N	N	N	Unclear	Unclear
Tu et al. [32]	Unclear	Unclear	N	N	N	Unclear	Unclear
G. L. Wang and J. Wang [104]	Unclear	Unclear	N	N	N	Unclear	Unclear
Wang [100]	Unclear	Unclear	N	N	N	Unclear	Unclear
Wang [39]	Random number table	Unclear	N	N	N	Unclear	Unclear
Wang [28]	Unclear	Unclear	N	N	N	Unclear	Unclear
Wang and Ye [87]	Unclear	Unclear	N	Y	N	Unclear	Unclear
Wang et al. [81]	Unclear	Unclear	N	N	N	Unclear	Unclear

Table 2: Continued.

Author Name	Sequence generation	Allocation concealment	Blinding	Incomplete outcome data	Selective outcome reporting	Other source of bias	Risk of bias
Wu and Duan [45]	Unclear	Unclear	Single-blind	Y	N	Unclear	Unclear
Wu and Wang [64]	Unclear	Unclear	N	N	N	Unclear	Unclear
Wu et al. [40]	Random number table	Unclear	N	N	N	Unclear	Unclear
Yang and Wu [82]	Unclear	Unclear	N	N	N	Unclear	Unclear
Yang et al. [54]	Unclear	Unclear	N	N	N	Unclear	Unclear
Yao and Lu [65]	Unclear	Unclear	N	N	N	Unclear	Unclear
Yin [83]	Unclear	Unclear	N	N	N	Unclear	Unclear
Yu et al. [84]	Unclear	Unclear	N	N	N	Unclear	Unclear
Yu [66]	Unclear	Unclear	N	N	N	Unclear	Unclear
Zhan and Yang [47]	Unclear	Unclear	N	Y	N	Unclear	Unclear
Zhang [79]	Unclear	Unclear	N	N	N	Unclear	Unclear
Zhang et al. [85]	Unclear	Unclear	N	N	N	Unclear	Unclear
Zhang [42]	odd and even number of ID	Unclear	N	N	N	Unclear	Unclear
Zhang and Pan [30]	Unclear	Unclear	N	N	N	Unclear	Unclear
Zhang [29]	Random number table	Unclear	N	N	N	Unclear	Unclear
Zhang [43]	Unclear	Unclear	Double-blind	N	N	Unclear	Unclear
Zhao et al. [11]	Random number table	Unclear	N	N	N	Unclear	Unclear
Zhao [91]	Unclear	Unclear	N	N	N	Unclear	Unclear
Zhou [59]	Unclear	Unclear	N	N	N	Unclear	Unclear
Zhou [19]	Drew lots	Unclear	N	N	N	Unclear	Unclear
Zhou et al. [31]	Unclear	Unclear	N	N	N	Unclear	Unclear
Zhu and Ma [105]	Unclear	Unclear	N	N	N	Unclear	Unclear
Zi and Li [41]	Random number table	Unclear	N	N	N	Unclear	Unclear
Guo et al. [22]	Unclear	Unclear	N	Y	N	Unclear	Unclear
Mo and Zhao [25]	Unclear	Unclear	N	N	N	Unclear	Unclear
Song and Zhang [33]	Unclear	Unclear	N	Y	N	Unclear	Unclear
Zeng et al. [34]	Unclear	Unclear	N	N	N	Unclear	Unclear
Zeng [26]	Unclear	Unclear	N	N	N	Unclear	Unclear
Zhang [35]	Unclear	Unclear	N	Y	N	Unclear	Unclear

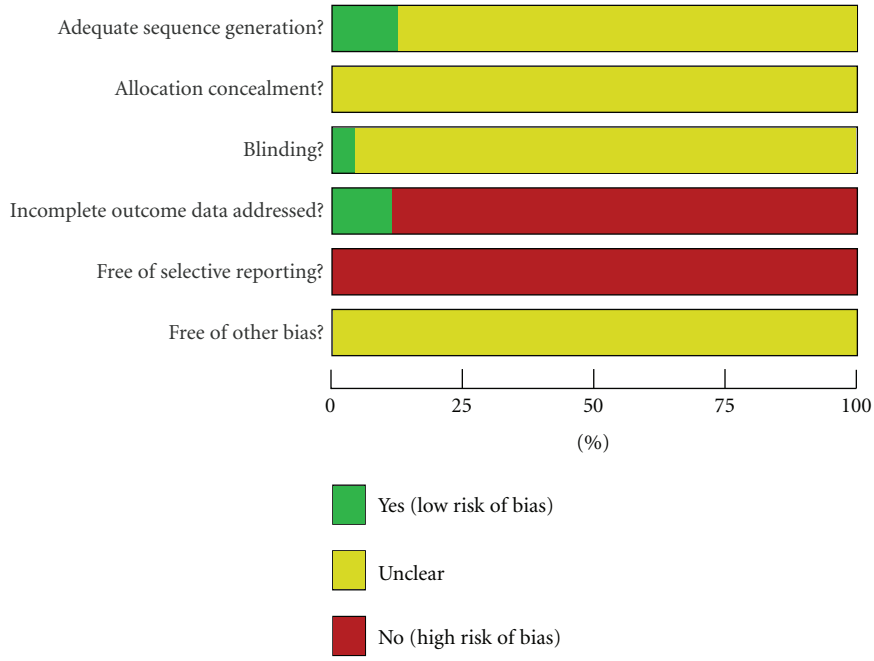

Figure 3: Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

Table 3: Adverse events.

Symptom	Reported trails	Cases reported
Dry mouth	4 [10, 16, 17, 60]	14
Fullness of the head	4 [9–12]	10
Dryness heat	2 [10, 13]	7
Insomnia	1 [13]	3
Dysphoria	1 [14]	2
Skin itching	1 [15]	1
Tachycardia	1 [16]	1
Feverish dysphoria	2 [17, 18]	5
Flushing of face and tidal fever	1 [19]	8
Dizziness due to low blood pressure	1 [20]	1
Gastrointestinal discomfort	1 [20]	1
Palpitation	1 [18]	2

than conventional medicine treatment did (Supplementary Figure 8).

LVDd is the end-diastolic dimension of the left ventricle. There was no statistical significance between SFI combined with conventional medicine treatment and conventional medicine treatment groups (WMD: −1.59; 95% CI [−5.29, 2.12], $P = 0.40$) (Supplementary Figure 9).

3.4.3. Quality of Life. None of the trials reported quality of life.

3.5. Publication Bias. Funnel plots based on the data of effect rate were elaborated in Figure 8. The figure was asymmetrical, which indicated that potential publication bias might influence the results of this paper. Although we conducted comprehensive searches and tried to avoid bias, since all trials were published in Chinese, we could not exclude potential publication bias.

3.6. Adverse Effect. Thirty seven out of ninety seven trials mentioned the adverse effect except in sixty-two trials which was unclear. Thirteen trials [9–20, 60] reported the following thirteen specific symptoms of side effects including dry mouth, dryness heat, fullness of the head, insomnia, dysphoria, skin itching, tachycardia, feverish dysphoria, flushing of face, tidal fever, dizziness due to low blood pressure, gastrointestinal discomfort, and palpitation. Among these side effects, dry mouth and fullness of the head were reported in 4 trails with 14 and 10 cases, respectively. These symptoms were regarded to be mild and recovered spontaneously after SFI withdrawal. Twenty four trials reported that no side effects were observed in the SFI group (Table 3).

The above side effects might be related to higenamine, which is the active ingredient of prepared aconite root. In TCM books and papers, prepared aconite root is frequently mentioned with adverse effects as dry mouth, dryness heat, fullness of the head, and dysphoria due to its strong effect of strengthening *yang*.

4. Discussion

In many years, western medicine has made tremendous progress and has become the dominating medical treatment worldwide. However, it has been increasingly recognized that

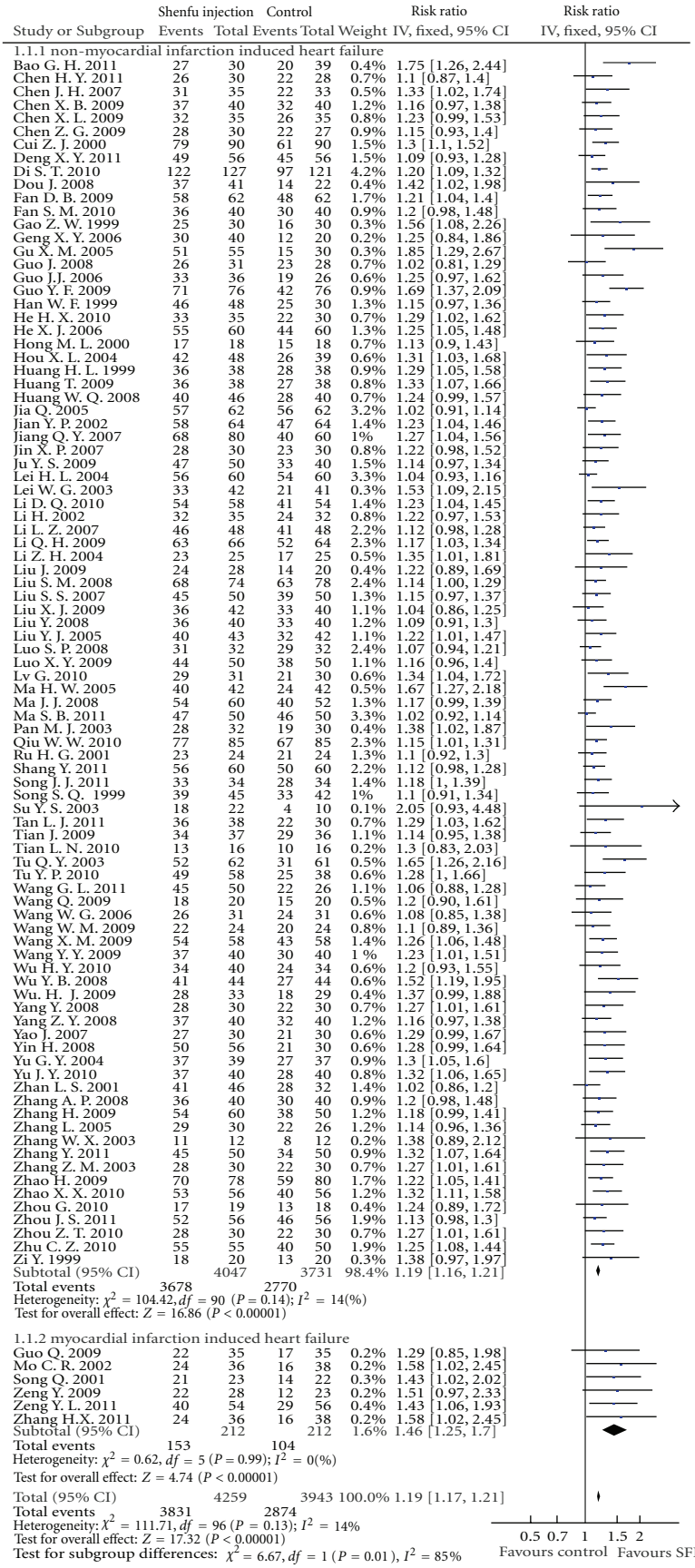

Figure 4: Forest plot of comparison: effect rate.

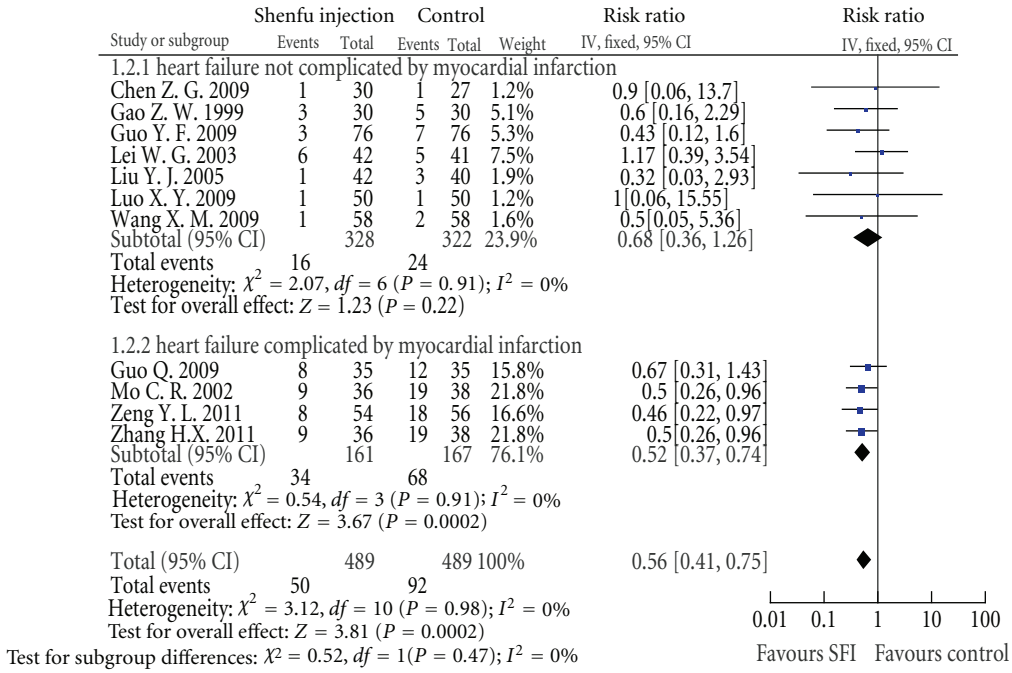

Figure 5: Forest plot of comparison: death.

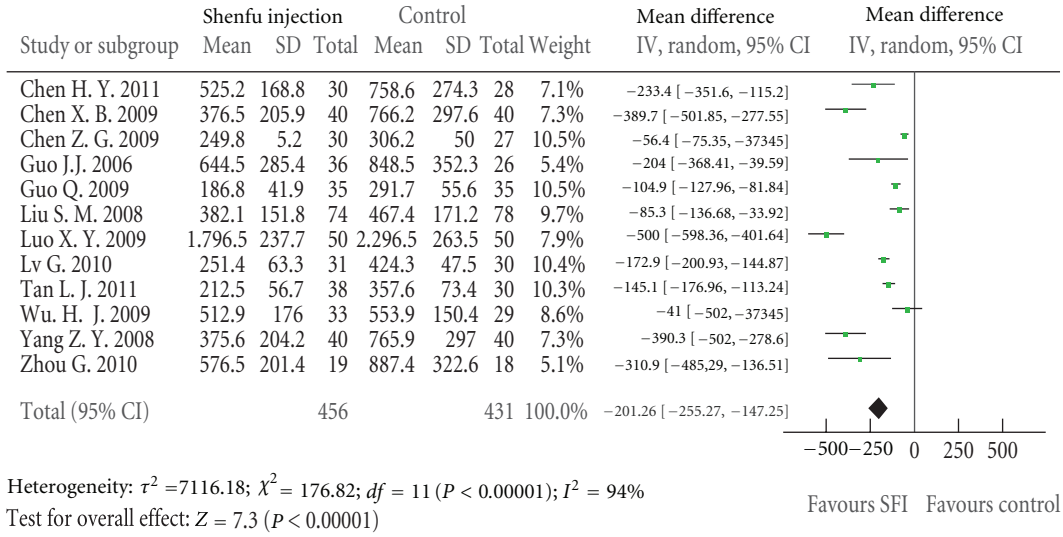

Figure 6: Forest plot of comparison: NT-proBNP.

western medicine may sometimes fail to treat an illness, whereas such illness is reportedly improved by the so-called complementary medicine based on a different theory [110, 111]. Although conventional therapeutic approaches were used in HF, it remained a cardiovascular disease with an increasing hospitalization burden and an ongoing drain on health care expenditures [2]. TCM plays an important role in treating HF in China. SFI was a traditional Chinese Patent Medicine based on TCM theory, which was approved by the Chinese State Food and Drug Administration. In recent 10 years, it has been widely used for HF in many hospitals and clinics. However, few RCTs of SFI were reported in English journals, and it was difficult for western doctors to accept SFI as an alternative medicine. Although there were two systematic reviews about SFI for HR published in Chinese journal [112, 113], only 16 and 8 trials were included in their study. Therefore, the present study aimed to systematically assess the efficacy and safety of SFI for HR.

Data from the 97 RCTs demonstrated that SFI combined with conventional medication may be more effective on HF than conventional medication only. With improvement of cardiofunction of patients, based on NYHA Classification of

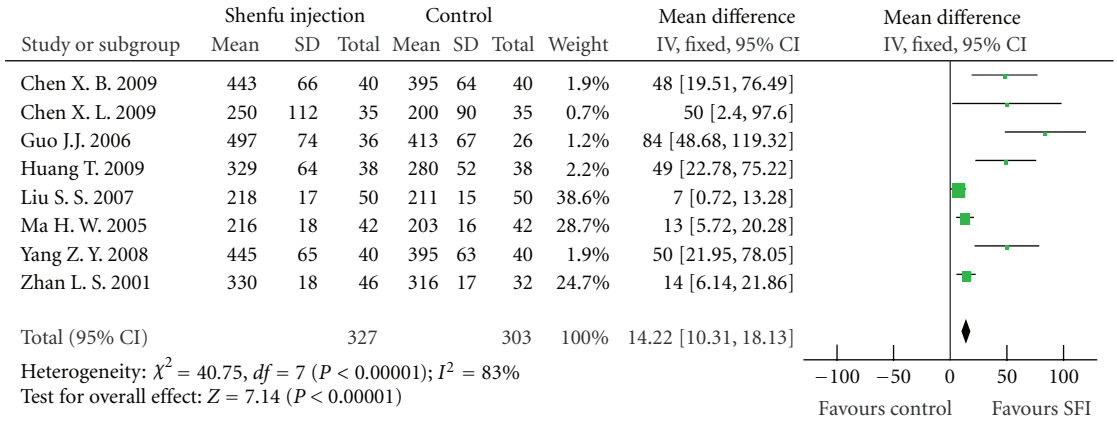

Figure 7: Forest plot of comparison: 6-MWD.

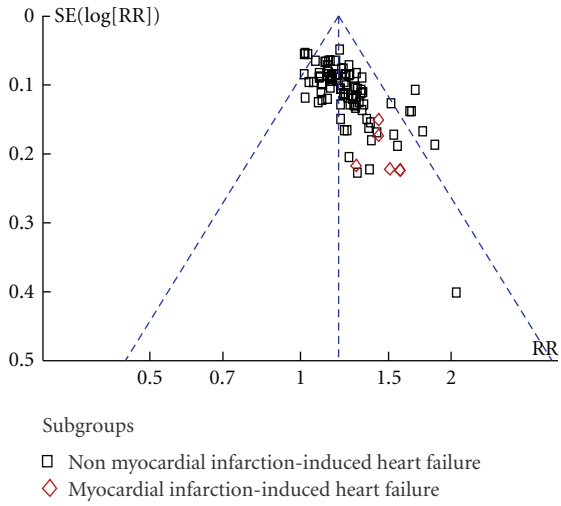

Figure 8: Funnel plot of comparison: effect rate.

Clinical Status and Killip's Rating Standards, the effect rate of SFI group was, on average, 17 percent more than control group (RR, 1.19; 95% CI, 1.17 to 1.21). Mortality data was another primary outcome. In eleven trials in which death was recorded, meta-analysis showed that mortality was significantly lower in SFI group than control group. This result was mainly contributed by subgroup of HF induced by myocardial infarction, for patients in this subgroup were more vulnerable.

Ultrasonic cardiography is widely used in inspection for HF patients. From results of ultrasonic cardiography, the systolic and diastolic functions of heart can be interpreted. LVEF, CO, CI, SV, LVDd, and E/A were reviewed by us, respectively. There was significant difference between SFI group and control group in all of the outcomes except LVDd. Since SV, CO, CI, and LVEF indicate heart systolic function, and E/A indicate heart diastolic function, conclusion can be drawn that SFI benefits both systolic and diastolic functions of heart. But it did not have significant effect on expansion of heart. NT-proBNP level in serum of SFI group was significantly lower than the control group, which is inconsistent with effect rate. 6-MWD results of patients of SFI group also are better than thos of control group. It indicates that SFI had a tendency to improve life status. Furthermore, heart rate was obviously reduced in SFI group, which could be related to alleviation of HF.

Meta-analysis on LVEF, CO, CI, SV, LVDd, E/A, heart rate, and NT-proBNP all showed significant heterogeneity. Several possible explanations can be given, for example, different complications, different instruments employed for test, and difference in methodological rigor.

However, we should consider the following limitations before accepting the findings of this paper.

Firstly, the methodological quality of the included studies is generally poor. Although all trials claimed to perform randomization, only eleven trials reported the procedure to generate the sequence, while the rest of trials did not give any details of the randomization method. Thus, whether randomization was effectively conducted in these trials was doubtful. Blinding was mentioned in four trials, with one trial blinded patients and outcome assessors [43] and three blinded patients only [44–46]. Neither of them described the methods of allocation concealment. Dropouts account and intention to treat analysis were not mentioned in all the trails. Due to inadequate reporting of methodological design, it was possible that there was performance bias and detection bias due to patients and researchers being aware of the therapeutic interventions for the subjective outcome measures. Therefore, we cannot draw a confident conclusion that there were significant beneficial effects of SFI combined with conventional medicine treatment compared with conventional medicine treatment.

Secondly, limited outcomes were reported, especially death and adverse events. Since HF is a disease with high mortality, death is the most important primary outcome. However, only eleven studies out of ninety seven trials reported death, and most of the eleven trials assessed mortality at the end of treatment, without followup. Another outcome was adverse events, to which more attention should be attached. Only 37.4% of the trials described the occurrence of adverse events, indicating an incomplete evaluation of the safety profile of SFI, as well as poor quality of reporting. In most trials, the duration of therapy and followup was

too short to achieve conclusive results, except that only one trial had a treatment of 10 months [47]. Only 6 included trials had a followup period (ranged from 3 to 12 months), while in rest of studies, the outcomes were evaluated at the end of the treatment (mostly range from 14 to 21 days). In order to evaluate drug efficacy for chronic HF, long-term improvement (at least 6 months) of chronic HF-specific clinical symptoms is needed [114], because some drugs have shown to increase mortality in the long-term application despite a short-term improvement in clinical symptoms [115]. In addition, long-term toxicity assessment was also important for drug safety evaluation.

Next, although irrespective of languages, all the trials included in this paper were published in Chinese journals, Zhang et al. and Liu et al. [115, 116] found that some Asian countries including China unusually publish high proportions of positive results. Wu et al. [117] and Jin et al. [118] accounted that RCTs in Chinese journals often had problems of low methodological quality and selective publication of positive results. Considering that all of the ninety seven trials were published in Chinese, the publication bias possibly existed.

Additionally, none of the ninety seven trials reported sample size calculation, and in most trials, the sample size was limited. Further high-quality studies with larger sample size are needed to confirm the effectiveness of SFI in treating HF. Quality of life was not reported in all the including trials. Although 6-MWD showed a tendency of SFI to improve life status for HF patients, we advise future RCTs to select outcomes of life quality according to international practice.

Considering that there was no sufficient amount of high-quality trials on SFI treating patients with HF, the effectiveness and safety of SFI need further rigorous trials to prove, which should be consistent with the CONSORT statement on the reporting of the results of randomized trials (http://www.consort-statement.org/).

5. Conclusion

The preliminary conclusion of the current study suggests that SFI might be beneficial to patients with HF. More rigorously designed trails with high methodological quality are necessary for further proof.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgments

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Research Article

The Effects of Velvet Antler of Deer on Cardiac Functions of Rats with Heart Failure following Myocardial Infarction

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Velvet antler of deer (VAD) is a commonly-used kidney-Yang supplementing traditional Chinese medication. According to the heart-kidney-related theory, heart Yang originates in kidney Yang and heart failure due to heart Yang deficiency can be treated by tonifying kidney Yang. In this study, we investigated therapeutic effects of VAD on cardiac functions in rats with heart failure following myocardial infarction. Forty-eight male Wistar rats were subjected either to left coronary artery ligation ($N = 36$) or to sham operation ($N = 12$). One week after the surgery, rats with heart failure received daily treatment of double-distilled water, captopril or VAD by gavage for consecutively four weeks, while sham-operated animals were given double-distilled water. Ultrasonic echocardiography was adopted to examine cardiac structural and functional parameters and serum brain natriuretic peptide (BNP) concentration was measured using radioimmunoassay. We found that VAD partially reversed changes in cardiac functional parameters and serum BNP levels in rats with heart failure. These results provide further evidence for the heart-kidney-related theory and suggest that VAD might be a potentially alternative and complementary medicine for the treatment of heart failure.

1. Introduction

Velvet antler of deer (VAD) is a precious traditional Chinese medication, warm in nature and sweet and salty in flavor, and is commonly used to treat various diseases by supplementing kidney Yang. According to TCM theories, kidney Yang deficiency can be presented in the symptoms including declining libido, soreness, or cold sensation in the knees and lumbar regions, spiritual fatigue, and so forth. Both clinical and animal studies have shown that VAD can promote the development of reproduction systems, relieve the pain of arthritis, nourish the neural cells, and so on [1]. These pharmacological effects provide empirical evidence for its role of tonifying kidney Yang.

Heart failure (HF) is a clinical syndrome which all types of heart diseases will eventually develop into. Evidence from epidemiological studies has demonstrated that the incidence of HF in Chinese adults was 0.9%: it reached 0.7% in males and 1.0% in females [2]. From the perspective of the traditional chinese medicine (TCM), the primary cause of HF is heart Yang deficiency that results from Qi inadequacy and blood stasis. According to the heart-kidney-related theory of TCM, heart Yang originates in kidney Yang, and therefore it is hypothesized that tonifying kidney Yang could be used to strengthen heart Yang and treat HF. Indeed, VAD has been reported to have protective effects on the damaged heart muscle cells in the animal models of myocardial infarction via various mechanisms, such as reducing the release of endothelin [3], promoting superoxide dismutase activities, and decreasing serum malondialdehyde levels [4], and increasing the levels of nitric oxide and calcitonin-gene-related peptide [5]. In addition, oral administration of VAD has been found

to strengthen the pulse, increase blood pressure, and enhance heart sounds for chronic circulatory disorders accompanied by hypotension [6]. However, to the best of our knowledge, the therapeutic effects of VAD on the heart failure have not been fully elucidated due to a lack of clinical and animal studies.

Left coronary artery ligation is one of the widely used animal models to mimic myocardial infarction and heart failure in patients [7]. Echocardiography and serum brain natriuretic peptide (BNP) levels are two commonly used indices to evaluate and diagnose heart failure [8]. To provide empirical evidence for VAD's clinical application in the treatment of heart diseases, in the current study, we investigated its therapeutic effects on heart failure following myocardial infarction with captopril as the positive control by evaluating echocardiographic parameters and serum BNP levels.

2. Methods and Materials

2.1. Animals and the Heart Failure Model. Male Wistar rats ($N = 48$, age 8 weeks, weighing 230 ± 20 g) were purchased from the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences, Beijing, China. They were housed four/cage in a controlled environment (23 ± 1°C; 45%–50% relative humidity; fixed 12/12 h light/dark cycle, lights on at 08:00 h) with food and water ad libitum. All procedures were performed in accordance with the National Institute of Health's Guide for the Use and Care of Laboratory Animals and were approved by the Committee on Animal Care and Use of the China-Japan Friendship Hospital.

After seven-day acclimation, animals had their body weights measured at first and then were anesthetized with intraperitoneal injection of 1% pentobarbital sodium. They were fixed on their backs, intubated using a 16-gauge catheter, and artificially ventilated (80 stokes/min, 0.4 L/250 g) with a pressure-controlled respirator for small animals (RSP1002, Kent Scientific Corporation, CT, USA). An incision of the skin and intercostal muscles was made between the third and fourth ribs. Under the monitor of a lead 2 electrocardiogram (XJJ-11, Kent Scientific Corporation, CT, USA), a thoracotomy was performed and the pericardium was opened, which left the heart adequately exposed. To induce myocardial infarction, the left coronary artery was ligated 1.5–2 mm from the aortic root between the pulmonary cone and the left auricular appendage with silk sutures. Then the muscles and skins were sutured and the ventilation was stopped when rats' heart rate and respiration went steady. The sham-operated animals underwent the same procedure except that the silk suture was placed around the left coronary artery without being tied. After the surgery, all animals were injected with penicillin for three days to prevent infection. Six rats with induced myocardial infarction died during the operation whereas two rats in the sham group died of infection. The operative mortality was 16.7%.

2.2. Drugs and Pharmacological Procedures. The powder of velvet antler of deer (VAD, The Scientific and Technological Development Center of Qingyuan Manchu Autonomous County, Liaoning Province, China) and captopril (CAP, Beijing Shuguang Pharmaceutical Co., Ltd., Beijing, China) were triturated and then well suspended in double-distilled water at the doses of 20 mg/mL and 1 mg/mL respectively. These doses were calculated according to the conversion table of animal doses to human equivalent doses based on body surface area [9]. Provided that the doses of rats should be seven times lower than those of humans, the clinical dose of VAD was 2 g/70 Kg and hence the equivalent dose for rats is 200 mg/Kg. Similarly, the clinical dose of captopril is 100 mg/70 Kg, and for rats the dose should be 10 mg/Kg. One week after the surgery, animals with myocardial infarction following left coronary artery ligation received daily treatment of double-distilled water (referred as the HF group), captopril (the HF + CAP group), or velvet (the HF + VAD group) by gavage (1 mL/100 mg body weight) for consecutively four weeks, while sham-operated animals (the SHAM group) were administered double-distilled water once daily for four weeks.

2.3. Measurement of Cardiac Structural and Functional Parameters. Echocardiography (HDI 5000, Philips) was performed before rats were sacrificed. Under anesthesia by intraperitoneal injection of 40 mg/kg pentobarbital sodium, rats were fixed on their backs with their fur shaved and skin cleaned. Using a high-frequency linear-array transducer (CL15-7), the structural parameters of the heart included left atrial diameter (LAD), left ventricular end-diastolic diameter (LVDd), left ventricular end-systolic diameter (LVDs), end-systolic interventricular septal thickness (IVSTs), end-diastolic interventricular septal thickness (IVSTd), left ventricular end-systolic posterior wall thickness (LVPWTs), and left ventricular end-diastolic posterior wall thickness (LVPWTd). Besides, two functional parameters were calculated by the following formulas: left ventricular short-axis fractional shortening (LVFS) = (LVDd-LVDs)/LVDd, left ventricular ejection fraction (LVEF) = ($LVDd^3$- $LVDs^3$)/$LVDd^3$.

2.4. Brain Natriuretic Peptide (BNP) Measurement. Blood samples were collected in EDTA tubes, which was then placed on ice and centrifuged within 30 min at 4°C. The plasma was stored at −80°C until assay. Radioimmunoassays for rat BNP were performed as previously reported [10]. Briefly, standards or samples were incubated with antibody for rat BNP for 24 hours at 4°C. ^{125}I-BNP (10 000 cpm) was then added, followed by additional incubation for 24 hours at 4°C. Then after 90 min of incubation with a secondary antibody, free and bound fractions were separated, and the radioactivity of the bound fraction was measured by a gamma counter (EG&G Wallac, USA).

2.5. Statistical Analysis. All data were expressed as means ± standard deviation. Group differences were evaluated using one-way analysis of variance (ANOVA). If the variance of the data was heterogeneous, the Kruskal-Wallis test was adopted, which was followed by the Nemenyi multiple comparison test. $P < 0.05$ was considered to be statistically significant.

Table 1: Comparison of cardiac structural parameters as revealed by echocardiography in rats ($\bar{x} \pm s$).

Group	N	LAD (mm)	IVSTd (mm)	IVSTs (mm)	LVPWTd (mm)	LVPWTs (mm)	LVDd (mm)	LVDs (mm)
sham	10	3.31 ± 0.15	1.22 ± 0.19	1.36 ± 0.20	2.13 ± 0.33	2.67 ± 0.33	5.79 ± 0.74	4.21 ± 0.64
HF	9	4.22 ± 0.23***	0.68 ± 0.14***	0.90 ± 0.17***	2.03 ± 0.32	2.58 ± 0.30	9.79 ± 0.66***	8.91 ± 0.71***
HF + CAP	11	4.42 ± 0.22***	0.66 ± 0.14***	0.91 ± 0.13***	1.88 ± 0.23	2.44 ± 0.27	10.28 ± 0.69***	9.18 ± 0.64***
HF + VAD	10	4.31 ± 0.30***	0.72 ± 0.13***	0.96 ± 0.12***	2.87 ± 0.33	2.48 ± 0.3	9.91 ± 0.71***	8.59 ± 0.63***

Notes: ***compared to the sham group, $P < 0.001$; HF = heart failure; CAP = captopril; VAD = velvet antler of deer.

Table 2: Comparison of functional parameters as revealed by echocardiography in rats ($\bar{x} \pm s$).

Group	N	LVFS (%)	LVEF (%)
sham	10	27.20 ± 6.16	60.60 ± 9.01
HF	9	9.11 ± 2.62***	24.67 ± 6.44***
HF + CAP	11	10.64 ± 2.11	32.82 ± 5.04Δ
HF + VAD	10	13.40 ± 2.91Δ	34.80 ± 6.84Δ

Notes: ***compared to the sham group, $P < 0.001$; Δ compared to the AMI group, $P < 0.05$. HF = heart failure; CAP = captopril; VAD = velvet antler of deer.

3. Results

3.1. Effects of VAD and CAP on the Cardiac Structural Parameters in Rats with Heart Failure following Myocardial Infarction (Table 1). Compared to the SHAM group, the diameters of left atrium and left ventricle (LAD, LVDd, and LVDs) of animals in the heart failure groups were significantly increased, the interventricular septal thickness (IVSTd and IVSTs) was decreased ($P < 0.001$) whereas no group differences were found with respect to LVPWTd and LVPWTs ($P > 0.05$). VAD and CAP treatment did fail to reverse the effects of myocardial infarction on cardiac structural parameters ($P > 0.05$).

3.2. Effects of VAD and CAP on the Cardiac Functional Parameters in Rats with Heart Failure following Myocardial Infarction (Table 2). Compared to the SHAM group, animals in the heart failure groups had significantly lower LVFS and LVEF ($P < 0.001$), indicating that cardiac function was severely impaired by myocardial infarction. Further comparisons with the heart failure groups showed that the LVFS of the VAD group was significantly elevated than that of the HF group ($P < 0.05$) whereas the similar trend in the CAP group did not reach significance ($P = 0.377$). The LVEFs of both treatment groups were higher than that of the AMI group (Ps < 0.05 for the VAD group and the CAP group). These results indicated that both VAD and CAP partially reversed the functional damage induced by myocardial infarction. No differences were found between the two treatment groups ($P > 0.05$).

3.3. Effects of VAD and CAP on Serum BNP Levels in Rats with Heart Failure following Myocardial Infarction (Table 3). The serum BNP levels of the HF group were significantly higher than the sham group ($P < 0.001$), a change that was reversed by both VAD and CAP treatments ($P < 0.05$). No differences were found between the two treatment groups ($P > 0.05$).

Table 3: Serum BNP levels of all groups ($\bar{x} \pm s$).

Group	N	BNP (pg/mL)
sham	10	0.89 ± 0.21
HF	9	1.31 ± 0.21***
HF + CAP	11	1.08 ± 0.16Δ
HF + VAD	10	1.10 ± 0.18Δ

Notes: *** compared to the sham group, $P < 0.001$; Δ compared to the AMI group, $P < 0.05$. HF = heart failure; CAP = captopril; VAD = velvet antler of deer.

4. Discussion

Although there is no such a term as "heart failure" (HF) in traditional Chinese medicine (TCM), its symptoms can be classified into the categories of dyspnea, palpitation, and edema. This disease is a condition of heart Qi deficiency and heart Yang devitalization due to Zang-Fu dysfunction that could be resulted from various factors such as improper diet, overstrain, and repeated invasion of exterior pathogens. The heart belongs to fire, governs blood and vessels, and controls spirit. It is a vital organ of the body just like a monarch to a country and plays a leading role in physical activities. The kidney belongs to water and is the organ which stores essence as well as true Yin and true Yang. It is the congenital origin and the root of life. Heart Yang originates in kidney Yang. Normal physical activities of the human body must rely on mutual coordination and constraints of these two organs. The outflow of heart Yang is the primary force that warms kidney Yang. The deficiency of Yang in the heart and kidney leads to weak contraction, which causes vascular obstruction and blood stasis, and hence the symptoms of heart failure appear. Just as *plain questions: Treatise on disharmony* said, "People who cannot lie down because that will make them gasp and cough are those who have too much water in their body. Water generates from fluid. Kidney is an organ of water, governing fluid flows and therefore controls lying down and gasping as well." Accordingly, deficiency of the heart- and

kidney Yang is the internal causes of heart failure and plays the pivotal role in the pathogenesis and prognosis of the disease.

Previous studies have shown that the TCM syndrome-type of rats with heart failure following myocardial infarction in the current study may belong to the syndrome of deficiency of Qi and blood stasis [11]. The pathogenesis mechanisms could be that coronary artery ligation caused blood stasis in the heart, which leads to Qi damage and produces symptoms of Qi deficiency in the organ and the whole body. Treatment that promotes blood circulation and replenishes Qi could improve cardiac function. Considering that Qi deficiency could develop into Yang deficiency and that kidney Yang is the origin of heart Yang, it is hypothesized that tonifying kidney Yang would strengthen heart Yang, which further supplements heart Qi and makes the heart beat stronger. These effects could be beneficial for the treatment of heart failure.

Based on the heart-kidney-related theory of TCM, in this study we investigated the therapeutic effects of VAD, a precious kidney Yang supplementing medicine, on cardiac structure and function as measured in echocardiography and serum BNP levels in rats with HF induced by myocardial infarction. BNP is a 32-amino-acid polypeptide secreted by the ventricles of the heart in response to excessive stretching of heart muscle cells and therefore has been used as an objective index reflecting the immediate condition of cardiac function and clinical severity of HF [8]. The finding that VAD treatment elevated LVFS/LVEF and decreased BNP levels provides evidence for the protective effects of VAD for the damaged heart. Recent studies have implicated multiple mechanisms of VAD in improving cardiac function. For instance, VAD could reduce the release of endothelin, prevent vascular contraction in the ischemic regions and enhance local coronary circulation [3]. VAD has also been reported to play a protective role on secondary injury of ischemic myocardium by promoting superoxide dismutase activities and decreasing serum malondialdehyde levels [4]. Lastly, VAD could elevate the levels of nitric oxide and calcitonin-gene-related peptide so as to modulate cardiomyocyte apoptosis [5].

We did not observe the therapeutic effects of VAD in cardiac structural parameters, a finding in contrast to a study showing that chronic treatment with VAD could significantly reverse the enlargement of left ventricle caused by myocardial infarction [12]. The inconsistency may be due to that our study used VAD powder whereas the latter study used the extracts of VAD, which contains a higher proportion of active components. Our finding that four-week treatment of VAD showed differential therapeutic effects on cardiac structure and function suggests that the treatment period of four weeks may be not long enough to induce structural changes of the heart as measured by echocardiography. According to our observation, VAD treatment can also reverse myocardial fibrosis of HF (unpublished data), which may serve as the structural basis of functional changes induced by VAD.

In the current study, we used captopril as a positive control and found that VAD and captopril showed comparable effects in reversing changes in functional parameters and BNP levels in rats with heart failure. Although VAD is more expensive than captopril, it has edges over captopril for patients with heart failure accompanied with lower blood pressure given the hypotensive side effect of captopril.

In conclusion, the kidney Yang supplementing drug, VAD, shows comparable therapeutic effects with captopril for heart failure induced by myocardial infarction as revealed by functional parameter changes in echocardiography and serum BNP levels. These results provide evidence for the heart-kidney-related theory of TCM and demonstrate that heart failure due to heart Yang deficiency can be treated by strengthening kidney Yang. Therefore, VAD might be a potentially alternative and complementary medicine used in the treatment of heart failure.

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Review Article

Outcome Measures of Chinese Herbal Medicine for Coronary Heart Disease: An Overview of Systematic Reviews

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Objective. The aim of this overview was to summarize the outcome measures of Chinese herbal medicine (CHM) as the treatment of coronary heart disease (CHD) based on available systematic reviews (SRs), so as to display the current situation and evaluate the potential benefits and advantages of CHM on CHD. *Methods*. An extensive search included the Cochrane Database of Systematic Reviews, MEDLINE, and 4 databases in Chinese. SRs of CHM for CHD were included. Besides evaluating and summarizing the outcome measures, we also estimated the quality of the included reviews by PRISMA (preferred reporting items for systematic reviews and meta-analyses). Data were extracted according to predefined inclusion criteria by two independent reviewers. *Results*. 46 articles were included. 20 kinds of CHM were reviewed. 7 SRs were concerned with myocardial infarction (MI), 38 SRs were related to angina pectoris. 11 SRs had primary endpoints, while others focused on secondary endpoints to evaluate CHM for CHD such as angina pectoris and electrocardiogram (ECG). One SR reported more adverse effects of CHM for CHD and of the SRs analyzed quality of life. Many CHM appeared to have significant effect on improving symptoms, ECG, biomarkers and so on. However, most SRs failed to make a definite conclusion for the effectiveness of CHM in CHD patients due specifically to the poor evidence. And according to PRISMA we found most of the trials in the SRs were of low quality. *Conclusion*. Primary endpoints were not used widely. The benefits of CHM for CHD need to be confirmed in the future with RCTs of more persuasive primary endpoints and high-quality SRs.

1. Introduction

Coronary heart disease (CHD) is the most common cause of death in western countries. With the infectious diseases controlled and improvement of people's living, the morbidity of CHD increases year by year in many developing countries. Acute myocardial infarction (AMI) and angina pectoris are the most important two types of CHD. Chinese herbal medicine (CHM) has a 3000-year-old history with unique theories for concepts of etiology and systems of diagnosis and treatment [1]. The interest in CHM is growing rapidly beyond China [2–5]. In recent years, some researchers have reported the effect of CHM on clinical symptoms, biomarkers and mortality in CHD patients. However, the evidence of CHM needs to be reviewed systematically and appraised critically.

High-quality systematic reviews (SRs) of randomized controlled trials (RCTs) are the sources of the best evidence [6]. Currently, there is an increasing number of SRs on studies of CHM, but few of them concluded that CHM was definitely effective for CHD due to the weak evidence. In addition to rigorous clinical design and standard reporting, the selection of outcome measures also plays an important role in drawing a more persuasive conclusion. The aim of this overview was to summarize the outcome measures of CHM as the treatment of CHD based on available SRs, so as to display the current situation and evaluate the potential benefits and advantages of CHM on CHD.

2. Methods

Electronic literature searches were performed to identify the maximum possible number of systematic reviews/meta-analyses of CHM for CHD. The following electronic databases were searched: (1) The Cochrane Database of

Systematic Reviews (Issue 10 of 12, Oct 2011); (2) MEDLINE (2001 to 2011); (3) Chinese Biomedical Database (CBM, 2001 to 2011); (4) China National Knowledge Infrastructure (CNKI, 2001 to 2011); (5) Wanfang Databases (2001 to 2011); (6) Chinese VIP Information (VIP, 2001 to 2011). CBM, CNKI, Wanfang, and VIP were databases in Chinese. We searched databases in Chinese because CHMs were researched in china mostly. And we searched papers from 2001 to 2011 for high-quality RCTs and SRs mainly focusing in recent ten years.

The strategy below was used to search The Cochrane Library and adapted appropriately for use in different electronic bibliographic databases: #1 herb*; #2 medic*; #3 (#1 and #2); #4 Chinese; #5 (#3 or #4); #6 cardiac; #7 heart; #8 circulation; #9 (#6 or #7 or #8); #10 (#5 and #9). To determine which article was we want, we scanned the title and abstract of each record independently by two reviewers (J. Luo and H. Xu). If the information included a systematic review or a meta-analysis of CHM for CHD, the full paper was obtained for further assessment. Papers were excluded when problems occurred with: repeat publication; methodological studies; quality assessment report; the interventions in the control groups were other Chinese herbs; research on acupuncture, qigong, massage, or other treatments (Figure 1).

We divided the outcome measures into primary endpoints and secondary endpoints [50, 51]. Primary endpoints include the mortality, AMI, restenosis after percutaneous coronary intervention (PCI), and recanalization. Secondary endpoints mainly indicate surrogate endpoints and laboratory measures, which include angina pectoris, arrhythmia, heart failure, consumption of nitroglycerine, electrocardiogram (ECG), ultrasonic cardiogram (UCG), Level of blood lipids, plasma endothelin, nitric oxide, myocardial enzyme, hemorheology, heart rate variability, and traditional Chinese medicine (TCM) syndrome.

In addition, we used PRISMA (preferred reporting items for systematic reviews and meta-analyses) as assessment tool to estimate the quality of the included reviews. This checklist includes 27 items of 7 key areas. And it describes the preferred way to present the abstract, introduction, methods, results, and discussion sections of a systematic review and a meta-analysis paper. It requires authors of each review to include a flow diagram that provides information about the number of studies identified, included, and excluded and the reasons for excluding them [52]. Information on each of the included reviews was imported into PRISMA statement for analysis. All data were extracted independently by two authors using predefined criteria. Disagreements were resolved by discussion between the authors. All inconsistencies were revised after a consensus was reached.

3. Results

46 articles were included (7 in English and 39 in Chinese). 39 SRs from the Chinese databases were published between 2004 and 2011. Since 2007, the number of SR increased markedly. 5 SRs from the Cochrane Database were published between 2006 to 2011 [8, 13, 26, 36, 44]. 2 SRs from MEDLINE were published between 2006 to 2011 [14, 45].

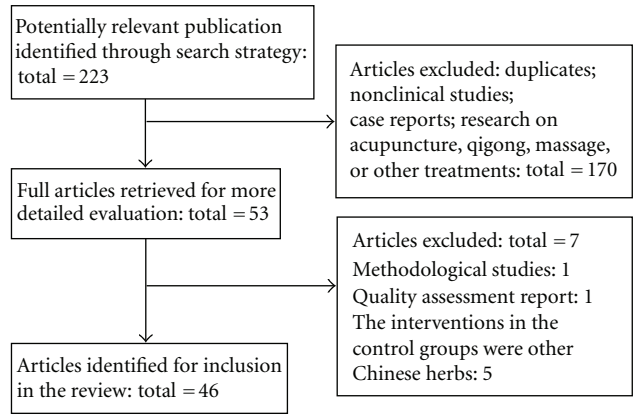

Figure 1: Flow-chart of SRs selection.

7 SRs were concerned with myocardial infarction (MI), 38 SRs were related to angina pectoris, and one SR was concerned with preventing and treating restenosis after PCI. The trials in SRs were mainly originated from china. The original trials included were called "RCTs" or "quasi-RCTs", but only a few of them were typical RCTs. Most of the trials in the SRs were of low quality, only 14 RCTs were high quality: one was concerned with MI, 12 were related to angina pectoris, and one was about preventing and treating restenosis.

20 kinds of CHM were reviewed, including injections, capsules, tablets, pellets, and herbal decoction as follows: Danshen preparations ($n = 13$) [8, 14, 20–22, 33, 38–41, 45–47], 7 of them were compound salvia pellet [14, 22, 33, 38, 45–47]; Tongxinluo Capsule ($n = 4$) [13, 22, 27, 37]; Yiqi huoxue (supplementing qi and activating blood circulation) products ($n = 3$) [10, 32, 49]; Xuefu zhuyu decoction ($n = 2$) [23, 30]; herbal products ($n = 4$) [11, 12, 17, 26]; Shengmai injection ($n = 2$) [9, 24]; Suxiao jiuxin wan ($n = 2$) [35, 36]; Gingko ($n = 2$) [28, 29]; Acanthopanax ($n = 2$) [53, 54]; Puerarin ($n = 2$) [15, 44]; Shexiang baoxin wan ($n = 2$) [34, 55]; Shenmai injection ($n = 1$) [7]; Tetramethylpyrazine ($n = 1$) [43]; Shuxuetong ($n = 1$) [48]; Xinkeshu ($n = 1$) [31]; Safflower injection ($n = 1$) [25]; Rhodiola ($n = 1$) [42]; Kudiezi injection ($n = 1$) [19]; Shuyu zaogan tablets ($n = 1$) [18]; Dengzhanhua injection ($n = 1$) [16].

11 SRs analyzed primary endpoints and the others all focused on secondary endpoints to evaluate CHM for CHD (Table 1). This was mainly based on whether there were available data in the original trials or not. Four primary endpoints were analyzed in the SRs including mortality, nonfatal myocardial infarction, restenosis after PCI, and recanalization. None of these SRs analyzed the quality of life. Angina pectoris was the most common secondary endpoint in the SRs. There was one SR without clear outcome measures [53], and 2 SRs only used "marked effective," "effective," "ineffective" as comprehensive outcome measures involving symptoms improvement and ECG changes [19, 54]. Many CHMs appear to have significant effect on improving symptoms, ECG, and level of blood lipids and

Table 1: Outcome Measures of CHM for CHD in systematic reviews.

Outcome measures (number of SR)	Condition (number of SR)	CHM	First author	Number of RCTs/total	Conclusion	Risk of publication bias
Primary Endpoints						
Mortality (7)	MI (6)	Shenmai injection	Zeng (2010) [7]	13/13	A	H
		Danshen preparations	Wu (2008) [8]	6/6	B	NA
		Shengmai injection	Gao (2008) [9]	4/4	A	NA
		Yiqi huoxue patent medicine	Zhang (2008) [10]	9/28	B	NA
		Herbal injection products	Zhen (2007) [11]	5/15	A	H
		Herbal products	Lin (2006) [12]	4/8	B	L
	Angina pectoris (1)	Tongxinluo capsule	Wu (2006) [13]	1/18	B	H
AMI (6)	MI (2)	Yiqi huoxue patent medicine	Zhang (2008) [10]	1/28	B	NA
		Herbal products	Lin (2006) [12]	2/8	A	L
	Angina pectoris (4)	Compound salvia pellet	Zhang (2008) [14]	1/17	B	H
		Puerarin	Wang (2008) [15]	1/11	B	H
		Tongxinluo capsule	Wu (2006) [13]	3/18	B	NA
		Dengzhanhua injection	Cao (2005) [16]	1/8	A	NA
Restenosis after PCI (1)	CHD (1)	Herbal products	Ren (2008) [17]	17/17	A	H
Recanalization (2)	MI (2)	Yiqi huoxue patent medicine	Zhang (2008) [10]	7/28	B	NA
		Herbal injection products	Zhen (2007) [11]	15/15	A	NA
Secondary Endpoints (ECG)						
ECG (34)	CHD (4)	Shuyu zaogan tablets	Zhang (2011) [18]	29/32	A	L
		Kudiezi injection	Zuo (2011) [19]	15/16	A	H
		Sodium tanshinone IIA Sulfonate	Wang (2011) [20]	17/29	A	H
		Danhong injection	Xu (2011) [21]	19/19	A	H
		Tongxinluo capsule and compound salvia pellet	Jia (2011) [22]	65/58	A	L
	Angina pectoris (30)	Xuefuzhuyu Decoction	Cui (2011) [23]	8/10	A	H
		Shengmai injection	Zhang (2010) [24]	8/13	A	H
		Safflower Injection	Wu (2010) [25]	2/6	B	NA
		Herbal products	Zhuo (2010) [26]	3/3	A	NA
		Tongxinluo capsule	Hao (2010) [27]	18/20	A	L
		Gingko	Zha (2010) [28]	36/50	A	L
		Gingko	Zhao (2010) [29]	9/23	A	L
		Xuefuzhuyu decoction	Song (2010) [30]	3/3	A	H

Table 1: Continued.

Outcome measures (number of SR)	Condition (number of SR)	CHM	First author	Number of RCTs/total	Conclusion	Risk of publication bias
		Xinkeshu	Chen (2010) [31]	12/18	A	H
		Yiqihuoxue	Long (2009) [32]	25/30	A	H
		Compound salvia pellet	Zhang (2009) [33]	5/8	A	L
		Compound salvia pellet	Zhang (2008) [14]	10/17	A	H
		Shexiang baoxin wan	Lin (2008) [34]	20/22	A	L
		Puerarin	Wang (2008) [15]	6/11	A	H
		Suxiao jiuxin wan	Wang (2008) [35]	14/14	A	L
		Suxiao jiuxin wan	Duan (2008) [36]	3/15	A	H
		Tong xin luo Capsule	He (2007) [37]	12/17	A	H
ECG (34)	Angina pectoris (30)	Compound salvia pellet	Jiang (2007) [38]	26/34	A	H
		Danshen preparations	Li (2007) [39]	7/13	A	L
		Compound preparation of salvia miltiorrhiza	Zhang (2007) [40]	30/33	A	H
		Danshen preparations	Li (2007) [41]	20/21	A	H
		Rhodiola L.	Wang (2006) [42]	7/8	A	L
		Tetramethylpyrazine	Zhang (2006) [43]	10/10	A	L
		Tongxinluo capsule	Wu (2006) [13]	10/18	A	NA
		Puerarin injection	Wang (2006) [44]	17/20	A	H
		Compound salvia pellet	Wang (2006) [45]	27/27	A	L
		Dengzhanhua injection	Cao (2005) [16]	8/8	A	NA
		Compound salvia pellet	Wang (2004) [46]	17/17	A	L
		Compound salvia pellet	Zhang (2004) [47]	19/22	A	L
		Secondry Endpoints (Angina Pectoris)				
		Shuyu zaogan tablets	Zhang (2011) [18]	21/22	A	L
Angina pectoris (30)	CHD (3)	Shengmai injection	Zhang (2010) [24]	10/13	A	H
		Compound salvia pellet	Zhang (2009) [33]	8/8	A	L

Table 1: Continued.

Outcome measures (number of SR)	Condition (number of SR)	CHM	First author	Number of RCTs/total	Conclusion	Risk of publication bias
		Sodium tanshinone IIA Sulfonate	Wang (2011) [20]	29/29	A	H
		Danhong injection	Xu (2011) [21]	19/19	A	H
		Tongxinluo capsule and compound salvia pellet	Jia (2011) [22]	65/65	A	L
		Kudiezi injection	Zuo (2011) [19]	16/16	A	H
		Shuxuetong	Li (2010) [48]	11/13	A	L
		Herbal products	Zhuo (2010) [26]	3/3	B	NA
		Tongxinluo capsule	Hao (2010) [27]	20/20	A	L
		Xinkeshu	Chen (2010) [31]	16/18	A	H
		Xuefuzhuyu decoction	Song (2010) [30]	3/3	A	H
		Gingko damo injection	Zha (2010) [28]	46/50	A	L
		Ginkgo extract	Zhao (2010) [29]	22/23	A	L
		Suxiao jiuxin wan	Duan (2008) [36]	1/15	A	H
Angina pectoris (30)	Angina pectoris (26)	Puerarin	Wang (2008) [15]	10/11	A	H
		Suxiao jiuxin wan	Wang (2008) [35]	14/14	A	L
		Compound salvia pellet	Zhang (2008) [14]	11/17	A	H
		Compound salvia pellet	Jiang (2007) [38]	34/34	A	H
		Compound preparation of salvia miltiorrhiza	Zhang (2007) [40]	32/33	A	H
		Danshen preparations	Li (2007) [41]	21/21	B	H
		Tetramethylpyrazine	Zhang (2006) [43]	8/10	A	L
		Rhodiola L.	Wang (2006) [42]	5/8	A	L
		Tongxinluo capsule	Wu (2006) [13]	5/18	A	NA
		Compound salvia pellet	Wang (2006) [45]	27/27	A	L
		Puerarin injection	Wang (2006) [44]	18/20	A	H
		Dengzhanhua injection	Cao (2005) [16]	8/8	A	NA
		Compound salvia pellet	Wang (2004) [46]	17/17	A	L
		Compound salvia pellet	Zhang (2004) [47]	20/22	A	L
	CHD after PCI (1)	Herbal products	Ren (2008) [17]	15/17	A	H

Table 1: Continued.

Outcome measures (number of SR)	Condition (number of SR)	CHM	First author	Number of RCTs/total	Conclusion	Risk of publication bias
Secondry End points (Others)						
Consumption of nitroglycerine (5)	Angina pectoris (5)	Herbal products	Zhuo (2010) [26]	2/3	A	NA
		Suxiao jiuxin wan	Duan (2008) [36]	1/15	A	H
		Rhodiola L.	Wang (2006) [42]	1/8	A	L
		Puerarin injection	Wang (2006) [44]	6/20	A	H
		Tongxinluo capsule	Wu (2006) [13]	1/18	A	NA
Level of blood lipids (4)	Angina pectoris (3)	Shuxuetong	Li (2010) [48]	4/13	A	H
		Compound salvia pellet	Zhang (2008) [14]	8/22	B	L
		Compound salvia pellet	Zhang (2004) [47]	4/8	A	L
	CHD (1)	Compound salvia pellet	Zhang (2009) [33]	4/17	A	L
Hemorheology (2)	Angina pectoris (1)	Safflower Injection	Wu (2010) [25]	2/6	A	NA
	CHD (1)	Shengmai injection	Zhang (2010) [24]	5/13	A	H
Heart failure (3)	MI (3)	Yiqi huoxue patent medicine	Zhang (2008) [10]	7/28	B	NA
		Danshen preparations	Wu (2008) [8]	1/6	B	NA
		Herbal products	Lin (2006) [12]	3/8	A	L
Arrhythmia (2)	MI (2)	Yiqi huoxue patent medicine	Zhang (2008) [10]	2/28	B	NA
		Herbal products	Lin (2006) [12]	2/8	B	L
UCG (2)	MI (2)	Yiqi huoxue herbal products	Song (2008) [49]	3/3	A	NA
		Herbal products	Lin (2006) [12]	4/8	A	L
Myocardial enzyme (1)	Angina pectoris (1)	Tongxinluo capsule	Wu (2006) [13]	1/18	B	NA
Level of plasma endothelin (2)	Angina pectoris (2)	Puerarin injection	Wang (2006) [44]	2/20	A	H
		Tongxinluo capsule	Wu (2006) [13]	4/18	A	NA
Level of nitric oxide (1)	Angina pectoris (1)	Tongxinluo capsule	Wu (2006) [13]	2/18	A	NA
Heart rate variability (1)	CHD (1)	Compound salvia pellet	Zhang (2009) [33]	3/8	A	L
TCM syndrome (1)	Angina pectoris (1)	Safflower Injection	Wang (2006) [25]	3/8	A	L

Notes: Yiqi huoxue: supplementing qi and activating blood circulation to patients with qi-deficiency and blood-stasis syndrome;
A: CHM may be or appears to be effective; B: The evidence is insufficient, inconclusive;
H: high; L: low; NA: not mentioned.

reducing the consumption of nitroglycerine, and so forth. Some SRs also reflected that CHM may be effective to reduce the risk of subsequent MI, heart failure, and arrhythmia. However, most SRs failed to draw a definite conclusion of the effectiveness of CHM for CHD due specifically to the poor evidence.

Adverse effects, which are important when evaluate a medicine, should be regarded as an essential outcome measure in clinical trials. However, only a few of the trials in the SRs had long-term data on adverse effects. Most of adverse effects of CHM were mentioned as "low adverse effect" or "none obvious". The adverse events reported majorly were abdominal complaints, nausea, and dyspepsia. One review reported more adverse reactions in treatment groups than in control groups [44]. Recently, several reviews have highlighted adverse reactions of CHM [56, 57].

Compared the usage of outcome measures between Cochrane and non-Cochrane reviews, we found that outcome measures of the included papers in Cochrane are more comprehensive. Every Cochrane review took primary endpoints, secondary endpoints, and safety as outcome measures. However, primary endpoints and safety are seldom taken as outcome measures in most of the non-Cochrane reviews. None of reviews analyzed quality of life or pay attention to medical economics.

According to PRISMA statement, we found that most of the included reviews are of low quality. The deficiencies are as follows: review methods in the abstracts and rationales for review were not well reported; only about half of the SRs reported the characteristics of included trials; just 5 SRs provided flow chart in the article, 2 in Chinese [22, 29], and 3 in English [8, 14, 26]; potential biases were not described well in the reports; most SRs lack in persuasive outcome measures.

4. Discussion

Our overview shows that primary endpoints and secondary endpoints are all used to evaluate the effect of CHM for CHD. Secondary endpoints are most commonly adopted in clinical trials due to their feasibility in small sample size and short-term clinical trials. They may signify future cardiovascular event to some extent and are sure to be valuable as surrogate endpoints. But it is clearly that primary endpoints are more persuasive in RCT of cardiovascular diseases. However, most of the outcome measures in the included SRs are angina pectoris and ECG. Primary endpoints such as mortality and major cardiovascular events are not used widely. Adverse effects, quality of life, and medical economics, which are also important when evaluate a medicine, should be taken as outcome measures too. All of these are the reasons why neither the trials nor the SRs of CHM for CHD could meet a sufficiently high standard to be broadly accepted by the Western medical community.

SRs of CHM with poor methodology and reporting quality have been reported [58]. According to PRISMA statement, we found that most of the included reviews have poor quality. Reviewers were not good at reporting how they avoided bias in selecting primary studies, how they extracted data, and how they evaluated the validity of the primary studies. Also, most of the reviewers chose less persuasive outcome measures, which reduced the persuasion of the interventions. So if reviewers did not master the method of performing SR, they could produce inaccurate or misleading conclusions for current clinical practice and even the future research. Although it appeared that CHM was effective for CHD in clinical use, such as compound salvia pellet, shengmai injection, suxiao jiuxin wan, and gingko, puerarin, most SRs were inconclusive that CHM had a definite effect for CHD owing to the poor evidence.

Before recommending the conclusion, we have to consider the following weaknesses in this overview. Firstly, data were abstracted from SRs instead of the original trials, and most of the included SRs have poor quality. Secondly, most of the RCTs in the SRs included are also of low quality due mainly to unclear randomization and blinding method, incomplete outcome reporting, publication bias, and so forth. Thirdly, we only selected SRs published in Chinese and English. SRs of CHM for CHD published in other language or originated from other countries might be omitted. Fourthly, we did not identify unpublished studies, thus negative trial might not be reported and could induce publication bias.

In conclusion, primary and secondary endpoints were all used to evaluate the effectiveness of CHM for CHD, but primary endpoints were not used widely. Although it appeared that CHM was effective for CHD in terms of some outcome measures, most SRs failed to draw a definite conclusion for the effectiveness of CHM in CHD patients due to the poor evidence. The benefits of CHM for CHD still need to be confirmed in the future with RCTs of more persuasive primary endpoints and high-quality SRs.

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Research Article

Aquatic Exercise Is Effective in Improving Exercise Performance in Patients with Heart Failure and Type 2 Diabetes Mellitus

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Background. Peak oxygen uptake (VO_{2peak}) and muscle function are more decreased in patients with a combination of chronic heart failure (CHF) and type 2 diabetes mellitus (2DM) compared to patients with only one of the conditions. Further, patients with 2DM have peripheral complications that hamper many types of conventional exercises. *Aim*. To evaluate the efficacy and applicability of eight-week aquatic exercise in patients with the combination of CHF and 2DM. *Methods*. Twenty patients (four women) with both CHF and 2DM (age 67.4 ± 7.1, NYHA II-III) were randomly assigned to either aquatic exercise or a control group. The patients exercised for 45 minutes 3 times/week in 33–34°C, swimming pool. *Results*. The training programme was well tolerated. Work rate (+11.7 ± 6.6 versus −6.4 ± 8.1 watt, $P < 0.001$) and VO_{2peak} (+2.1 ± 0.8 versus −0.9 ± 1.4 $mL \cdot kg^{-1} \cdot min^{-1}$, $P < 0.001$) and walking capacity ($P = 0.01$) increased significantly in the training group. Muscle function was also significantly improved and Hba1c decreased significantly ($P < 0.01$) during training, while fasting glucose, insulin, c-peptide, and lipids were unchanged . Training also increased vitality measured by SF-36 significantly ($P = 0.05$). *Conclusion*. Aquatic exercise could be used to improve exercise capacity and muscle function in patients with the combination of CHF and 2DM.

1. Introduction

Up to 25% of patients with chronic heart failure (CHF) have type 2 diabetes mellitus (2DM) [1], and it could be foreseen that this combination will be increasingly common [2]. 2DM entails a markedly increased risk of developing cardiovascular diseases [3]. Further, in all types of cardiovascular disease patients with 2DM have a significantly higher rate of mortality and morbidity than patients without [4]. On the contrary, CHF results in an insulin resistance syndrome which in turn could lead to the development of 2DM [5]. Both patients with CHF and 2DM, separately or in combination, suffer from reduced physical function, with decreased oxygen uptake and poor muscle function [6, 7]. In both conditions, similar pathological consequences are found in the skeletal muscle, such as an increased number of type II muscle fibres, low capillary density, and decreased oxidative capacity [8, 9]. Other similarities are signs of impaired endothelial function, which might be important to muscle function and physical performance [10, 11]. However, the impaired endothelial function might be corrected by exercise training [12]. The diseases do often impact negatively on activity of daily living and quality of life. These patients risk in a greater extent to develop depression and anxiety in comparison with a healthy population [13, 14]. There are consistent reports of improvement in physical performance and psychological function after aerobic and/or resistance exercise training in CHF as well as in 2DM [15, 16]. Since the prevalence of both conditions increases with age it is not unusual that patients also have other disabilities

that further restrain physical ability. Aquatic exercise, that is, physical training in warm water, is an alternative exercise regimen, and we have recently shown positive effects of aquatic exercise in patients with CHF without 2DM [17]. To confirm the results of our previous study using aquatic exercise and to assess the efficacy of such training in patients with the combination of CHF and 2DM, this study was performed.

The hypothesis was that training in warm water would be safe and result in improvement in physical performance, muscle function, and metabolic control in patients with CHF and 2DM.

Therefore the aim of this study was to investigate the effect of aquatic exercise in patients with CHF and 2DM.

2. Methods

2.1. Patients. Twenty patients (four women) with stable CHF and 2DM in NYHA class II or III, ejection fraction (EF) <50%, age above 55 years were included. Heart failure medication had to be stable for the previous three months. Exclusion criteria were peripheral artery disease, chronic pulmonary disease, status after stroke, or other disabling diseases that might interfere with the exercise protocol. The process of patient recruitment is described in Figure 1. After baseline testing, patients were randomised, using a 1 : 1 ratio, in a stratified according to order to 8 weeks of aquatic exercise ($n = 10$), or to a control period ($n = 10$). The patients were stratified according to age, NYHA class and gender. Baseline characteristics of the study population are given in Table 1. The study complied with the Declaration of Helsinki. The Ethics committee of Gothenburg University approved the research protocol, and informed consent was obtained from each subject. The testing procedures were repeated after eight weeks of training or control period, respectively.

2.2. Procedure. All patients performed the below given tests within 10 days before the randomisation and then during the last 10 days of the study period. Patients started on the first day with venous blood samples followed by an acquaintance test on the ergospirometer. Thereafter, questionnaires were filled out and the six-minute walk test performed. Finally patients performed on day 4–6 the maximal test on the ergospirometer and on day 8–10 the muscle tests.

2.3. Assessments

2.3.1. Exercise Capacity. Work rate and peak oxygen uptake (VO_{2peak}) were measured on an ergometer, using a ramp protocol with a 10-watt increase every minute until exhaustion. Expired gas was measured breath by breath using a *V*-max system (Sensor Medics, USA) as previously described [17].

2.3.2. Six-Minute Walking Test. A standardised six-minute walking test was used to assess exercise capacity related to activities of daily living. The patients were asked to walk as far as possible during six minutes on a premarked 30-meter walkway [17, 18].

Table 1: Demographic data of patients with chronic heart failure and type 2 diabetes mellitus.

Variables	Training ($n = 10$)	Control ($n = 10$)	*P* value
Age (years)	65.8 ± 5.8	69 ± 8.2	ns
Sex (F/M)	2/8	2/8	ns
Weight (kg)	93.6 ± 16.2	86.6 ± 24.2	ns
Height (cm)	176.1 ± 10	174 ± 8.8	ns
Duration of CHF (years)	5.3 ± 2.6	6.0 ± 5.2	ns
Duration of 2DM (years)	7.2 ± 5.8	6.9 ± 4.4	ns
LVEF (%)	34.1 ± 9.8	34.8 ± 9.1	ns
Etiology of CHF (IHD/DCM/HT)	8/1/1	4/4/2	ns
NYHA class (II/III)	5/5	3/7	ns
Chronic atrial fibrillation (*n*)	5	3	ns
Beta blockers (*n*)	9	8	ns
ACE-inhibitors (*n*)	8	9	ns
Diuretics (*n*)	9	9	ns
Digitalis (*n*)	2	5	ns
Insulin (*n*)	5	3	ns
Anti diabetics (*n*)	5	8	ns
Acetyl-salicylic acid (*n*)	5	8	ns
Warfarin (*n*)	5	3	ns

F/M: female and male, LVEF: left ventricular ejection fraction, NYHA: New York Heart Association classification, IHD: ischemic heart disease, DCM: dilated cardiomyopathy, HT: hypertension, ACE: angiotensin converting enzyme, *n*: number, ns: not significant.

2.3.3. Muscle Strength and Endurance. For measurement of isometric and isotonic strength and isotonic endurance the Biodex III (Biodex medical systems, New York, USA) was used. The test was preceded by a 5-minute warmup on a test bicycle. The subjects sat with a hip angle of 90°, and the right leg was attached to the lever arm of the dynamometer. Isometric knee extension strength was measured at a 60° knee angle. Isokinetic concentric strength was measured at 60°/s and at 180°/s for knee extensors. Isokinetic endurance was evaluated as the reduction of torque (in percent) between the first and the last three extensions in a series of 50 maximal contractions with an angle of 180°/s. Handgrip strength, the maximum grip force, and the mean value of the 10-second sustained grip was assessed by Grippit (AB Detector, Göteborg, Sweden). Clinical endurance tests, that is, unilateral isotonic heel-lift, bilateral isometric shoulder abduction and unilateral isotonic shoulder flexion were also measured. The test procedures have been described previously [17].

2.3.4. Quality of Life. Health-related quality of life was measured using the Medical Outcome Short Form—36 (SF-36) [19] and disease-specific quality of life with the Minnesota living with heart failure questionnaire (LHFQ) [20]. Hospital

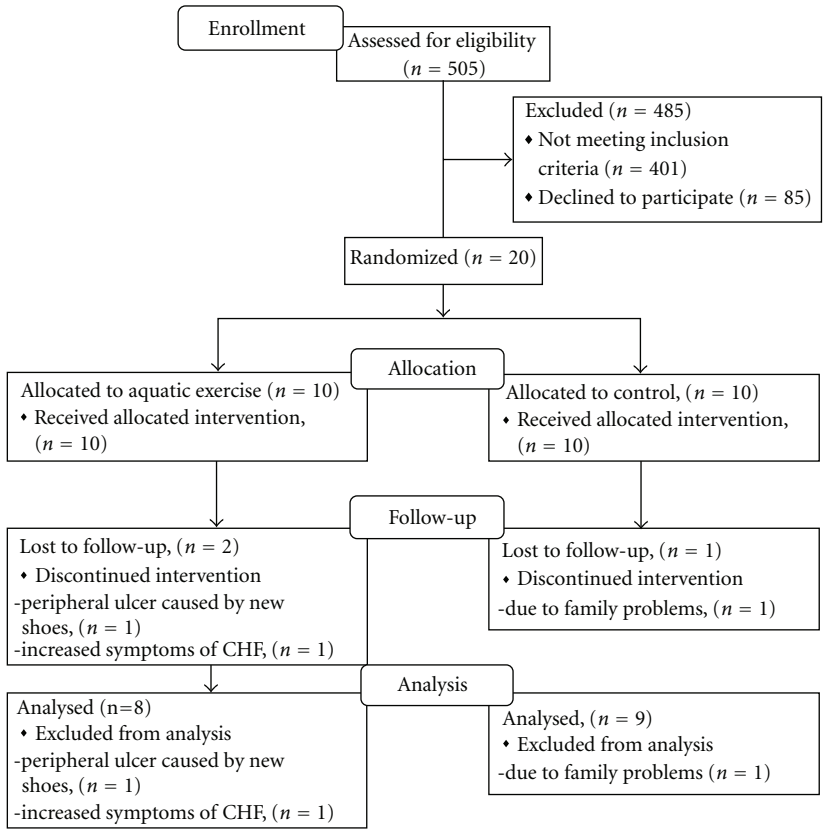

Figure 1: The inclusion process of patients.

anxiety and depression scale (HAD) was used to assess the level of anxiety and depression [21].

2.3.5. Metabolic Function. Venous blood samples for assessing plasma glucose, HbA1c, serum insulin, serum C-peptide, and serum lipids were taken before and after the intervention period after an overnight fast and analysed according to the European Accreditation system [22].

2.3.6. Training Programme. The training programme comprised 45-minute sessions in a heated pool (33°-34°C), three times a week over an eight-week period. The patients trained as a group following a low-to-moderate exercise level, that is, 40 to 75% of maximal heart rate reserve (HRR). The basis posture was standing with water just below neck level. The programme focused on peripheral muscle training but central circulatory exercises were also included as earlier described [17]. The control group was instructed to live their life as normal for eight weeks and was not allowed to increase their habitual physical activity during this period.

2.3.7. Statistics. The SPSS 12.0 for Windows (Chicago, IL, USA) was used to analyse the data.

Ratio and interval data are given as mean (±1 SD or 95% CI) and ordinal data as median and range. Wilcoxon-matched pairs signed rank sum test was used for comparisons of paired observations within each study group. The Mann Whitney U-test was used to assess differences between groups. Nominal data between groups was compared by Chi-squared test or Fisher's exact test. A P value ≤ 0.05 was considered significant. A per-protocol design was used on all data.

3. Results

Aquatic exercise was well tolerated by the patients and no adverse events occurred during the aquatic exercise. Two patients in the training group were withdrawn, due to a peripheral ulcer caused by new shoes, increased symptoms of CHF, respectively. One patient in the control group was withdrawn, due to family problems. The average adherence (total number of attended sessions) was 92%. HRR during training ranged between 40% and 60% during peripheral muscle training exercises and between 55% and 75% during the aerobic exercises. In the training group two patients needed to reduce their insulin and one to take away the oral antidiabetics due to hypoglycaemia.

3.1. Exercise Capacity and Muscle Function. Physical performance was significantly improved in the training group

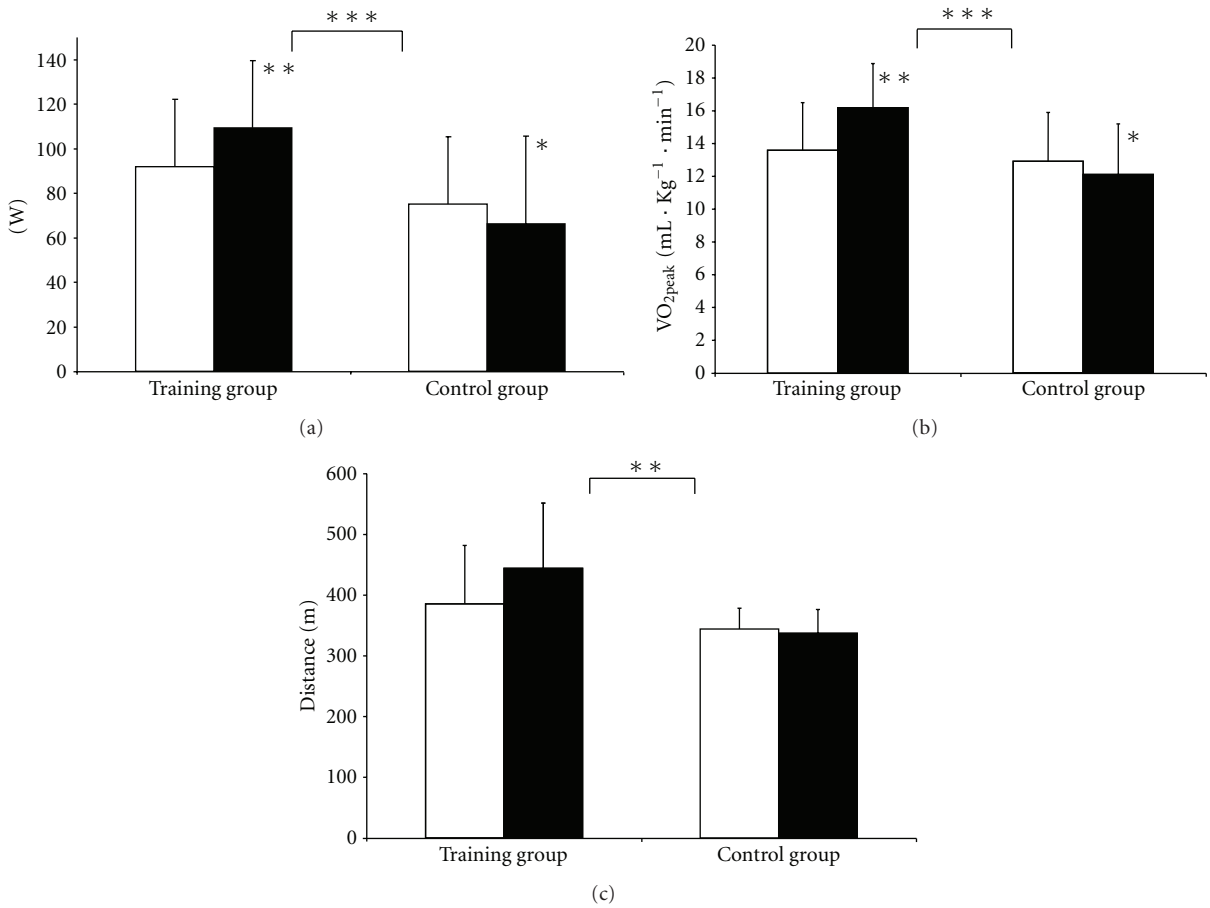

Figure 2: Work rate (a), peak oxygen uptake VO_{2peak} (b), and distance walked (c) in six minute walk test before □ (n = 10 and 10) and after ■ (n = 8 and 9) eight weeks of aquatic exercise.

compared with the control group, regarding work rate, VO_{2peak} ($P < 0.001$), and walking capacity ($P = 0.01$) (Figure 2(a)–2(c)). There were no significant differences in knee extension regarding isometric strength, isotonic strength 60°/s, or isotonic endurance, neither in handgrip strength or endurance. However, a significant increase in isokinetic strength 180°/s ($P < 0.001$), isotonic heel lift ($P = 0.01$), shoulder flexion ($P < 0.05$), and isometric shoulder abduction ($P < 0.001$) was found in the training group after aquatic exercise (Table 2).

3.2. Metabolic Function. Hba1c decreased during aquatic exercise, but there was no significant improvement in fasting plasma glucose, insulin, c-peptide, or blood lipids after eight weeks of training (Table 3).

3.3. Health Related Quality of Life. Compared to a Swedish reference population [23], our patients with CHF and 2DM had lower SF-36 scores in all domains except for bodily pain, Figure 3(a). There was a significant difference in vitality scoring after aquatic exercise, whereas other domains were unchanged, Figure 3(b). Disease specific quality of life and grade of anxiety was unchanged in both groups after the intervention period, Table 4.

4. Discussion

This is the first study to show that aquatic exercise could be used as an effective tool to improve physical function in patients with the combination of CHF and 2DM. Further, the study confirms the results of our previous study with warm water training in elderly patients, supporting that this training is safe for patients with CHF.

4.1. Exercise Performance. A number of studies have demonstrated that exercise training on land, aerobic and resistance exercise improve function in patients with either CHF or with 2DM [15, 16]. VO_{2peak} is an important prognostic marker in these patients [24, 25] and it is significantly more reduced in patients with a combination of 2DM and CHF, than in patients with only one of the diseases [26]. It was therefore an important finding in this study that aquatic exercise was associated with a significant improvement in VO_{2peak}. Improved general performance was also shown as an increase in work rate and walking capacity. The physiological

Table 2: Muscle function before and after aquatic exercise in patients with chronic heart failure and type 2 diabetes mellitus.

Knee extension in Biodex III. Isokinetic		Before ($n = 10/10$)	After ($n = 8/9$)	P value within the group	P value versus the control group
Peak torque (60°s Nm) right leg	T	122 ± 41	127 ± 34	ns	ns
	C	102 ± 30	98 ± 32	ns	
Peak torque (180°s Nm) right leg	T	88 ± 28	119 ± 54	0.02	<0.001
	C	66 ± 22	64 ± 24	ns	
Endurance decline in %, left leg	T	46 ± 17	44 ± 13	ns	ns
	C	51 ± 14	52 ± 16	ns	
Isometric					
Peak torque	T	136 ± 41	136 ± 40	ns	ns
60° (N) right leg	C	109 ± 37	101 ± 32	ns	
Hand strength					
Peak force (N)	T	342 ± 121	385 ± 106	ns	ns
Right hand	C	248 ± 82	221 ± 62	0.04	
Peak force 10 s (N)	T	289 ± 108	323 ± 89	ns	ns
Right hand	C	207 ± 77	187 ± 62	ns	
Clinical endurance tests					
Heel lift (n.o)	T	14 ± 7	18 ± 6	0.01	0.01
	C	14 ± 4	14 ± 5	ns	
Shoulder flexion (n.o)	T	26 ± 11*	36 ± 12	0.02	0.03
	C	17 ± 8	17 ± 28	ns	
Shoulder abduction (s)	T	75 ± 25	89 ± 27	0.01	<0.001
	C	64 ± 26	56 ± 22	0.03	

T: Training group, C: control group, ns: not significant, n.o.: number of, *: $P \leq 0.05$ at baseline between training and control group.

Table 3: Metabolic function before and after aquatic exercise in patients with chronic heart failure and type 2 diabetes mellitus.

Variables		Before ($n = 10/10$)	After ($n = 8/9$)	P value within the group	P value versus the control group
Hba1c (%)	T	7.9 ± 2.9	7.2 ± 0.9	0.01	ns
	C	6.9 ± 2.0	6.7 ± 3.2	ns	
P-Fasting glucos (mmol/L)	T	10.2 ± 2.9	9.3 ± 2.6	ns	ns
	C	7.8 ± 3.3	6.9 ± 2.0	ns	
S-Insulin (mU/L)	T	20 ± 5.7	20.1 ± 11.5	ns	ns
	C	19.2 ± 14.9	16.1 ± 14.0	ns	
S-C-peptide (nmol/L)	T	0.8 ± 0.4	1.1 ± 0.5	ns	ns
	C	1.0 ± 0.9	1.4 ± 1.5	ns	
S-Triglycerides (mmol/L)	T	2.4 ± 3.4	2.2 ± 2.1	ns	ns
	C	1.9 ± 1.0	1.6 ± 0.8	ns	
S-Cholesterol (mmol/L)	T	4.2 ± 1.0	4.3 ± 0.9	ns	ns
	C	4.2 ± 1.1	4.1 ± 0.5	ns	

P: plasma, S: serum, T: training group, C: control group.

reason for this improvement was not investigated, but others have shown that exercise in the two diseases separately results in cardiac and peripheral muscle function enhancement which improves cardiac output and arteriovenous oxygen difference. Elevated peripheral resistance and poor endothelial function are factors that might contribute to reduced exercise capacity; however it could be enhanced by physical training [15, 16]. Immersion in warm water results in immediate improvement in cardiac function, probably mediated by peripheral vasodilation and unloading of left ventricular function [27, 28]. Whether such effects would be more beneficial during long-term treatment, compared with

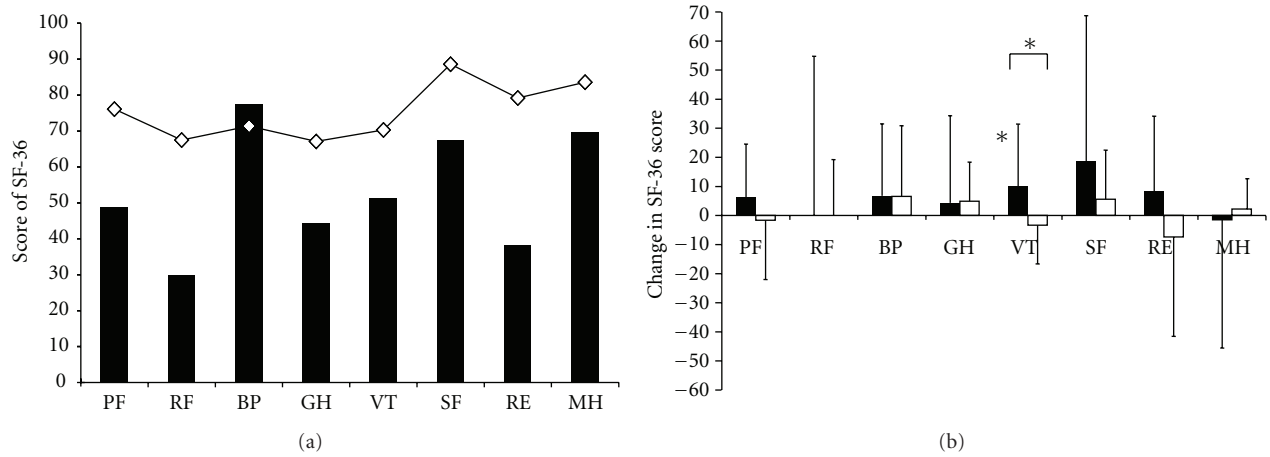

Figure 3: (a) Scores of SF-36 in all patients with chronic heart failure and type 2 diabetes mellitus, ■, ($n = 20$) compared to a Swedish healthy reference population -◇-. (b) Change in SF-36 after aquatic exercise, training group, ■, ($n = 8$) control group, □, ($n = 9$)*, $P = 0.05$.

Table 4: No significant changes in disease specific quality of life and grade of anxiety and depression occurred after eight weeks of aquatic exercise.

LHFQ		Before ($n = 10/10$)	After ($n = 8/9$)	HAD	Before ($n = 10/10$)	After ($n = 8/9$)
Total score	T	48 ± 22	43 ± 15	Anxiety	5.1 ± 4.8	5.4 ± 3.7
	C	35 ± 13	35 ± 16		3.1 ± 1.8	3.5 ± 3.0
Physical dimension	T	22 ± 14	16 ± 8	Depression	3.5 ± 3.2	3.6 ± 2.3
	C	19 ± 11	20 ± 13		4.2 ± 2.7	4.9 ± 3.7
Emotional dimension	T	10 ± 8	7 ± 6			
	C	5 ± 4	5 ± 4			

LHFQ: Minnesota living with heart failure questionnaire HAD: hospital anxiety and depression scale. T: Training group, C: control group.

training on land, would need further studies comparing the two exercise regimens.

4.2. Muscular Performance. Isokinetic strength in knee extensors was merely significantly improved at 180°/s and not in isokinetic strength at 60°/s or in isokinetic endurance and isometric strength. We have previously been hypothesised that an absent improvement in knee muscle function during aquatic exercise is due to the difficulty to gain enough resistance for this large muscle group in water [17]. However, the sensitivity of the test does also have large impact of the test results after training. Studies have shown that isotonic knee extensor training did not result in isokinetic knee extensor improvement [29, 30]. The increment in knee extension at 180°/s might be due to an enhanced neural adaptation since this test mirrors the ability to develop power [31, 32]. It seems less likely that this improvement should be attributable to an increase in the amount of type II fibres, after a relatively short endurance training of eight weeks. Aquatic exercise resulted also in increased isometric and isotonic muscle endurance measured by clinical endurance tests. These tests were performed exactly the way as it was trained. Specific adaptations in skeletal muscle after exercise seem to benefit patients with 2DM since the active muscle tissue reveals a higher metabolic rate in glucose metabolism [15]. An important finding in this study was that the training maintained and improved endurance in both upper and lower body muscle groups, which is important for older people to prevent falls and to accomplish daily tasks of living requiring both static and dynamic efforts [33].

4.3. Metabolic Control. No specific advice concerning diet or diabetic treatment was given during this study. Diabetic therapy was supplied by the patient's ordinary health care and was not part of the study. A positive finding was the decrease in HbA1c after training. However, other markers of metabolic control did not change. It was not the scope of this study to investigate insulin resistance, and others have shown signs of decreased insulin resistance after exercise in 2DM [34] however, the effect of training is unclear in CHF [35]. We could not confirm that immersion in warm water solely could enhance metabolic function in patients with 2DM, as shown by Hooper [36].

4.4. Quality of Life Measurements. The size of the population in this study was inadequate to show unequivocal changes in quality of life. Of the instruments used, only an index in SF-36, vitality increased after aquatic exercise. Since the level of anxiety and depression was low among most of our patients at baseline no effect was seen in HAD scores.

4.5. Aquatic Exercise. Aquatic exercise enables a combination of aerobic and resistance exercises and is especially suitable for patients with advanced age, obesity, peripheral neuropathy, orthopaedic problems, or other comorbidity that hampers exercises on land. Due to the buoyancy effect in water weight bearing activities are much more effortless to perform in water [37]. For example, it is more uncomplicated for a patient with peripheral neuropathy to walk in water.

The rate of adherence in this supervised short-term exercise study was high, which is in accordance with several other studies in patients with CHF [17, 38, 39] as well as with 2DM [24]. However, the long-term adherence in nonsupervised exercise has been reported low by others [40, 41]. A "smorgasbord" of physical training regimen to the patient's disposal might enhance the rate of adherence to prescribed exercise.

4.6. Limitations. Similar to many other exercise studies in patients with CHF, our study was performed in a limited number of patients which may restrict external validity. A marked difficulty was to recruit patients that were free from other disabling and complicating disorders like peripheral ulcers, infections, or problems with glycaemic control which are more common in patients with the combination of CHF and 2DM. Further, these patients have a higher morbidity that increases the risk of withdrawal during the study period. In clinical practice, these conditions might temporarily hinder participation in training programmes. However, a temporary stop in the programme should not exclude these patients from the beneficial effects of physical training in the long run.

5. Conclusion

Aquatic exercise is safe and effective to improve physical and metabolic function in patients with the combination of CHF and 2DM. Whether conventional exercise on land is equally effective has not been shown and would need further studies. Training in water is especially beneficial for those patients with other disabilities that obstruct exercises on land.

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Review Article

Astragalus Injection for Hypertensive Renal Damage: A Systematic Review

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Objective. To evaluate the effectiveness of astragalus injection (a traditional Chinese patent medicine) for patients with renal damage induced by hypertension according to the available evidence. *Methods*. We searched MEDLINE, China National Knowledge Infrastructure (CNKI), Chinese VIP Information, China Biology Medicine (CBM), and Chinese Medical Citation Index (CMCI), and the date of search starts from the first of database to August 2011. No language restriction was applied. We included randomized controlled trials testing astragalus injection against placebo or astragalus injection plus antihypertensive drugs against antihypertensive drugs. Study selection, data extraction, quality assessment, and data analyses were conducted according to the Cochrane review standards. *Results*. 5 randomized trials (involving 429 patients) were included and the methodological quality was evaluated as generally low. The pooled results showed that astragalus injection was more effective in lowering β_2-microglobulin (β_2-MG), microalbuminuria (mAlb) compared with placebo, and it was also superior to prostaglandin in lowering blood urea nitrogen (BUN), creatinine clearance rate (Ccr). There were no adverse effects reported in the trials from astragalus injection. *Conclusions*. Astragalus injection showed protective effects in hypertensive renal damage patients, although available studies are not adequate to draw a definite conclusion due to low quality of included trials. More rigorous clinical trials with high quality are warranted to give high level of evidence.

1. Introduction

Hypertensive renal damage has been defined as being characterized by the changes in renal structure and function which was caused by hypertension. Renal damage is one of three hypertensive complications. Hypertension could cause renal damage in early stage, and the renal damage often happens insidiously and persists many years without any typical clinical symptoms. In the past ten years, the incidence of end-stage renal disease (ESRD) was rising at an annual rate of 9% and 28% was caused by hypertension [1]. In recent years, the incidence of ESRD caused by hypertension was also increased in China [2].

At present, antihypertensive drugs have been shown to be effective in lowering blood pressure and thus reducing morbidity and mortality of cardiovascular diseases. Angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor blocker (ARB) could also exert kidney protective effect by dilating efferent arterioles more than afferent arteriole, decreasing urinary albumin, and inhibiting the glomerulosclerosis. However, the treatment of hypertensive renal damage still needs to be further improved even on the basis of ACEI or ARB. Astragalus injection is a preparation of an extract of Radix Astragali. The major components are astragalosides [3], and the other pharmacological ingredients include polysaccharides, flavones, and aminoacids. Modern pharmacological research has indicated that astragalus injection could enhance myocardial contractility, improve circulation, protect myocardial cells and regulate immune function [4, 5]. Recent reviews [6–8] further indicated the potential benefit of astragalus injection in the treatment of hypertensive renal damage. The following systematic review aims to test whether astragalus injection is effective and safe in treating hypertensive renal damage.

2. Methods

2.1. Database and Search Strategies. We searched MEDLINE, China National Knowledge Infrastructure (CNKI), Chinese VIP Information, China Biology Medicine (CBM), and Chinese Medical Citation Index (CMCI). The date of search was from the first of database start to August 2011. No language restrictions were applied. We used the terms "hypertensive renal damage", "hypertensive renal injury", "astragalus injection", and "Huangqi injection". Various combinations of the terms were used, depending on the database searched.

2.2. Inclusion Criteria. (1) Randomized controlled trials (RCT); (2) male or female patients, of any age or ethnic origin, who had hypertensive renal damage. Hypertensive renal damage was diagnosed on the basis of: (i) a history of essential hypertension, (ii) persistent proteinuria, (iii) hypertensive retinopathy, (iv) primary renal diseases or other secondary renal disease was excluded; (3) the intervention measure was astragalus injection, astragalus injection plus placebo, or astragalus injection plus antihypertensive drugs; (4) all trials had to report clinically relevant outcome measures of hypertensive renal damage; (5) the treatment should be at least two weeks. Outcome measures include results of blood pressure, renal function, clinical comprehensive effect, and Traditional Chinese medicine (TCM) syndrome differentiation. Duplicated publications reporting the same groups of participants were excluded.

2.3. Data Extraction and Quality Assessment. Two reviewers (T. Sun, H. Xu) extracted data independently. We assessed the methodological quality of all included trials by using the table of risk of bias provided by RevMan 5.1.0. The scale consists of seven items pertaining to description of random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias.

2.4. Data Synthesis. We used RevMan 5.1.0 provided by Cochrane Collaboration to analyse the data. Dichotomous data were expressed as relative risk (RR) and continuous outcomes as weighted mean difference (WMD), both with 95% confidence intervals (CI). Heterogeneity was assessed using the I^2 test with the significance level set at I^2 over 50% or $P < 0.1$. In the absence of significant heterogeneity, we pooled data using a fixed-effect model ($I^2 < 50\%$), otherwise we using random effects model ($I^2 > 50\%$) [9].

3. Results

3.1. Description of Included Trials. Our search identified 32 references. We excluded 27 of these articles. Flow diagram of the article selection for this study is shown in Figure 1.

The search yielded 5 eligible trials, which were all conducted and published in China. A total of 429 participants with renal damage induced by hypertension were included in the 5 trials. The proportion of male participants was 66.8%. All the trials included inpatients, and the average size of the trials was 86 patients (ranging from 48 to 127 participants). Five trials enrolled patients with renal damage induced with hypertension. The diagnostic criteria of trials were based on the guidelines for prevention and treatment of hypertension in China [4, 5], clinical manifestations, and laboratory tests [3, 6, 7]. Three trials were astragalus injection combined with antihypertensive drugs against antihypertensive drugs, one trial was astragalus injection against placebo, and one trial was astragalus injection against prostaglandin. No trial reported outcomes of the incidence of complications, health economic costs, quality of life, or adverse effects. The outcomes that were reported included twenty-four hours urinary protein content, microalbuminuria (mAlb), β_2-microglobulin (β_2-MG), blood urea nitrogen (BUN), serum creatinine (Scr), and creatinine clearance rate (Ccr). Characteristics of included studies were shown in Table 1.

3.2. Methodological Quality of Included Trials. The methodological quality of all the five trials was very low (Figure 2): none of trials reported sample calculation, the sample size of trials was small. These trials provided limited information on allocation concealment and blinding, and they were all lack of description of the allocation sequence generation. All the trials did not mention followup. We contacted the author for further information but regrettably no information has been provided to date.

3.3. Effect of Interventions. Three trials [3, 5, 7] gave biochemical indices to analyse the effective of astragalus injection. One trial [4] only gave the number of patients who had symptomatic improvement, and one trial [6] gave both biochemical indices and the number of patients who had symptomatic improvement. All were showed in Tables 2 and 3.

3.3.1. The Analysis of Improvement of Renal Damage Indices. It was not possible to pool the data on renal damage indicators, since the results describing varied indicators to prove the curative effect of astragalus injection.

In the Ji trial [10], the experimental group used astragalus injection ($n = 54$), while glucose injection was administered in the control group. Astragalus injection showed significant effect on indicators of β_2-MG (MD −15.14, 95%CI −21.61 to −8.67) and mAlb (MD −28.41, 95%CI 47.67 to −9.15).

Xu trial [11] used astragalus injection combined with Telmisartan and Plendil in the experimental group ($n = 26$), while Telmisartan and Plendil were administered in the control group. Only indicators of pulse pressure (MD −7.00, 95%CI −11.56 to −2.44), systolic blood pressure (SBP) (MD −21.70, 95%CI −31.24, −12.16), and twenty-four hours urinary protein content (MD −0.05, 95%CI −0.07, −0.04) showed significant differences.

In He trial [12], the experimental group used astragalus injection ($n = 50$), and prostaglandin was used in the control group. Astragalus injection showed significant effect on indicator of BUN (MD −7.39, 95%CI −9.83, −4.95) and Ccr (MD 6.84, 95%CI 4.57, 9.11).

Table 1: Characteristics of Included Studies.

Study ID	Gender male/female	Base-line information	Average age (years)	Interventions	Control	Duration of treatment (days)	Outcome measures
Ji and Yin 2006 [10]	59/35	Age, sex, condition	45.2	Astragalus injection (250 mL i.d, qd)	5% glucose injection	30	β_2-MG, mAlb
Hua et al. 2009 [17]	84/43	Age, sex, blood pressure, duration	44.3	Astragalus injection (250 mL i.d, qd) calcium channel blockers (CCB), ACEI/ARB, thiazine diuretics	CCB,ACEI/ARB, thiazine diuretics	20	Twenty-four hours urinary protein content, blood pressure, urinalysis, renal function
Xu et al. 2008 [11]	28/20	Age, sex, blood pressure	63.6 ± 14.1	Astragalus injection (250 mL i.d, qd) Telmisartan, Plendil	Telmisartan, Plendil	21	Twenty-four hours urinary protein content, blood pressure, mAlb, serum potassium, pulse pressure, estimated glomerular filtration rate (eGFR)
He 2004 [12]	78/18	Age, sex	74.2	Astragalus injection (500 mL i.d, qd)	Prostaglandin (PGE1)	15	BUN, Scr, Ccr
Yao et al. 2002 [13]	38/26	Age, sex, blood pressure	64.7 ± 13.7	Astragalus injection (250 mL i.d, qd) Lotensin, Plendil	Lotensin, Plendil	21	Twenty-four hours urinary protein content

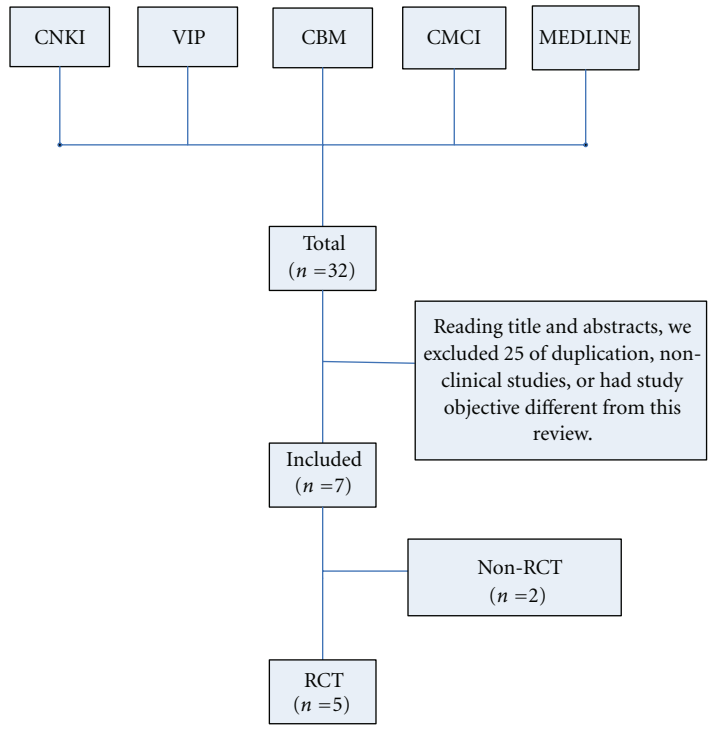

Figure 1: Flow diagram of the article selection for this study.

Table 2: The Analysis of Improvement of renal damage indices.

Renal damage indices and comparison between the groups	No. of studies	WMD [95% CI]	P value
β_2-MG			
Astragalus versus glucose injection [10]	1	−15.14 [−21.61, −8.67]	$P < 0.00001$
mAlb			
Astragalus versus glucose injection [10]	1	−28.4 [−47.67, −9.15]	$P = 0.004$
Astragalus plus Telmisartan, Plendil versus Telmisartan, and Plendil [11]	1	−4.20 [−7.47, −0.93]	$P = 0.01$
eGFR			
Astragalus plus Telmisartan, Plendil versus Telmisartan, and Plendil [11]	1	4.10 [−2.38, 10.58]	$P = 0.21$
pulse pressure			
Astragalus plus Telmisartan, Plendil versus Telmisartan, and Plendil [11]	1	−7.00 [−11.56, −2.44]	$P = 0.003$
SBP			
Astragalus plus Telmisartan, Plendil versus Telmisartan, and Plendil [11]	1	−21.70 [−31.24, −12.16]	$P < 0.00001$
DBP			
Astragalus plus Telmisartan, Plendil versus Telmisartan, and Plendil [11]	1	−4.20 [−11.02, 2.62]	$P = 0.23$
serum potassium			
Astragalus plus Telmisartan, Plendil versus Telmisartan, and Plendil [11]	1	−0.08[−0.28, 0.12]	$P = 0.44$
twenty-four hours urinary protein content			
Astragalus plus Telmisartan, Plendil versus Telmisartan, and Plendil [11]	1	−0.05 [−0.07, −0.04]	$P < 0.00001$
Astragalus plus Lotensin, Plendil versus Lotensin, and Plendil [13]	1	−0.21 [−0.50, 0.08]	$P = 0.15$
BUN			
Astragalus versus prostaglandin [12]	1	−7.39 [−9.83, −4.95]	$P < 0.00001$
Scr			
Astragalus versus prostaglandin [12]	1	−3.37 [46.05, 39.31]	$P = 0.88$
Ccr			
Astragalus versus prostaglandin [12]	1	6.84 [4.57, 9.11]	$P < 0.00001$

Table 3: The analysis of comprehensive therapeutic effect.

Symptom and sign	No. of studies	Intervention *(n/N)*	Control *(n/N)*	RR [95%CI]	*P* value
Astragalus plus thiazine diuretics versus thiazine diuretics	1	10/64	32/64	0.18 [0.08, 0.41]	$P < 0.00001$
Astragalus versus prostaglandin	1	2/50	13/46	0.11 [0.02, 0.50]	$P = 0.005$

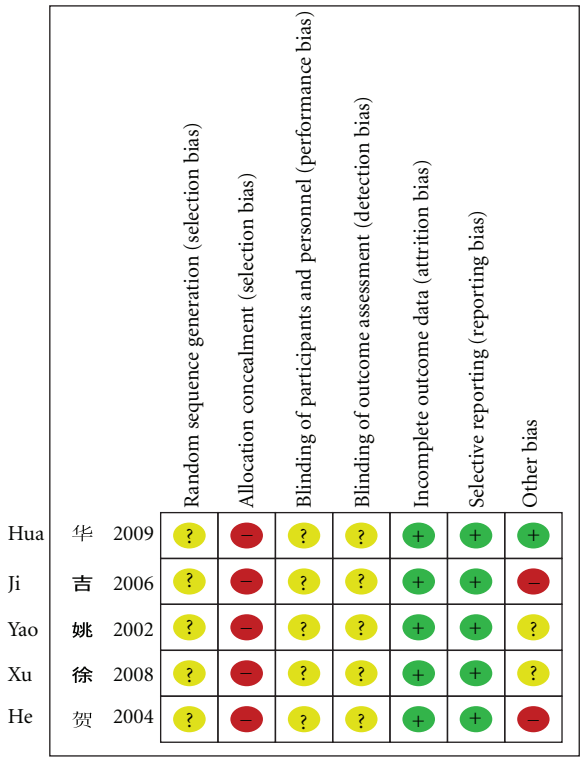

Figure 2: Risk of bias summary.

In Yao trial [13], there was no significant difference between astragalus injection plus Lotensin and Plendil group and Lotensin and Plendil group, according to indicator of twenty-four hours urinary protein content.

3.3.2. Symptoms and Signs. There were only two trials who reported the improvement on symptoms and signs (Table 3). However, they were all for comprehensive therapeutic effect. We cannot obtain the number of patients with individual symptoms and the data of individual symptoms improvement after treatment. So we cannot get the analysis of comparison between groups.

3.4. Final Indicator at Endpoint. None of the trial reported the mortality rate or the incidence of complication.

3.5. Sensitivity Analysis, Subgroup Analysis, and Publication Bias. The number of trials was too small to conduct any sufficient additional analysis of sensitivity, subgroup, and publication bias.

3.6. Adverse Reaction. None of the trial reported the observation of side effects.

4. Discussion

Our systematic review suggested that astragalus injection may be effective on laboratory indices of renal damage (β_2-MG, mAlb, pulse pressure, SBP, BUN, Ccr) or improvement of symptoms and signs. However, according to potential publication bias and low-quality trials, available data are not adequate to draw a definite conclusion of astragalus injection in treating renal damage induced by hypertension. More specifically, the positive findings should be interpreted conservatively due to the following facts.

The five trials included in this paper had risk of bias in terms of design, reporting, and methodology. They provided only limited descriptions of study design, allocation concealment, and baseline data. All the five RCTs prohibited us from performing meaningful sensitivity analysis. The included trials were heterogeneous in the populations (adults, elderly people) and the reported outcomes. All the included trials were not multicenter, large scale RCTs.

The primary goal of treatment for renal damage induced by hypertension is to prevent death or progression to complications. The outcomes from all the included trials are mainly laboratory indices and symptom improvement. There is a lack of data from all the trials on clinically relevant outcomes such as the mortality, incidence of complications, and quality of life.

Nevertheless, astragalus injection is administered for treating renal damage induced by hypertension in China. We have identified more than 30 randomized trials on this topic until now. However, most of them are not eligible for the review due to inadequate design, conducting, and reporting of the trials. Chinese researchers must be aware of the need to design and use appropriate statistical methods in future RCTs of astragalus injection and to measure clinical outcomes rather than physiological (surrogate) outcomes.

All the five trials did not report that adverse events. A conclusion about the safety of astragalus injection cannot be made. In China, it is widely believed that it is safe to use herbal medicines for various conditions. All the trials did not report that adverse events may reflect current situation. However, the safety of herbal medicines needs to be monitored carefully and reported appropriately in the future clinical trials. In fact, we found that some reports [14–16] indicated that astragalus injection had adverse outcomes.

Although we conducted comprehensive searches, we only identified and included trials published in Chinese. Most of the trials are small sample with positive findings. We tried to

avoid language bias and location bias, but we cannot exclude potential publication bias. We have conducted extensive searches for unpublished material, but at the same time we cannot neglect the fact that trials with negative findings remain unpublished.

Based on this systematic review, the effectiveness and safety of astragalus injection in patients with hypertensive renal damage is uncertain. The evidence is inconclusive due to poorly designed and low-quality trials. There is a need for additional RCTs that emphasize not only good clinical design but also more elaborated description of the intervention and clinically relevant outcomes including the mortality, incidence of complications, and quality of life.

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Review Article

A Systematic Review of Xuezhikang, an Extract from Red Yeast Rice, for Coronary Heart Disease Complicated by Dyslipidemia

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Objective. This systematic review aims to evaluate the benefit and side effect of Xuezhikang for coronary heart disease (CHD) complicated by dyslipidemia. *Methods*. All randomized clinical trials (RCTs) with Xuezhikang as a treatment for CHD combined with dyslipidemia were considered for inclusion. Data extraction and analyses and quality assessment were conducted according to the Cochrane standards. *Results*. We included 22 randomized trials. Xuezhikang showed significant benefit on the incidence of all-cause deaths, CHD deaths, myocardial infarction, and revascularization as compared with placebo based on conventional treatment for CHD. It remarkably lowered total cholesterol (TC), triglyceride (TG), and low-density lipoprotein-cholesterol (LDL-C) as compared with the placebo or inositol nicotinate group, which was similar to statins group. Xuezhikang also raised high-density lipoprotein cholesterol (HDL-C) compared to placebo or no intervention, which was similar to Inositol nicotinate and slightly inferior to statins. The incidence of adverse events did not differ between the Xuezhikang and control group. *Conclusions*. Xuezhikang showed a comprehensive lipid-regulating effect and was safe and effective in reducing cardiovascular events in CHD patients complicated by dyslipidemia. However, more rigorous trials with high quality are needed to give high level of evidence.

1. Introduction

Coronary heart disease (CHD) is one of the most serious diseases with high incidence and mortality. Dyslipidemia contributes greatly to the formation and progression of atherosclerosis (AS), which plays a dominant role in leading to CHD. Patients with CHD are also commonly complicated with dyslipidemia. Modulating dyslipidemia actively, especially lowering low-density lipoprotein-cholesterol (LDL-C) by statins, has been demonstrated to be very crucial to prevent AS and reduce the morbidity and mortality of CHD. Most recently, the updated ESC/EAS guidelines for management of dyslipidemia [1] further highlighted the aggressive lipid-lowering strategy in subjects with documented coronary vascular disease (CVD) or previous myocardial infarction (MI). However, the application of statins might be restricted by the adverse effect on the liver function and creatine kinase, especially in patients with old age, multiple comorbid diseases, high-dose statins, or a combination lipid-lowering therapy. Thus it is of great clinical significance to find an effective but safer alternative therapy in CHD patients complicated by dyslipidemia.

Xuezhikang is a partially purified extract of fermented red yeast rice (Monascus purpureus). It is composed of 13 kinds of natural statins, unsaturated fatty acids, ergosterol, amino acids, flavonoids, alkaloid, trace element, and so forth. The health enhancing qualities of this yeast have been introduced and used in China for over two thousand years. At latest systematic review indicated the beneficial effects of Xuezhikang in the treatment of hyperlipidemia [2]. Therefore, Xuezhikang has been recommended in a guideline for China adult dyslipidemia prevention [3]. Recently, clinical benefits of Xuezhikang were also found in CHD patients combined with dyslipidemia in some randomized controlled trials [4–6]. This systematic review aims to evaluate the benefit and side effect of Xuezhikang, a potential

Table 1: Definition of dyslipidemia or treatment goal of patients with CHD or equivalents on serum lipid level.

Orgination	Definition of dyslipidemia or treatment goal of Patients with CHD or equivalents on serum lipid level
ATP I 1988 [14]	Ideal lipid level: TC < 5.17 mmol/L (200 mg/dL); LDL-C < 3.36 mmol/L (130 mg/dL). Patients with HDL-C < 0.9 mmol/L (35 mg/dL) were defined unmoral. The definition of dyslipidemia was according to the level of LDL-C
ATP II 1993 [15]	Treatment goal: LDL-C $\leqq$ 2.6 mmol/L (100 mg/dL)
Ministry of Health of the People's Republic of China 1993 [8]	The treatment goal was not introduced
CADPS 1997 [16]	Treatment goal: TC < 4.68 mmol/L (180 mg/dL); TG < 1.7 mmol/L (150 mg/dL); LDL-C < 2.6 mmol/L (100 mmol /L)
ATP III 2001 [17]	Treatment goal: LDL-C < 2.6 mmol/L (100 mg/dL)
Implication of ATP III 2004 [18]	Treatment goal: LDL-C < 2.6 mmol/L (100 mg/dL); the optional goal: LDL-C < 1.8 mmol/L (70 mg/dL)
AHA/ACC Guideline 2006 [19]	Treatment goal: LDL-C < 2.6 mmol/L (100 mmol/L), and it is seasonal for lower than 1.8 mmol/L (70 mg/dL)
CADPG 2007 [3]	Treatment goal: TC < 4.14 mmol/L (160 mg/dL) and LDL-C < 2.59 mmol/L (100 mg/dL) for CHD or equivalents Treatment goal: TC < 3.11 mmol/L (120 mg/dL); LDL-C < 2.07 mmol/L (80 mg/dL) for ACS or ischemic cardiovascular disease complicated with diabetes mellitus Suitable scope of HDL-C: $\geqslant$1.04 mmol/L (40 mg/dL); suitable scope of TG: <1.7 mmol/L (150 mg/dL)
ESC/EAS 2011 [1]	In patients at very high CV risk (established CVD, type 2 diabetes, type I diabetes with target organ damage, moderate to severe CKD or a score level $\geqq$ 10%), the LDL-C goal is <1.8 mmol/L (70 mg/dL) and/or $\geqslant$50% LDL-C reduction when target level cannot be reached (I A recommendation)

alternative drug of statins, for CHD patients complicated by dyslipidemia, and thus provide further evidence for clinical application.

2. Methods

2.1. Inclusion Criteria. Randomized controlled trials (RCTs) comparing Xuezhikang with placebo, no intervention, or established lipid-lowing agents in English or Chinese were considered. Quasirandomized trials were excluded, and the duration of the intervention was no less than four weeks. Participants of all age with CHD complicated by dyslipidemia meeting with at least one of the current or past definitions or guidelines of CHD [including acute coronary syndrome (ACS)] [7–13] and dyslipidemia (treatment goal as the lower limit, see Table 1) [14–20] were considered. Those who did not introduce diagnostic criteria in the text but stated patients with definite CHD or dyslipidemia were also included. Secondary dyslipidemia, high serum lipid level after meal, serious heart failure, and serious hepatic or renal failure were excluded.

Outcome measures include primary outcomes (including all-cause mortality, CHD mortality, incidence of MI, revascularization, and rehospitalization for unstable angina) and secondary outcomes [including serum total cholesterol (TC), triglyceride (TG), LDL-C, and-high density lipoprotein cholesterol (HDL-C)].

2.2. Search Strategy. Two reviewers searched the following databases up to September 2011 independently for the identifications of trials (publication or nonpublication): The Cochrane Library, Pubmed, Chinese Biomedical Database (CBM), China National Knowledge Infrastructure (CNKI), Chinese VIP Information (VIP), and Wanfang Databases. We used the terms as follows: coronary heart disease, CHD, coronary artery disease, angina pectoris, myocardial infarction, acute coronary syndrome, cardi*, and Xuezhikang, red yeast rice, monascus. Because of different characteristics of various databases, MeSH terms and free text terms were used regardless of the report types in full text, title, keyword, subject terms, or abstract.

2.3. Data Extraction and Quality Assessment. Two reviewers (Shang QH, Liu ZL) independently extracted data according to a data extraction form made by the authors. Disagreements were resolved by consensus or consultation from a third reviewer (Liu JP). The methodological quality of trials was assessed independently using criteria from the Cochrane Handbook for Systematic Review of Interventions, Version 5.0.1 (Shang QH, Liu ZL) [20]. We contacted with the authors if there was any doubt in randomization and blinding method. The items included random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other bias. We judged each item from three levels ("Yes" for a low of bias, "No" for a high risk of bias, "Unclear" otherwise), and then we assessed the trials and categorized them into three levels: low risk of bias (all the items were in low risk of bias), high risk of bias (at least

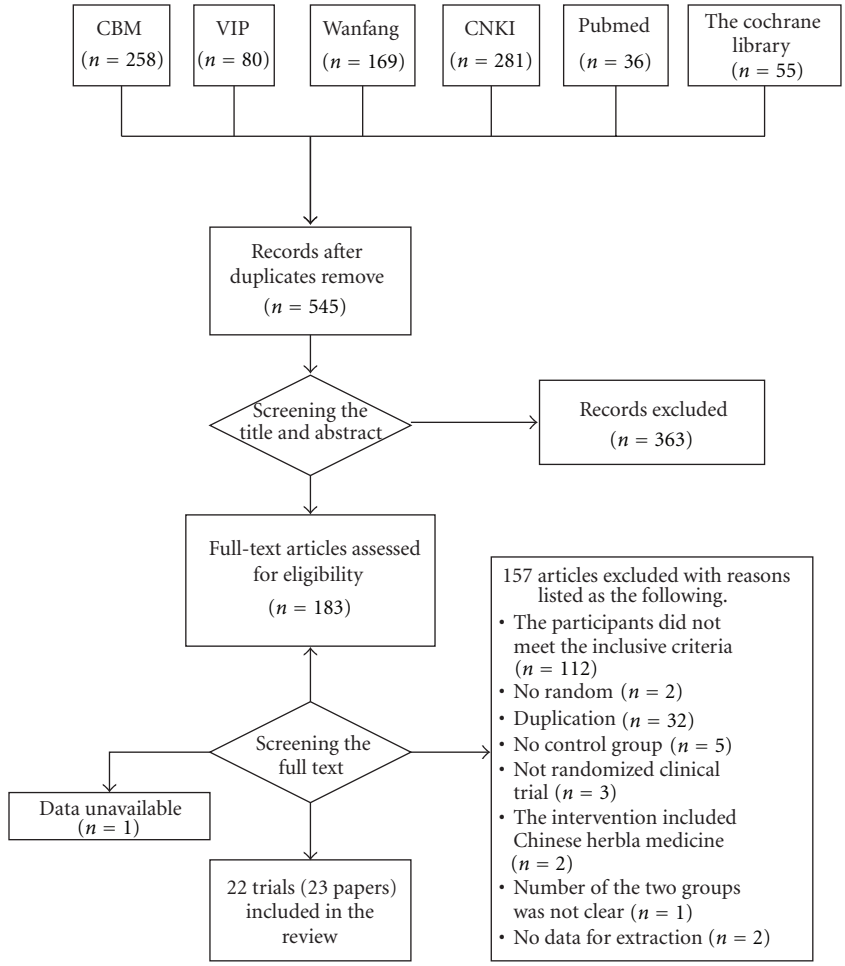


Figure 1: Flow chart of study selection.

one item was in high risk of bias), unclear risk of bias (at least one item was in unclear).

2.4. Data Synthesis. We used Revman 5.1 software provided by the Cochrane Collaboration for data analyses. Studies were stratified by the types of comparisons. We will express dichotomous data as risk ratio (RR) and its 95% confidence intervals (CI). Continuous outcome will be presented as mean difference (MD) and its 95% CI. Heterogeneity was recognized significant when $I^2 \geqq 50\%$. Fixed effects model was used if there is no significant heterogeneity of the data; random effects model was used if significant heterogeneity existed ($50\% < I^2 < 85\%$). Publication bias was explored using a funnel plot.

3. Results

3.1. Description of Included Trials. 22 RCTs (23 papers) [4–6, 21–39] were included, 21 papers were published in Chinese, one paper published in English, and one was unpublished as a postgraduate dissertation. The whole process of trials selection was demonstrated in Figure 1. The characteristics of included trials were listed in Table 2.

6520 Participants were included (3264 in intervention group and 3256 in control group). Two of the trials did not report the gender, and 4905 male and 1538 female were included in the other 20 trials. A total of 7 criteria of CHD (including ACS) were selected, but 6 trials did not introduce criteria of CHD but mentioned "patients with CHD were eligible to include." 3 criteria of dyslipidemia were used for 11 trials, and the other 11 trials only reported the serum lipid levels, which were categorized to dyslipidemia according to the previous and current definitions and guidelines Table 1. One trial [4] included patients with MI; five of the trials [5, 27, 28, 34, 39] included patients with unstable angina; two of the trials [6, 38] included patients with ACS; three of the trials [21, 22, 31] included patients with stable angina. The other 11 trials [23–26, 29, 30, 32, 33, 35–37] did not introduce the types of CHD or all types were included.

Patients in 19 trials prescribed Xuezhikang 600 mg QD (regulation was conducted for adverse events), one trial used Xuezhikang 600 mg TID if the serum TC or TG still higher

after having been prescribed for 6 weeks (600 mg BID in previous 6 weeks) [30], one trial [37] prescribed Xuezhikang 300 mg TID, and one trial [31] prescribed Xuezhikang 1200 mg QN. The duration of treatment ranged from 4 weeks to 7 years.

There were five comparisons in the review according to various control groups. (1) Xuezhikang and conventional therapy versus conventional therapy (8 trials) [5, 6, 24, 29, 33, 34, 38, 39]; (2) Xuezhikang and conventional therapy versus placebo and conventional therapy (2 trials) [4, 35]; (3) Xuezhikang and conventional therapy versus statin and conventional therapy (9 trials) [21–23, 25, 26, 28, 31, 37, 39]; (4) Xuezhikang and statin and conventional therapy versus statin and conventional therapy (2 trials) [27, 36]; (5) Xuezhikang and aspirin versus inositol nicotinate and aspirin (1 trials) [32]. One trial [39] was designed as three groups with two comparisons and Xuezhikang and conventional therapy versus conventional therapy; Xuezhikang and conventional therapy versus atorvastatin and conventional therapy.

3.2. Methodological Quality of Included Trials. According to the criteria introduced above, no trial was evaluated as having a low risk of bias. Only one trial of the 22 trials reported the method to generate the allocation sequence (random number table) in the paper [6]. After we contacted with the authors, six trials announced a correct method for allocation sequence [4–6, 31, 33, 35]. One trial was assessed as having adequate concealment [35]. Two trials applied double-blinding [4, 35], and two trials used single-blinding but did give us objective to be blinded [25, 37]. One trial blinded the outcome assessors [4]. One trial reported prior sample size estimation and mentioned intention-to-treat analysis [4]. Five trials reported information on withdrawal/dropout [4, 6, 22, 29, 32]. 18 trials [4–6, 22–27, 29, 31–33, 35–39] provided baseline data for the comparability among groups. The results of the assessment of risk of bias are presented in a "risk of bias summary" figure produced by Revman 5.1 automatically Figure 2.

3.3. Effect Estimates of Outcomes (Tables 3 and 4)

3.3.1. All-Cause Mortality. There was only 1 trial [4] reported the all-cause mortality in the comparisons of Xuezhikang and conventional therapy versus placebo and conventional therapy [RR 0.67; 95% CI 0.54 to 0.83; 1 trial, $n = 4870$].

3.3.2. Mortality of CHD. There were 5 studies [4, 22, 27, 28, 32] that presented the effect of Xuezhikang in reducing the mortality of CHD. Compared to placebo on the basis of conventional therapy, Xuezhikang showed a reduction of mortality of CHD (RR 0.69; 95% CI 0.54 to 0.89; 1 trial, $n = 4870$) [4]. Compared to simvastatin on the basis of conventional therapy, Xuezhikang showed no significant difference in mortality of CHD (RR 0.26; 95% CI 0.06 to 1.21; 2 trial, $n = 220$) [22, 28]. Compared to no treatment on the basis of simvastatin and conventional therapy, Xuezhikang showed no effect in reducing mortality of CHD (RR 0.33; 95% CI 0.01 to 7.80; 1 trial, $n = 48$) [27].

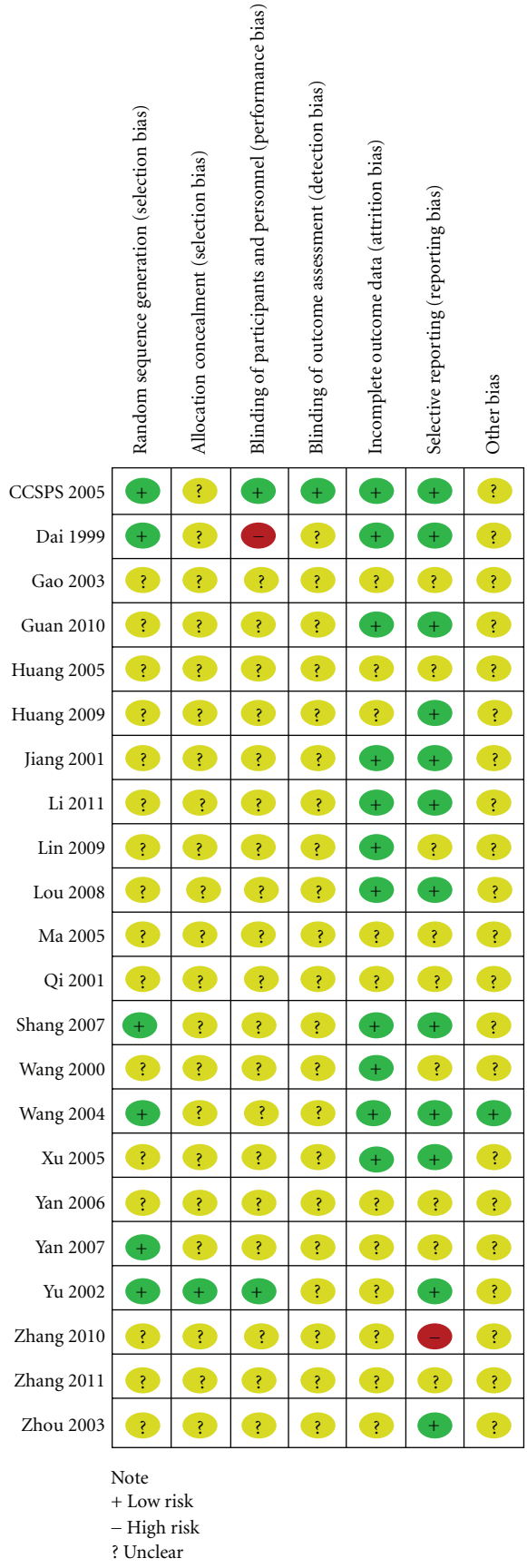


Figure 2: Risk of bias summary.

Compared with inositol nicotinate on the basis of aspirin, Xuezhikang showed no significant difference in mortality of CHD (RR 0.15; 95% CI 0.02 to 1.18; 1 trial, $n = 122$) [32].

3.3.3. Incidence of MI. There were 3 studies reporting CHD events in 3 different comparisons. Compared with placebo on the basis of conventional therapy, Xuezhikang showed a reduction of morbidity of MI (RR 0.39; 95% CI 0.28 to 0.55; 1 trial, $n = 4870$) [4]. Compared with simvastatin on the basis of conventional therapy, Xuezhikang showed no significant difference (RR 0.95; 95% CI 0.30 to 3.05; 1 trial, $n = 84$) [28]. In comparisons of Xuezhikang and simvastatin and conventional therapy versus simvastatin and conventional therapy, Xuezhikang showed no effect in reducing incidence of MI (RR 0.20; 95% CI 0.01 to 3.96; 1 trial, $n = 48$) [27].

3.3.4. Revascularization. Revascularization included percutaneous coronary intervention (PCI) and coronary artery bypass graft (CABG). There were 2 studies [4, 28] reporting revascularization in 2 different comparisons. Compared with placebo on the basis of conventional therapy, Xuezhikang showed a significant reduction of revascularization (RR 0.67; 95% CI 0.50 to 0.89; 1 trial, $n = 4870$) [4]. Compared with simvastatin on the basis of conventional therapy, Xuezhikang showed no significant difference (RR 1.14; 95% CI 0.38 to 3.46; 1 trial, $n = 84$) [28].

3.3.5. Rehospitalization for Unstable Angina. There were 2 trials [27, 28] reporting rehospitalization in 2 different comparisons. Compared with simvastatin on the basis of conventional therapy, Xuezhikang showed no significant difference in the number of rehospitalization (RR 1.02; 95% CI 0.57 to 1.84; 1 trial, $n = 84$) [28]. Compared with no treatment on the basis of simvastatin and conventional therapy, Xuezhikang showed no effect in reducing rehospitalization (RR 0.20; 95% CI 0.03 to 1.59; 1 trial, $n = 48$) [27].

3.3.6. Serum TC Level. There were 21 studies that reported the level of total cholesterol Table 4, but one trial only reported the serum lipid level of the treatment group [30]. (1) Compared to no treatment with cointervention of conventional therapy, Xuezhikang showed a reduction of TC level (MD −0.97 mmol/L; 95% CI −1.24 to −0.71; 8 trials, $n = 500$) [5, 6, 24, 29, 33, 34, 38, 39]. (2) There were two trials that reported Xuezhikang versus placebo on the basis of conventional therapy, meta-analysis was not used for significant difference, and, in this comparison, Xuezhikang showed a reduction of TC level (MD −0.57 mmol/L; 95% CI −0.61 to −0.53; 1 trial, $n = 4870$) [4] and (MD −2.62 mmol/L; 95% CI −2.98 to −2.26; 1 trial, $n = 62$) [35]. (3) There was no significant difference on serum TC level of Xuezhikang comparing to statins on the basis of conventional therapy (MD 0.19 mmol/L; 95% CI −0.22 to 0.59; 8 trial, $n = 633$) [21, 23–25, 28, 31, 37, 39]. Since there was significant heterogeneity in the comparison, we examined the data carefully and found that data of two trials deviated from the others. After looking over the papers, one of the two trial [26] with an unclear conventional therapy and the other used Xuezhikang 300 mg tid in the whole trial [37]. Sensitive analysis was used and got a similar conclusion (MD 0.02 mmol/L; 95% CI −0.03 to 0.06; 6 trial, $n = 489$) after excluded the two trials [26, 37]. (4) Compared with no treatment on the basis of statins and conventional therapy, Xuezhikang showed a reduction of TC level (MD −0.96 mmol/L; 95% CI −1.33 to −0.58; 2 trial, $n = 108$) [27, 36]. (5) Compared to inositol nicotinate on the basis of aspirin, Xuezhikang showed a significant difference in the reduction of TC level (MD −1.05 mmol/L; 95% CI −1.46 to −0.64; 1 trial, $n = 105$) [32].

3.3.7. Serum TG Level. There were 20 studies that reported the level of TG (see Table 4), but one trial only reported the serum lipid level of the treatment group [30]. (1) Compared to no treatment with cointervention of conventional therapy, Xuezhikang showed a reduction of TG level (MD −0.49 mmol/L; 95% CI −0.58 to −0.39; 7 trial, $n = 412$) [5, 6, 24, 29, 33, 38, 39]. (2) There were two trials that reported Xuezhikang versus placebo on the basis of conventional therapy, meta-analysis was not used for significant difference, and, in this comparison, Xuezhikang showed a reduction of TG level (MD −0.17 mmol/L; 95% CI −0.22 to −0.12; 1 trial, $n = 4870$) [4] and (MD −1.29 mmol/L; 95% CI −1.57 to −1.01; 1 trial, $n = 62$) [35]. (3) There was no significant difference on serum TG level of Xuezhikang comparing to statins on the basis of conventional therapy (MD −0.05 mmol/L; 95% CI −0.12 to 0.02; 8 trial, $n = 633$) [21, 23–25, 28, 31, 37, 39]. (4) Compared with no treatment on the basis of fluvastatin and conventional therapy, Xuezhikang showed a reduction of TG level (MD −0.27 mmol/L; 95% CI −0.35 to −0.19; 1 trial, $n = 60$) [36]. (5) Compared to inositol nicotinate on the basis of aspirin, Xuezhikang showed a significant difference in the reduction of TG level (MD −0.60 mmol/L; 95% CI −0.95 to −0.25; 1 trial, $n = 105$) [32].

3.3.8. Serum LDL-C Level. There were 21 studies that reported the level of LDL-C (see Table 4), but one trial only reported the serum lipid level of the treatment group [30]. (1) Compared to no treatment with cointervention of conventional therapy, Xuezhikang showed a reduction of LDL-C level (MD −0.78 mmol/L; 95% CI −1.19 to −0.38; 7 trial, $n = 444$) [5, 6, 24, 33, 34, 38, 39]. (2) There were two trials that reported Xuezhikang versus placebo on the basis of conventional therapy, meta-analysis was not used for significant difference, and, in this comparison, Xuezhikang showed a reduction of LDL-C level (MD −0.57 mmol/L; 95% CI −0.62 to −0.52; 1 trial, $n = 4870$) [4] and (MD −1.82 mmol/L; 95% CI −2.01 to −1.63; 1 trial, $n = 62$) [35]. (3) There was no significant difference on serum LDL-C level of Xuezhikang comparing to statins on the basis of conventional therapy (MD 0.03 mmol/L; 95% CI −0.10 to 0.25; 8 trial, $n = 633$) [21, 23–25, 28, 31, 37, 39]. Because there was significant heterogeneity in the comparison, we examined the data carefully and found that data of two trials deviated from the others. After looking over the papers, one of the two trials [26] with an unclear conventional therapy and the other used Xuezhikang 300 mg tid in the whole trial [37].

Table 2: Characteristics of included trials.

ID	Diagnostic criteria of CHD (ACS)	Diagnostic criteria of dyslipidemia	Types of CHD	Sample size (I/C)	Age (y, I/C)	Interventions group	Control group	Duration of treatment	Outcomes evaluation	Balance report of baseline
CCSPS 2005 [4]	Not specified	TC: 4.40–6.47	MI	2441/2429	(Male: 58.1 ± 9.9; female: 62.9 ± 6.7)/ (male: 58.0 ± 9.7; female: 62.6 ± 7.4)	Xuezhikang 600 mg BID + conventional therapy (no detail)	Placebo + conventional therapy (no detail)	4 year in average	Serum lipid level (TC, TG, LDL-C, HDL-C), all-cause mortality, cardiovascular events, serum lipid level (TC, TG, HDL-C, LDL-C), ADs	Yes
Dai et al. 1999 [5]	WHO 1979 and Gao 1994	Ministry of Health of the People's Republic of China 1993	Unstable angina	33/25	(57 ± 9)/(56 ± 8)	Xuezhikang 600 mg, BID + control	Nitrate esters 10 mg BID + nifedipine GIFTS 30 mg QD/diltiazem 30 mg tid + metoprolol 12.5 mg BID + aspirin 50 mg QD	8 weeks	Serum lipid level (TC, TG, HDL-C, LDL-C), ADs	Yes
Gao and Liao 2003 [21]	Not specified	TC ≥ 5.2 mmol/L, LDL-C ≥ 3.12 mmol/L, TG ≥ 1.7 mmol/L	Stable Angina	30/30	53–85, 67.5 in average	Xuezhikang 600 mg BID + conventional therapy (no detail)	Fluvastatin (Lescol see fluvastatin) 20 mg QD + conventional therapy (no detail)	4 weeks	Serum lipid level (TC, TG, LDL-C, HDL-C)	Unclear
Guan 2010 [22]	Not specified	TC > 7.08 mmol/L; TG > 3.34; LDL-C > 4.2; HDL < 0.93. Two items of the above were included	Stable Angina	72/64	49–76, 62 in average	Xuezhikang 600 mg BID	Simvastatin 10 mg QN	1 year	CHD mortality, ADs	Yes
Huang et al. 2005 [23]	WHO 1979	CADPS 1997	OMI and UA	45/63	44–72	Xuezhikang 600 mg BID	Simvastatin 20 mg QN	6 weeks	Serum lipid level (TC, TG, LDL-C, HDL-C)	Yes
Huang et al. 2009 [24]	WHO 1979	CADPS 1997	Unclear	43/42	65.78 ± 4.62	Xuezhikang 600 mg, BID + control	Nitroglycerine 20 mg BIDIV + 10% KCL + insulinIV QD	12 weeks	Serum lipid level (TC, TG, HDL-C, LDL-C)	Yes

Table 2: Continued.

ID	Diagnostic criteria of CHD (ACS)	Diagnostic criteria of dyslipidemia	Types of CHD	Sample size (I/C)	Age (y, I/C)	Interventions group	Control group	Duration of treatment	Outcomes evaluation	Balance report of baseline
Jiang and Cai 2001 [25]	Not specified	CADPS 1997	Unclear	30/45	51 ± 8	Xuezhikang 600 mg BID + conventional therapy (as same as B)	Simvastatin 10 mg QN + conventional therapy (nitrate esters 10 mg tid, aspirin 100 mg QD or anticoagulation drugs or thrombolytic drug or hypoglycemic)	8 weeks	Serum lipid level (TC, TG, LDL-C, HDL-C), ADs	Yes
Li et al. 2011 [26]	References [12, 13]	As same as Guan 2010	Unclear	32/32	(46.9 ± 14.5)/ (50.7 ± 15.1)	Xuezhikang 600 mg BID	Lovastatin 40 mg QD (20 mg QD if the ALT or AST was 3 times higher than the normal)	8 weeks	Serum lipid level (TC, TG, LDL-C, HDL-C), ADs	Yes
Lin et al. 2009 [27]	Chinese Society of cardiology 2000	TC ≥ 4.68 mmol/L or LDL-C ≥ 2.6 mmol/L	Unstable angina	24/24	35–71, 55.4 in average	Xuezhikang 600 mg, BID + control	Simvastatin 60 mg QN + conventional therapy (nitrate esters, β adrenergic blocking agent, CCB, aspirin, low molecular heparin and et al.	6 months	Serum lipid level (TC, LDL-C), CHD events	Yes
Lou et al. 2008 [28]	Chinese society of cardiology 2000	TC > 3.64 mmol/L and TG > 3.9 mmol/L and LDL-C > 2.6	Unstable angina	43/41	65 ± 10	Xuezhikang 600 mg BID + conventional therapy (as same as B)	Simvastatin 20 mg QD + conventional therapy (anticoagulation drugs, nitrate esters, β adrenergic blocking agent, ACEI, CCB and et al.)	6 months	Serum lipid level (TC, TG, LDL-C, HDL-C), Cardiovascular events, ADs	Unclear
Ma and Teng 2005 [29]	WHO 1979	CADPS 1997	Unclear	29/28	(62.7 ± 6.5)/ (61.2 ± 7.1)	Xuezhikang 600 mg BID + control	Conventional therapy (nitrate esters, β adrenergic blocking agent, ACEI, CCB and et al.)	8 weeks	Serum lipid level (TC, TG)	Yes

Table 2: Continued.

ID	Diagnostic criteria of CHD (ACS)	Diagnostic criteria of dyslipidemia	Types of CHD	Sample size (I/C)	Age (y, I/C)	Interventions group	Control group	Duration of treatment	Outcomes evaluation	Balance report of baseline
Qi et al. 2001 [30]	WHO 1979	TC > 6.0 mmol/L	Unclear	60/60	60.6 ± 12.3	Xuezhikang 600 mg, BID (600 mg TID if the lipid level was still higher than the treatment goal) + control	Conventional therapy (nitrate esters, β adrenergic blocking agent, ACEI, CCB, and et al.)	12 weeks	Serum lipid level (TC, TG), ADs	Unclear
Shang 2007 [31]	WHO 1979	CADPS 1997	Stable Angina	65/65	(51 ± 10)/(55 ± 10)	Xuezhikang 1200 mg QN + conventional therapy (as same as control group)	Atorvastatin 10 mg QN + conventional therapy (aspirin, nitrate esters, β adrenergic blocking agent, ACEI, and et al.)	2 months	Serum lipid level (TC, TG, LDL-C, HDL-C)	Yes
Wang and Xiao 2000 [32]	WHO 1979	CADPS 1997	MI, UA, CHD with no symptoms	65/57	49–76, 62 in average	Xuezhikang 600 mg BID + aspirin 50 mg QD	Inositol niacinate 400 mg TID + aspirin 50 mg QD	1 year	Serum lipid level (TC, TG, LDL-C, HDL-C), cardiovascular evnets, ADs	Yes
Wang et al. 2004 [6]	ACC/AHH 2000	CADPS 1997	ACS	26/26	(60.1 ± 8.9)/ (59.7 ± 8.6)	Xuezhikang 600 mg BID + control	Conventional therapy (aspirin, nitrate esters, β adrenergic blocking agent, ACEI, and et al.)	12 weeks	Serum lipid level (TC, TG, LDL-C, HDL-C), ADs	Yes
Xu 2005 [39]	Chinese Society of cardiology 2000	Not specified	UA	12/13/10	Unclear	Xuezhikang 600 mg BID + control group (1)	(1) Conventional therapy (isosorbide dinitrate 10 mg tid, betaloc 25–50 mg BID/TID, aspirin 50–150 mg QD, low molecular heparin 0.4–0.6 mL Q12H or diltiazem 30 mgtid/qid, or plendil 5 mg QD/BID or captopril 12.5–25 mg TID or nitroglycerine) (2) Conventional therapy (as same as (1)) and atorvastatin 20 mg Qn	1 month	Serum lipid level (TC, TG, LDL-C, HDL-C)	Yes

Table 2: Continued.

ID	Diagnostic criteria of CHD (ACS)	Diagnostic criteria of dyslipidemia	Types of CHD	Sample size (I/C)	Age (y, I/C)	Interventions group	Control group	Duration of treatment	Outcomes evaluation	Balance report of baseline
Yan 2006 [34]	Chinese Society of cardiology 2000	LDL-C: 1.84–4.12 mmol/L	UA	44/44	56.8 ± 8.6	Xuezhikang 600 mg BID + control	magnesium polarizing liquorIV + heparinIH + Aspirin, Nitrate esters, β adrenergic blocking agent CCB and et al.	8 weeks	Serum lipid level (TC, TG, LDL-C, HDL-C), ADs	Unclear
Yan and Li 2007 [33]	WHO 1979	CADPS 1997	Unclear	28/28	(66.68 ± 4.23)/ (66.79 ± 4.48)	Xuezhikang 600 mg, BID + control	Nitroglycerine 20 mg BID.iv + 10% KCL + insulinIV QD	8 weeks	Serum lipid level (TC, TG, LDL-C, HDL-C)	Yes
Yu et al. 2002 [35]	WHO 1979	CADPS 1997	Unclear	32/30	(53.5 ± 10.8)/ (50.6 ± 6.7)	Xuezhikang 600 mg, BID + conventional therapy (as same as control)	Placebo + conventional therapy (aspirin, nitrate esters, CCB and et al.)	8 weeks	Serum lipid level (TC, TG, LDL-C, HDL-C)	Yes
Zhang 2010 [36]	Reference [8]	CADPS 1997	Unclear	30/30	(58–80, 72.3 in average)/(59–82, 73.1 in average)	Xuezhikang 600 mg, BID + control	Fluvastatin 40 mg QD	4 weeks	Serum lipid level (TC, TG, LDL-C, HDL-C)	Yes
Zhang 2011 [37]	Unclear	CHOL > 5.72 mmol/L or LDL-C > 3.64 mmol/L complicated with high TG level	Unclear	40/40	(50 ± 13)/ (45 ± 15)	Xuezhikang 300 mg TID	Atorvastatin 20 mg/d QD	8 weeks	Serum lipid level (TC, TG, LDL-C), ADs	Yes
Zhou et al. 2003 [38]	Unclear	TC > 6.0 mmol/L and (or) LDL-C > 4.2 mmol/L or complicate with >1.92 mmol/L	ACS		60.8 ± 10.6	Xuezhikang 600 mg BID + control	Conventional therapy (nitrate esters, β adrenergic blocking agent, CCB, anticoagulation drugs, thrombolytic drug, PTCA and et al.)	8 weeks	Serum lipid level (TC, TG, LDL-C)	Yes

Table 3: Analysis of clinical events.

Outcomes (comparisons)	Treatment group (n/N)	Control group (n/N)	RR	95% CI
(1) All-cause mortality				
Xuezhikang capsule and conventional therapy versus placebo and conventional therapy				
CCSPS 2005 [4]	126/2429	189/2441	0.67	[0.54, 0.83]
(2) Mortality of CHD				
(2.1) Xuezhikang capsule and conventional therapy versus placebo and conventional therapy				
CCSPS 2005 [4]	92/2429	134/2441	0.69	[0.54, 0.89]
(2.2) Xuezhikang and conventional therapy versus simvastatin and conventional therapy				
Guan 2010 [22]	1/72	6/64	0.15	[0.02, 1.20]
Lou et al. 2008 [28]	1/43	1/41	0.95	[0.06, 14.75]
	Overall (FEM, I^2 = 13%)		**0.26**	[**0.06**, **1.21**]
(2.3) Xuezhikang and simvastatin and conventional therapy versus simvastatin and conventional therapy				
Lin et al. 2009 [27]	0/24	1/24	0.33	[0.01, 7.8]
(2.4) Xuezhikang and aspirin versus inositol nicotinate and aspirin				
Wang and Xiao 2000 [32]	1/65	6/57	0.15	[0.02, 1.18]
(3) Myocardial infarction				
(3.1) Xuezhikang and conventional therapy versus placebo and conventional therapy				
CCSPS 2005 [4]	47/2429	120/2441	0.39	[0.28, 0.55]
(3.2) Xuezhikang and conventional therapy versus simvastatin and conventional therapy				
Lou et al. 2008 [28]	5/43	5/41	0.95	[0.30, 3.05]
(3.3) Xuezhikang and simvastatin and conventional therapy versus simvastatin and conventional therapy				
Lin et al. 2009 [27]	0/24	2/24	0.2	[0.01, 3.96]
(4) Revascularization				
(4.1) Xuezhikang capsule and conventional therapy versus placebo and conventional therapy				
CCSPS 2005 [4]	73/2429	110/2441	0.67	[0.50, 0.895]
(4.2) Xuezhikang and conventional therapy versus simvastatin and conventional therapy				
Lou et al. 2008 [28]	6/43	5/41	1.14	[0.38, 3.46]
(5) Rehospitalization				
(5.1) Xuezhikang and conventional therapy versus simvastatin and conventional therapy				
Lou et al 2008 [28]	15/43	14/41	1.02	[0.57, 1.84]
(5.2) Xuezhikang and simvastatin and conventional therapy versus simvastatin and conventional therapy				
Lin et al. 2009 [27]	1/24	5/24	0.2	[0.03, 1.59]

Sensitive analysis was used and got a similar conclusion (MD 0.05 mmol/L; 95% CI −0.09 to 0.19; 6 trial, n = 489) after excluded the two trials [26, 37]. (4) Compared with no treatment on the basis of statins and conventional therapy, Xuezhikang showed a reduction of LDL-C level (MD −0.44 mmol/L; 95% CI −0.57 to −0.31; 2 trial, n = 108) [27, 36]. (5) Compared to inositol nicotinate on the basis of aspirin, Xuezhikang showed a significant difference in the reduction of LDL-C level (MD −0.88 mmol/L; 95% CI −1.27 to −0.48; 1 trial, n = 105) [32].

3.3.9. Serum HDL-C Level. There were 19 studies that reported the level of HDL-C (see Table 4), but one trial only reported the serum lipid level of the treatment group [30]. (1) Compared to no treatment with cointervention of conventional therapy, Xuezhikang showed a beneficial effect of HDL-C level (MD 0.24 mmol/L; 95% CI 0.08 to 0.40; 6 trial, n = 364) [5, 6, 24, 33, 34, 39]. (2) There were two trials that reported Xuezhikang versus placebo on the basis of conventional therapy, meta-analysis was not used for significant difference, and, in this comparison, Xuezhikang showed a beneficial effect of HDL-C level (MD 0.05 mmol/L; 95% CI 0.03 to 0.07; 1 trial, n = 4870) [4] and (MD 0.48 mmol/L; 95% CI 0.37 to 0.59; 1 trial, n = 62) [35]. (3) There was a lower effect on serum HDL-C level of Xuezhikang comparing to statins on the basis of conventional therapy (MD −0.10 mmol/L; 95% CI −0.19 to −0.01; 8 trial, n = 633) [21, 23–25, 28, 31, 37, 39]. Because there was significant heterogeneity in the comparison, we examined the data carefully and found that data of one trials deviated from the others. After looking over the papers, we found that the trial used Xuezhikang 300 mg tid [37]. Sensitive analysis was used and got a similar conclusion (MD −0.10 mmol/L; 95% CI −0.11 to −0.08; 7 trial, n = 553) after excluded the trial [37]. (4) Compared with no treatment on the basis of

Table 4: Analysis of serum lipid level.

Serum lipid level (comparison)	Intervention group		Control group		Weight (%)	MD	95% CI
	Mean	SD	Mean	SD			
(1) TC (mmol/L)							
(1.1) Xuezhikang and conventional therapy versus conventional therapy							
Dai et al. 1999 [5]	5.41	0.87	6.54	0.89	11.40	−1.13	[−1.59, −0.67]
Huang et al. 2009 [24]	4.98	0.79	5.99	0.87	13.30	−1.01	[−1.36, −0.66]
Ma and Teng 2005 [29]	5.30	1.30	6.30	1.00	9.00	−1.00	[−1.61, −0.39]
Wang et al. 2004 [6]	4.33	0.96	6.30	0.79	11.10	−1.97	[−2.45, −1.49]
Xu 2005 [39]	5.49	1.12	6.20	0.93	6.60	−0.71	[−1.52, 0.10]
Yan 2006 [34]	4.90	0.10	5.50	0.20	17.30	−0.60	[−0.67, −0.53]
Yan and Li 2007 [33]	4.90	0.13	5.93	0.23	17.00	−1.03	[−1.13, −0.93]
Zhou et al. 2003 [38]	4.30	0.54	4.84	0.78	14.30	−0.54	[−0.83, −0.25]
			Overall (REM, I^2 = 92%)		**100**	**−0.97**	**[−1.24, −0.71]**
(1.2) Xuezhikang and conventional therapy versus placebo and conventional therapy							
CCSPS 2005 [4]	4.65	0.67	5.22	0.88	—	−0.57	[−0.61, −0.53]
Yu et al. 2002 [35]	4.10	0.58	6.72	0.85	—	−2.62	[−2.98, −2.26]
(1.3) Xuezhikang and conventional therapy versus statin and conventional therapy							
(1.3.1) Xuezhikang and conventional therapy versus lovastatin and conventional therapy							
Li et al. 2011 [26]	4.57	1.42	5.32	1.72	9.5	−0.75	[−1.52, 0.02]
(1.3.2) Xuezhikang and conventional therapy versus simvastatin and conventional therapy							
Huang et al. 2005 [23]	4.62	0.63	4.36	0.60	13.8	0.26	[0.02, 0.50]
Jiang and Cai 2001 [25]	5.19	0.90	4.91	0.66	12.8	0.28	[−0.10, 0.66]
Lou et al. 2008 [28]	5.4	0.12	5.40	0.11	14.4	0.00	[−0.05, 0.05]
	Subgroup		**Overall (REM, I^2 = 69%)**			**0.14**	**[−0.08, 0.35]**
(1.3.3) Xuezhikang and conventional therapy versus fluvastatin and conventional therapy							
Gao and Liao 2003 [21]	4.05	0.74	3.63	0.59	13.1	0.42	[0.08, 0.76]
(1.3.4) Xuezhikang and conventional therapy versus atorvastatin and conventional therapy							
Shang 2007 [31]	4.65	0.79	4.88	0.85	13.5	−0.23	[−0.51, 0.05]
Xu 2005 [39]	5.49	1.12	5.50	0.92	8.8	−0.01	[−0.86, 0.84]
Zhang 2011 [37]	4.51	0.38	4.00	3.35	14.1	1.16	[0.99, 1.33]
	Subgroup		**Overall (REM, I^2 = 97%)**			**0.33**	**[−0.77, 1.43]**
After sensitive analysis	**Subgroup**		**Overall (FEM, I^2 = 0%)**			**−0.21**	**[−0.48, 0.06]**
	Total		**Overall (REM, I^2 = 96%)**			**0.19**	**[−0.22, 0.59]**
After sensitive analysis	**Total**		**Overall (REM, I^2 = 66%)**			**0.02**	**[−0.032, 0.06]**
(1.4) Xuezhikang and statin and conventional therapy versus statin and conventional therapy							
(1.4.1) Xuezhikang and simvastatin and conventional therapy versus simvastatin and conventional therapy							
Lin et al. 2009 [27]	4.30	0.71	5.00	0.81	35.6	−0.70	[−1.13, −0.27]
(1.4.2) Xuezhikang and fluvastatin and conventional therapy versus fluvastatin and conventional therapy							
Zhang 2010 [36]	4.60	0.10	5.70	0.24	64.4	−1.10	[−1.19, −1.01]
	Total		**Overall (REM, I^2 = 68%)**			**−0.96**	**[−1.33, −0.58]**
(1.5) Xuezhikang and aspirin versus inositol nicotinate and aspirin							
Wang and Xiao 2000 [32]	5.20	0.80	6.00	0.70	—	−1.05	[−1.46, −0.64]
2. TG (mmol/L)							
(2.1) Xuezhikang and conventional therapy versus conventional therapy							
Dai et al. 1999 [5]	1.84	0.68	2.30	0.87	5.50	−0.48	[−0.87, −0.05]
Huang et al. 2009 [24]	1.49	0.31	1.97	0.37	44.40	−0.48	[−0.63, −0.33]
Ma and Teng 2005 [29]	1.70	0.40	2.30	0.70	10.50	−0.60	[−0.90, −0.30]

Table 4: Continued.

Serum lipid level (comparison)	Intervention group		Control group		Weight (%)	MD	95% CI
	Mean	SD	Mean	SD			
Wang et al. 2004 [6]	1.88	0.5	2.2	0.76	7.70	−0.32	[−0.67, 0.03]
Xu 2005 [39]	2.70	0.92	2.52	1.67	0.90	0.18	[−0.87, 1.23]
Yan and Li 2007 [33]	1.54	0.10	2.02	0.59	19.10	−0.48	[−0.70, −0.26]
Zhou et al. 2003 [38]	1.20	0.66	1.80	0.61	12.10	−0.60	[−0.88, −0.32]
			Overall (FEM, I^2 = 0%)		**100%**	**−0.49**	**[−0.58, −0.39]**
(2.2) Xuezhikang and conventional therapy versus placebo and conventional therapy							
CCSPS 2005 [4]	1.58	0.78	1.75	0.88	50.80	−0.17	[−0.22, −0.12]
Yu et al. 2002 [35]	2.22	0.71	3.51	0.36	49.20	−1.29	[−1.57, −1.01]
(2.3) Xuezhikang and conventional therapy versus statin and conventional therapy							
(2.3.1) Xuezhikang and conventional therapy versus lovastatin and conventional therapy							
Li et al. 2011 [26]	3.75	1.17	3.82	1.29	1.3	−0.07	[−0.67, 0.53]
(2.3.2) Xuezhikang and conventional therapy versus simvastatin and conventional therapy							
Huang et al. 2005 [23]	1.85	0.81	1.92	0.72	5.5	−0.07	[−0.37, 0.23]
Jiang and Cai 2001 [25]	1.9	0.72	2.11	0.91	3.5	−0.21	[−0.58, 0.16]
Lou et al. 2008 [28]	3.1	0.2	3.2	0.33	35.2	−0.11	[−0.21, 0.00]
	Subgroup		**Overall (FEM, I^2 = 0%)**		**44.3**	**0.11**	**[−0.21, −0.00]**
(2.3.3) Xuezhikang and conventional therapy versus fluvastatin and conventional therapy							
Gao and Liao 2003 [21]	1.01	0.63	1.42	0.46	6.2	−0.41	[−0.69, −0.13]
(2.3.4) Xuezhikang and conventional therapy versus atorvastatin and conventional therapy							
Shang 2007 [31]	1.61	0.53	1.57	0.55	14.1	0.04	[−0.15, 0.23]
Xu 2005 [39]	2.7	0.92	2.22	0.73	1.0	0.48	[−0.21, 1.17]
Zhang 2011 [37]	1.64	0.33	1.61	0.21	33.0	0.03	[−0.09, 0.15]
	Subgroup		**Overall (FEM, I^2 = 0%)**		**48.1**	**0.04**	**[−0.06, 0.14]**
	Total		**Overall (FEM, I^2 = 45%)**		**100**	**−0.05**	**[−0.12, 0.02]**
(2.4) Xuezhikang and statin and conventional therapy versus statin and conventional therapy							
Zhang 2010 [36]	1.58	0.20	1.85	0.10	—	−0.27	[−0.35, −0.19]
(2.5) Xuezhikang and aspirin versus inositol nicotinate and aspirin							
Wang and Xiao 2000 [32]	1.70	0.90	2.30	0.90	—	−0.60	[−0.95, −0.25]
(3) LDL-C (mmol/L)							
(3.1) Xuezhikang and conventional therapy versus conventional therapy							
Dai et al. 1999 [5]	3.42	0.96	3.93	0.81	13.50	−0.51	[−0.97, −0.05]
Huang et al. 2009 [24]	2.88	0.91	3.96	0.96	14.10	−1.08	[−1.48, −0.68]
Wang et al. 2004 [6]	2.21	0.4	3.87	0.56	15.20	−1.66	[−1.92, −1.40]
Xu 2005 [39]	2.82	0.95	3.7	0.95	10.50	−0.88	[−1.63, −0.13]
Yan 2006 [34]	2.89	0.44	2.9	0.6	15.50	−0.01	[−0.23, 0.21]
Yan and Li 2007 [33]	2.97	0.10	3.88	0.20	16.20	−0.91	[−0.99, −0.83]
Zhou et al. 2003 [38]	3.22	0.6	3.68	0.71	15.00	−0.46	[−0.75, −0.17]
			Overall (REM, I^2 = 94%)		**100**	**−0.78**	**[−1.19, −0.38]**
(3.2) Xuezhikang and conventional therapy versus placebo and conventional therapy							
CCSPS 2005 [4]	2.66	0.85	3.23	0.85	50.30	−0.57	[−0.62, −0.52]
Yu et al. 2002 [35]	2.48	0.39	4.30	0.39	49.70	−1.82	[−2.01, −1.63]
(3.3) Xuezhikang and conventional therapy versus statin and conventional therapy							
(3.3.1) Xuezhikang and conventional therapy versus lovastatin and conventional therapy							
Li et al. 2011 [26]	2.45	0.72	3.25	0.84	10.6	−0.80	[−1.18, 0.42]

Table 4: Continued.

Serum lipid level (comparison)	Intervention group		Control group		Weight (%)	MD	95% CI
	Mean	SD	Mean	SD			
(3.3.2) Xuezhikang and conventional therapy versus simvastatin and conventional therapy							
Huang et al. 2005 [23]	2.68	0.55	2.52	0.49	13.9	0.16	[−0.04, 0.36]
Jiang and Cai 2001 [25]	3.1	0.41	2.90	0.90	12.2	0.20	[−0.10, 0.50]
Lou et al. 2008 [28]	2.8	0.09	2.9	0.1	15.7	−0.10	[−0.14, −0.06]
	Subtotal		**Overall (REM, I^2 = 79%)**		**41.8**	**0.06**	[**−0.17, 0.28**]
(3.3.3) Xuezhikang and conventional therapy versus fluvastatin and conventional therapy							
Gao and Liao 2003 [21]	2.13	0.58	2.08	0.61	12.2	0.05	[−0.25, 0.35]
(3.3.4) Xuezhikang and conventional therapy versus atorvastatin and conventional therapy							
Shang 2007 [31]	2.54	0.56	2.44	0.52	14.2	0.10	[−0.09, 0.29]
Xu 2005 [39]	2.82	0.95	2.93	0.52	6.9	−0.11	[−0.74, 0.52]
Zhang 2011 [37]	3.04	0.48	2.51	0.32	14.3	0.53	[0.35, 0.71]
	Subtotal		**Overall (REM, I^2 = 84%)**		**35.4**	**0.23**	[**−0.14, 0.60**]
After sensitive analysis	**Subtotal**		**Overall (FEM, I^2 = 0%)**			**0.08**	[**−0.10, 0.26**]
	Total		**Overall (REM, I^2 = 90%)**			**0.03**	[**−0.10, 0.25**]
After sensitive analysis	**Total**		**Overall (REM, I^2 = 64%)**			**0.05**	[**−0.09, 0.19**]
(3.4) Xuezhikang and statin and conventional therapy versus statin and conventional therapy							
(3.4.1) Xuezhikang and simvastatin and conventional therapy versus simvastatin and conventional therapy							
Lin et al. 2009 [27]	2.10	0.78	2.60	0.80	8.4	−0.50	[−0.95, −0.05]
(3.4.2) Xuezhikang and fluvastatin and conventional therapy versus fluvastatin and conventional therapy							
Zhang 2010 [36]	2.87	0.32	3.30	0.20	91.6	−0.43	[−0.57, −0.29]
	Total		**Overall (FEM, I^2 = 0%)**			**−0.44**	[**−0.57, −0.31**]
(3.5) Xuezhikang and aspirin versus inositol nicotinate and aspirin							
Wang and Xiao 2000 [32]	2.70	0.70	3.40	0.90	100	−0.88	[−1.27, −0.48]
(4) HDL-C (mmol/L)							
(4.1) Xuezhikang and conventional therapy versus conventional therapy							
Dai et al. 1999 [5]	1.71	0.42	1.04	0.49	14.60	0.67	[−0.43, 0.91]
Huang et al. 2009 [24]	1.12	0.3	0.82	0.2	19.50	0.3	[0.19, 0.41]
Wang et al. 2004 [6]	1.44	0.38	1.31	0.27	17.00	0.13	[−0.05, 0.31]
Xu 2005 [39]	1.67	0.51	1.68	0.75	7.10	−0.01	[−0.51, 0.49]
Yan 2006 [34]	1.04	0.10	1.04	0.20	20.60	0.00	[−0.07, 0.07]
Yan and Li 2007 [33]	1.09	0.09	0.80	0.07	21.10	0.29	[0.25, 0.33]
			Overall (REM, I^2 = 93%)		**100**	**0.24**	[**0.08, 0.40**]
(4.2) Xuezhikang and conventional therapy versus placebo and conventional therapy							
CCSPS 2005 [4]	1.24	0.31	1.19	0.31	50.80	0.05	[0.03, 0.07]
Yu et al. 2002 [35]	1.45	0.25	0.97	0.19	49.20	0.48	[0.37, 0.59]
(4.3) Xuezhikang and conventional therapy versus statin and conventional therapy							
(4.3.1) Xuezhikang and conventional therapy versus lovastatin and conventional therapy							
Li et al. 2011 [26]	1.12	0.38	1.06	0.36	11.4	0.16	[−0.33, 0.65]
(4.3.2) Xuezhikang and conventional therapy versus simvastatin and conventional therapy							
Huang et al. 2005 [23]	1.85	0.81	1.92	0.72	6.4	−0.09	[−0.47, 0.29]
Jiang and Cai 2001 [25]	1.16	0.17	1.21	0.12	19.0	−0.05	[−0.12, 0.02]
Lou et al. 2008 [28]	0.8	0.03	0.9	0.03	21.4	−0.10	[−0.11, −0.09]
			Overall (FEM, I^2 = 0%)			**−0.10**	[**−0.11, −0.09**]
(4.3.3) Xuezhikang and conventional therapy versus fluvastatin and conventional therapy							
Gao and Liao 2003 [21]	1.14	0.27	1.30	0.45	11	−0.16	[−0.35, 0.03]

Table 4: Continued.

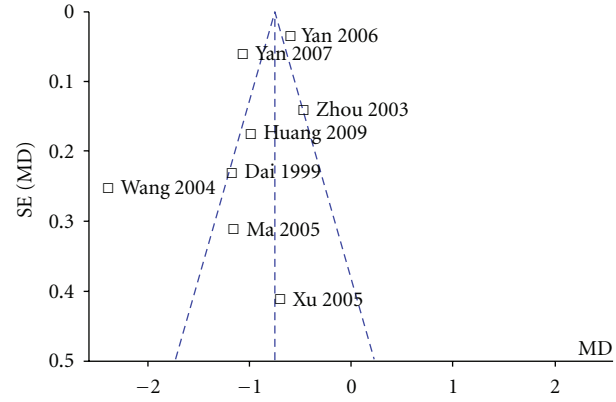
Serum lipid level (comparison)	Intervention group		Control group		Weight (%)	MD	95% CI
	Mean	SD	Mean	SD			
(4.3.4) Xuezhikang and conventional therapy versus atorvastatin and conventional therapy							
Shang 2007 [31]	1.45	0.41	1.44	0.33	14.9	0.01	[−0.12, 0.14]
Xu 2005 [39]	1.67	0.51	1.53	0.48	3.8	0.14	[−0.27, 0.55]
Zhang 2011 [37]	1.09	0.48	1.62	0.27	12.1	−0.53	[−0.70, −0.36]
	Subtotal		**Overall (REM, I^2 = 93%)**		**30.9**	**−0.15**	[**−0.57, 0.28**]
After sensitive analysis	**Subtotal**		**Overall (FEM, I^2 = 0%)**			**0.02**	[**−0.10, 0.14**]
	Total		**Overall (REM, I^2 = 79%)**			**−0.10**	[**−0.19, −0.01**]
After sensitive analysis	**Total**		**Overall (FEM, I^2 = 35%)**			**−0.10**	[**−0.11, −0.08**]
(4.4) Xuezhikang and fluvastatin and conventional therapy versus fluvastatin and conventional therapy							
Zhang 2010 [36]	0.97	0.28	0.82	0.06	100	0.15	[0.05, 0.25]
(4.5) Xuezhikang and aspirin versus inositol nicotinate and aspirin							
Wang and Xiao 2000 [32]	0.95	0.22	0.91	0.25	100	0.17	[−0.21, 0.55]

Note: FEM: fixed effects model; REM: random effects model.

fluvastatin and conventional therapy, Xuezhikang showed a beneficial of HDL-C level (MD 0.15 mmol/L; 95% CI 0.05 to 0.25; 1 trial, $n = 60$) [36]. (5) Compared with inositol nicotinate on the basis of aspirin, Xuezhikang showed no significant difference on HDL-C level (MD 0.17 mmol/L; 95% CI −0.21 to 0.55; 1 trial, $n = 105$) [32].

3.4. Publication Bias. A funnel plot analysis of the 8 trials in comparison of Xuezhikang and conventional therapy versus conventional therapy on serum TC level was conducted and shown in Figure 3.

3.5. Adverse Events. There were 17 trials that reported adverse events (Ads); see Table 5. 4 of the 17 trials [5, 24, 33, 37] indicated no Ads in the duration of treatment, and 2 trials [23, 34] only introduced that there was no difference of the two groups. The most commonly reported Ads in the 10 trials were intestinal disturbance (abdominal distension, constipation, and diarrhea), dizziness, high serum alanine aminotransferase (ALT), high serum creatine kinase (CK), high serum creatinine, high blood urea nitrogen (BUN), and skin itch. All of Ads were not significantly different between the Xuezhikang group and control group. One trial [4] reported that there was significant difference between the two groups on sexual dysfunction ($P = 0.0253$) in the paper, but after we import the data into Revman 5.1, there was no difference (RR 0.09, 95% CI [0.01, 1.64]) between the two groups. CCSPS [4] reported the clinical total Ads number (intestinal disturbance, allergy and et al.) in each group (treatment group 43; control group 39), and there was no significant difference between the two groups, this trial also reported death in other reason, which was introduced in all-cause mortality, and the difference between the two groups was not significant.



Note: The funnel plot presented 8 trials in the comparison of Xuezhikang and conventional therapy versus conventional therapy on the effect of TC

Figure 3: The funnel plot for assessing reporting bias.

4. Discussion

This systematic review included 22 randomized trials and a total of 6520 participants. Xuezhikang showed significant benefit on the incidence of all-cause deaths, CHD deaths, myocardial infarction, and revascularization as compared with placebo or no intervention based on conventional treatment for CHD. It remarkably lowered TC, TG, and LDL-C as compared with the placebo or inositol nicotinate group, which was similar to statins group. Xuezhikang also significantly raised HDL-C compared to placebo or no intervention, which was similar to inositol nicotinate and slightly inferior to statins. The incidence of adverse events did not differ between the Xuezhikang and control group. The results showed the comprehensive lipid-regulating effect of Xuezhikang and indicated that it was safe and effective in reducing cardiovascular events in CHD patients complicated by dyslipidemia.

Table 5: Adverse Events.

Ads/ID	Comparison	Treatment group (n/N)	Control group (n/N)	RR	95% CI
Loss of followup					
Guan 2010 [22]	Xuezhikang versus simvastatin	16 (72)	15 (64)	0.95	[0.51, 1.76]
CCSPS 2005 [4]	Xuezhikang and conventional therapy versus placebo and conventional therapy	37 (2441)	28 (2429)	1.31	[0.81, 2.14]
Ma and Teng 2005 [29]	Xuezhikang and conventional therapy versus conventional therapy	1 (29)	No report		
Intestinal disturbance					
Guan 2010 [22]	Xuezhikang versus simvastatin	5 (72)	2 (64)	2.22	[0.45, 11.06]
Ma and Teng 2005 [29]	Xuezhikang and conventional therapy versus conventional therapy	2 (29)	No report		
Wang et al. 2004 [6]	Xuezhikang and conventional therapy versus conventional therapy	2 (26)	No report		
Jiang and Cai 2001 [25]	Xuezhikang and conventional therapy versus simvastatin and conventional therapy	0 (30)	1 (45)	0.49	[0.02, 11.75]
Shang 2007 [31]	Xuezhikang and conventional therapy versus atorvastatin and conventional therapy	No report	1 (65)		
Wang and Xiao 2000 [32]	Xuezhikang and aspirin versus inositol nicotinate and aspirin	5 (65)	2 (57)	2.19	[0.44, 10.87]
Headache					
Jiang and Cai 2001 [25]	Xuezhikang and conventional therapy versus simvastatin and conventional therapy	1 (30)	0 (45)	4.45	[0.19, 105.77]
Dizziness					
Guan 2010 [22]	Xuezhikang and conventional therapy versus simvastatin and conventional therapy	0 (72)	10 (64)	0.04	[0.00, 0.71]
Jiang and Cai 2001 [25]	Xuezhikang and conventional therapy versus simvastatin and conventional therapy	1 (30)	1 (45)	1.5	[0.10, 23.07]
		Overall (REM, I^2 = 72%)		**0.26**	[**0.01**, **10.49**]
Skin itech					
Guan 2010 [22]	Xuezhikang versus simvastatin	0 (72)	3 (64)	0.13	[0.01, 2.42]
Wang and Xiao 2000 [32]	Xuezhikang and aspirin versus inositol nicotinate and aspirin	0 (65)	3 (57)	0.13	[0.01, 2.38]
Sexual dysfunction					
CCSPS 2005 [4]	Xuezhikang and conventional therapy versus placebo and conventional therapy	0 (1996)	5 (1990)	0.09	[0.01, 1.64]
High serum ALT					
CCSPS 2005 [4]	Xuezhikang and conventional therapy versus placebo and conventional therapy	15 (2441)	22 (2429)	0.68	[0.35, 1.30]
Lou et al. 2008 [28]	Xuezhikang and conventional therapy versus simvastatin and conventional therapy	No report	1 (41)		
High serum CK					
CCSPS 2005 [4]	Xuezhikang and conventional therapy versus placebo and conventional therapy	0 (2441)	3 (2429)	0.14	[0.01, 2.75]
High serum CR					
CCSPS 2005 [4]	Xuezhikang and conventional therapy versus placebo and conventional therapy	104 (2441)	89 (2429)	1.16	[0.88, 1.53]
High BUN					
CCSPS 2005 [4]	Xuezhikang and conventional therapy versus placebo and conventional therapy	124 (2441)	131 (2429)	0.94	[0.74, 1.20]

Due to the potential side effects of statins, natural products have raised more and more attention worldwide. The health-enhancing qualities of red yeast rice have been introduced and used in China for over two thousand years. A meta-analysis of randomized controlled trials on Chinese red yeast rice for primary hyperlipidemia showed a significant reduction in serum levels of TC, TG, LDL-C, and an increase in HDL-C levels compared with placebo. The lipid modification effects appeared to be similar to pravastatin, simvastatin, atorvastatin, lovastatin, or fluvastatin [40]. A latest systematic review also indicated the beneficial effects of Xuezhikang in the treatment of hyperlipidemia [2]. The lipid-regulating effects of Xuezhikang in these reviews were similar to our findings. In addition, some cardioprotective effects of Xuezhikang have been investigated in recent years [41–43]. We further demonstrated the benefit of Xuezhikang in reducing cardiovascular events in CHD patients complicated by dyslipidemia, or even CHD with normal blood lipid level but failed to reach the lipid-lowering goal. However, current evidence comparing the effectiveness and Ads between Xuezhikang and statins in CHD patients was not enough to draw the conclusion.

It is worth mentioning China Coronary Secondary Prevention Study (CCSPS) [4], which was the largest RCT included in this review. This multicenter, randomized, and placebo-controlled study aimed to demonstrate the long-term therapeutic effect and safety of Xuezhikang in the second prevention of CHD. 4870 cases in 66 medical centers were enrolled and followed up for an average of 4.5 years. The results showed that Xuezhikang significantly decreased the recurrence of coronary events and the occurrence of new cardiovascular events and deaths, improved lipoprotein regulation, and was safe and well tolerated [4]. The study was the first large-scale clinical trial in eastern population who suffered from mild or moderate degree of hyperlipidemia and previous MI. The CCSPS study is quite comparable with (Cholesterol and Recurrent Events) CAREs study [44] in terms of the target population, sample size, baseline lipid and follow-up time. However, Xuezhikang in CCSPS lowered less lipid level as compared with pravastatin in CARE but seemed to gain more benefit in reducing the cardiovascular events. Since the effect of Xuezhikang is partially attributed to the presence of statins, it has been hypothesized that relatively high concentrations of unsaturated fatty acids and other natural compounds found in Xuezhikang may work in concert with the statins to provide additional health benefits [45]. Therefore, a large-scale RCT comparing directly the effectiveness and safety of long-term use of Xuezhikang and statins is warranted.

Before recommending the conclusion of this review to clinical practicers, we have to consider the following weaknesses in this review. (1) Firstly, the "randomization" was not clear in most of the trials for insufficient reporting of generation methods of the allocation sequence and allocation concealment. Most trials stated only that patients were randomly assigned. (2) Secondly, most of trials did not introduce double blind in this review, and only one trial introduced blinding of outcome assessment, therefore, in non-placebo-controlled and non-double-blind trials, placebo effects may add to the complexity of interpreting the conclusion. (3) Most of the trials did not introduce the study plan, attrition bias and selective reporting bias might exist in this conclusion. (4) Thirdly, funnel plot indicated that publication bias would exist in this review. The reasons are as follows. We only selected trials published in Chinese and English, and trials published in other language or originated from other countries might be omitted; we only identified unpublished studies from conference paper or academic thesis, and negative trials might not be reported and induced publication bias.

Therefore, further rigorously designed trials are still needed before Xuezhikang could be recommended to patients with CHD complicated by dyslipidemia, especially as an alternative to statins. Whether or not long-term medication of Xuezhikang could provide similar benefit to statins for CHD secondary prevention with less adverse events? Is it related to the target lipid value? All of these need to be answered in the future investigation.

5. Conclusion

Xuezhikang showed a comprehensive lipid-regulating effect and was safe and effective in reducing CHD mortality, the incidence of myocardial infarction and revascularization in CHD patients complicated by dyslipidemia. However, the small sample size and potential bias of most trials influence the convincingness of this conclusion. Before recommending Xuezhikang as an alternative to statins in CHD patients, more rigorous trials with high quality are needed to give high level of evidence, especially for comparing the effectiveness and safety between Xuezhikang and statins.

Authors' Contributions

J. Liu and H. Xu conceived and designed the review and performed interpretation of the review; Q. Shang, Z. Liu developed the search strategy and did the literature search, study selection, data extraction, data analyses and interpretation; K. Chen provided methodological perspectives and revised review. All of authors contributed to writing the review.

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Research Article

In Silico Syndrome Prediction for Coronary Artery Disease in Traditional Chinese Medicine

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Coronary artery disease (CAD) is the leading causes of deaths in the world. The differentiation of syndrome (ZHENG) is the criterion of diagnosis and therapeutic in TCM. Therefore, syndrome prediction *in silico* can be improving the performance of treatment. In this paper, we present a Bayesian network framework to construct a high-confidence syndrome predictor based on the optimum subset, that is, collected by Support Vector Machine (SVM) feature selection. Syndrome of CAD can be divided into asthenia and sthenia syndromes. According to the hierarchical characteristics of syndrome, we firstly label every case three types of syndrome (asthenia, sthenia, or both) to solve several syndromes with some patients. On basis of the three syndromes' classes, we design SVM feature selection to achieve the optimum symptom subset and compare this subset with Markov blanket feature select using ROC. Using this subset, the six predictors of CAD's syndrome are constructed by the Bayesian network technique. We also design Naïve Bayes, C4.5 Logistic, Radial basis function (RBF) network compared with Bayesian network. In a conclusion, the Bayesian network method based on the optimum symptoms shows a practical method to predict six syndromes of CAD in TCM.

1. Introduction

Coronary artery disease (CAD), which is a narrowing of the small blood vessels that supply the heart with blood, oxygen, and nutrients, is the most common cardiovascular disease (CVD). It is the leading cause of death in the world. According to the newest World Health Organization reports, an estimated 17.3 million people died from CVD in 2008, representing 30% of all global deaths [1]. CAD is responsible for a large proportion of CVD, accounting for an estimated 7.3 million (12.57%) [2].

CAD is caused by many factors such as genetics, the environment, harmful use of alcohol, unhealthy diet, tobacco, and others. In western medicine, CAD is treated by surgical operation, pharmaceutical drugs, physical activity, and other interventional therapies. These achievements typically lead to good outcomes by decreasing rates of death related to CAD. However, these methods generally focus on the structure and function of the heart, but ignore differences in systematic functions, curative reaction, and the individual. Since western medicine faces problems such as high cost and significant side effects, Traditional Chinese Medicine (TCM) can be a complementary alternative to overcome these defects. In TCM, CAD belongs to the scope of chest heartache and cardiodynia [3]. TCM, which has a history of thousands of years, makes significant contributions to people worldwide, especially in Asia. The TCM approach is fundamentally different from that of western medicine [4]. In TCM, the human body is based on the holistic understanding of the universe and is recognized by system discrimination in a cybernetic way [5]. Zheng (syndrome) is the key pathological principle of TCM. All diagnostic and therapeutic methods in TCM are based on the differentiations of syndrome (ZHENG), and this concept has been used for thousands of years in China [6, 7]. A syndrome is constituted by a set of symptoms, including subjective feeling and objective signs. It is the integrative response of the body state in the body's internal and external environment. In the process of disease development, syndromes changes dynamically with rise or fall of corresponding occurrence of evidence. A disease

is the nature of a comprehensive reflection of one or more syndromes in the different stages of pathology. In the process of development of CAD, syndromeprediction *in silico* is a potentially logical choice for prevention and treatment.

In order to achieve an effective and objective standard of syndrome prediction, many researchers have used a data mining approach to construct the classifier for the TCM dataset [8, 9]. Syndrome prediction is regarded as supervised classification analysis: the class label is the diagnosis, and features are the symptoms of the patient. Because clinical diagnosis datacontain irrelevant features and noise, the identification of the related symptoms is an important problem in syndrome prediction aside from classifying the syndrome.

In recent years, there has been remarkable progress in thesyndrome predictionof TCM. Data have focused on two aspects: feature selection (symptom selection) and syndrome prediction (syndrome classification). Jie et al. investigated syndrome factors of CAD by using the support vector machine (SVM) method on the basis of 15 typical medical records from prominent TCM doctors. Eight syndromes were drawn, including blood stasis, turbid phlegm, Qi deficiency, Yang insufficiency, Yin deficiency, inner heat, blood deficiency, and Qi stagnation [10]. Li et al. compared the cold and hot syndrome networks through literature searches and found that hormones are predominant in the Cold ZHENG network, immune factors are predominant in the Hot ZHENG network, and these two networks are connected by neurotransmitters [6]. Zhou et al. developed a clinical data warehouse system including medical knowledge discovery and TCM clinical decision support to use variousclassification methods, namely, machine SVM decision tree and Bayesian network, to look at syndrome differentiation [11]. Chen et al. proposed a novel pattern discovery algorithm based on revised mutual information to discover syndromesfor chronic renal failure [12]. In regards to CAD, Liu et al. designed standardization scale on inquiry diagnosis and constructed this diagnostic model by using the method of multilabel learning [3]. In addition, many techniques of data mining are applied to syndromes in TCM [9, 13–26].

Though many achievements have been made in syndrome prediction, there are still some problems left, which deserve discussion [8]. Our research is focused on discovering symptoms of TCM, and lab-measured indexes are rarely included. The characteristics of CAD syndrome are usually not considered when the classifier is built. First, we used symptoms including TCM and western symptoms for identifying syndromes of CAD. Second, we constructed six predictors to classify six syndromes of CAD. Third, the related symptoms were selected based on characteristics of syndromes of CAD and were placed into three classes: sthenia, asthenia, or both.

In this paper, 987 CAD cases were used for selecting related symptoms and building the predicting model of CAD syndrome. Based on symptoms, we propose a syndrome prediction method which integrates SVM feature selection and Bayesian network classifier to improve the predictive performance of the classifier.

The rest of this paper is organized as follows. Section 2 describes materials and methods including data description, preprocessing and symptom selection method, syndrome prediction method. Experimental results and discussions are shown in Section 3. Section 4 draws conclusions from this paper.

2. Material and Methods

2.1. Material. In this paper, the cases were collected from two provinces including 5 clinical centers from June 2005 to October 2008, where patients who suffered from CAD were surveyed. Each patient was diagnosed by western doctors by means of coronary artery angiography.

Inclusion criteria are as follows [24].

(1) Each case must have been diagnosed with CAD defined by the American College of Cardiology (ACC) together with American Heart Association (AHA) in 2002.

(2) Each case was verified by coronary artery angiography as having at least one branch of the coronary artery main branch with stenosis larger than 70% or coronary artery left diameter stenosis greater than 50%.

(3) Each case must have included an attached informed consent signed by each patient.

(4) Each patient was greater than 35 years of age.

In western medicine, the diagnosis of patients was in accordance with the "Guidelines for the diagnosis and management of chronic angina pectoris, unstable angina pectoris, and non-ST elevation myocardialinfarction" released by the ACC/AHA, and "Recommendation about Diagnosis of Diagnosing Unstable Angina Pectoris" released by Chinese Society of Cardiology in 2000. In TCM, syndrome diagnosis was in accordance with the foundation theory of TCM. For example, the diagnosis of blood stasis was judged by "Standard of Blood Stasis Diagnosis" (1986.11, Guangzhou); the diagnosis of deficiency was treated by "Standard of TCM Syndrome Differentiation of Deficiency" (1986.5); the diagnosis of turbid phlegmwas decided by "Classification Code of TCM Diseases"; the others depended on the teaching materials ("*Diagnosis of TCM*").

There were two exclusion criteria [24]:

(1) any patient with acute ST-segment elevation myocardial infarction, and

(2) any patient who also suffers from concomitant serious diseases such as liver orkidney disease.

Each symptom has four levels: none, light, middle, and severe. Each case was diagnosed as a syndrome by experienced TCM experts. Each symptom was considered a feature; the diagnosed syndrome was taken as a response.

In total, we evaluated 1,008 cases of patients, including the diagnosis results of western medicine and TCM, and over 100 symptoms of both western medicine and TCM. Data were compiled according to the characteristics of

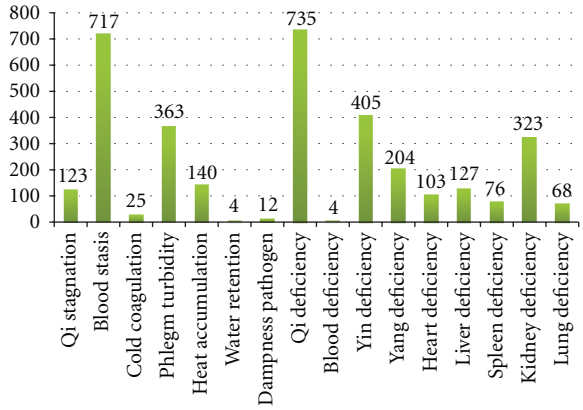

Figure 1: Histogram of syndromes of TCM.

syndromes of CAD, sthenia and asthenia syndromes follow CAD. In regards to the foundation and practice of TCM, sthenia syndromes include Qi stagnation, blood stasis, cold coagulation, phlegm turbidity, heat accumulation, water retention, and dampness pathogen; asthenia syndromes include Qi deficiency, blood deficiency, Yin deficiency, Yang deficiency, heart deficiency, liver deficiency, spleen deficiency, kidney deficiency, and lung deficiency.

2.2. Data Preprocessing. In every case, there were over 70 diagnostic symptoms in TCM and above 30 lab-measured symptoms in the western medicine information. For TCM diagnosis, there was Qi stagnation, blood stasis, cold coagulation, phlegm turbidity, heat accumulation, Qi deficiency, Yin deficiency, and so on. A histogram of syndromes of TCM diagnosis results is shown in Figure 1.

In the process of medical surveys, there inevitably exists missing data. Cases were discarded if the missing data frequency rate of it symptom was more than 70%. Some symptoms which were not treated by data mining technique were removed. If its syndrome was not in the top six syndromes, the case was discarded. Overall, there were 113 features including 78 TCM symptoms and 35 lab-measured indexes. Details of the symptoms are shown in Table 1.

2.3. Method of Syndrome Prediction of CAD. In general, syndrome prediction of CAD included the symptom selection phase and syndrome prediction phase. Symptom selection was regarded as the problem of feature selection, and syndrome prediction was regarded as supervised pattern classification in data mining fields. In the feature selection phase, mingling symptoms including TCM symptoms and western symptoms were selected to be used as feature of the syndrome prediction model. In the syndrome prediction phase, every case was classified as blood stasis, phlegm turbidity, Qi deficiency, Yin deficiency, Yang deficiency, and kidney deficiency based on the syndrome prediction model.

2.3.1. Symptom Selection. Symptoms are essential to diagnose CAD for everyone from TCM doctors to western medicine doctors. Therefore, a strong predicting model of syndrome is based on key symptoms. In this phase, we investigated which symptoms influence the predicted syndromes most. We propose two feature selection methods to discover critical symptoms. In this paper, we design SVM and Markov blanket feature selection methods to identify the optimal symptom subset.

SVMs have been an acknowledged tool with high accuracy and efficiency for data classification. The basic idea is to map data into a high dimensional space and find a separating hyperplane with the maximal margin [27]. Given the training vectors $x_k \in R^n$, $k = 1, 2, \ldots, m$ in two classes, and a vector of labels $y \in R^m$ such that $y_k \in \{-1, 1\}$, SVM solves a quadratic optimization problem [28, 29]:

$$\begin{aligned} \min_{\omega,b,\xi} \quad & \frac{1}{2}\omega^T\omega + C\sum_{k=1}^{m}\xi_k \\ \text{subject to} \quad & y_k\left(\omega^T\phi(x_k) + b\right) \geq 1 - \xi_k \\ & \xi_k \geq 0, \quad k = 1, \ldots, m, \end{aligned} \tag{1}$$

where training data are mapped to a higher dimensional space by the function ϕ, and C is a penalty parameter on the training error. For any training instance x, the decision function (predictor) is

$$f(x) = \operatorname{sgn}\left(\omega^T\phi(x) + b\right). \tag{2}$$

Generally, the nonlinear mapping function $\phi(\cdot)$ is represented by a kernel function $k(x, x') = \phi(x)^T\phi(x')$. Several kernels are commonly used such as Gaussian kernel, polynomial kernel, spline kernel, and RBF kernel.

Together with penalty function or optimization objective, SVM can be exploited to select appropriate features or optimal feature groups. As for the feature selection problem, there are two alternative situations [30]: (1) given a fixed $p \ll n$ (number of features much less than dimension of feature space), find the p features that gives the smallest expected generalization error, or (2) given a maximum allowable generalization error, find the smallest p. The former situation will be discussed below, while the latter one can always be formulated as the dual of the former.

One may distinguish between the two types of methods to solve the problem of filter and wrapper methods [31]. The filter method actually performs a procedure of subtractive iterations which removes the least relevant feature iteratively [32]. The wrapper method, on the other hand, is a searching process which starts from a null feature set and chooses the best feature into the feature set in each iteration [33].

Several existing strategies have been combined with SVM for feature selection. Given training vectors x_k, $k = 1, 2, \ldots, m$, if the positive and negative instances are n_+ and n_-, respectively, then the F-score of the ith feature is defined as

Table 1: Symptom list.

Symptoms of comprehensive subset					
Symptoms of TCM subset				Symptoms of western medicine	
(1) Chest pain	(21) Sighing	(41) Frothy sputum	(61) Red eye	(79) ST normal	(97) Ef
(2) Oppression in chest	(22) Depression	(42) Pharyngeal foreign body	(62) Deep-colored eye weeks	(80) ST lower than 0.1	(98) A/e
(3) Shortness of breath	(23) Inappetence	(43) Thirst without large fluid intake	(63) Eyelids swelling	(81) ST greater than 0.1	(99) Wall motion
(4) Palpitation	(24) Abdominal distension	(44) Tastelessness	(64) Dark red lip and gingivitis	(82) ST limb breast high	(100) Valve regurgitation
(5) Cough	(25) Ruffian of epigastrium	(45) Bitter taste in mouth	(65) Light-colored lip and methyl	(83) ECG	(101) Regurgitant degree
(6) Chilly sensation and the cold limbs	(26) Belching	(46) Sweet taste in mouth	(66) Deep-colored palate mucosa	(84) Q wave	(102) Leukocyte
(7) Tiredness and fatigue	(27) Nausea and vomiting	(47) Salty taste in mouth	(67) Less abdominal pressure	(85) Frequent extrasystole	(103) Neutral %
(8) Spontaneous sweating	(28) Loose stool	(48) Sticky and greasy sensation in mouth	(68) Lower extremity edema	(86) High left ventricular voltage	(104) Lymph %
(9) Night sweating	(29) Constipation	(49) Morning diarrhea	(69) Faint low voice	(87) T wave	(105) Erythrocyte
(10) Dysphoria with feverish sensation in chest, palms, and soles	(30) Soreness and weakness of waist and knees	(50) Powerless in defecation	(70) Atrophy	(88) Diameter of main root	(106) Hemoglobin
(11) Dry eyes	(31) Frequent urination at night	(51) Deep-colored urine	(71) Tongue quality	(89) Main pulmonary	(107) Platelet
(12) Dry mouth	(32) Limb numbness	(52) Clear urine in large amounts	(72) Patchy petechia and ecchymosis	(90) Left atrial dimension	(108) Fasting plasma glucose
(13) Dizziness	(33) Heel pain	(53) Residual urine	(73) Tongue body	(91) Interventricular septum thickness	(109) TG
(14) Amnesia	(34) Hemiplegic limbs	(54) Coldness in abdomen and waist	(74) Quality of tongue coating	(92) Pulsatile range	(110) TG
(15) Vertigo	(35) Subcutaneous ecchymosis	(55) Heavy limbs	(75) Color of tongue coating	(93) End-diastolic diameter	(111) HDL
(16) Tinnitus	(36) Rough skin	(56) Pale complexion	(76) Body fluid on tongue coating	(94) Systolic diameter	(112) LDL
(17) Facial flush	(37) Obesity	(57) Suddenly white complexion	(77) Vein color	(95) Right ventricular diameter	(113) Fibrinogen
(18) Insomnia	(38) White phlegm	(58) Darkish complexion	(78) Vein type	(96) Outflow tract	
(19) Fussy temper and irascibility	(39) Yellow phlegm	(59) Sallow complexion			
(20) Distending pain in the hypochondria	(40) Blood in the sputum	(60) Flushing			

$$F(i) = \frac{\left(\overline{x}_i^{(+)} - \overline{x}_i\right)^2 + \left(\overline{x}_i^{(-)} - \overline{x}_i\right)^2}{(1/(n_+ - 1)) \sum_{k=1}^{n_+} \left(x_{k,i}^{(+)} - \overline{x}_i^{(+)}\right)^2 + (1/(n_- - 1)) \sum_{k=1}^{n_-} \left(x_{k,j}^{(-)} - \overline{x}_i^{(-)}\right)^2}, \tag{3}$$

where $\overline{x}_i$, $\overline{x}_i^{(+)}$, $\overline{x}_i^{(-)}$ are the average of the ith feature of the whole, positive, and negative data sets, respectively; $x_{k,i}^{(+)}$ is the ith feature of the kth positive instance, and $x_{k,i}^{(-)}$ is the ith feature of the kth negative instance.

Table 2: Ranked symptoms by means of SVM feature selection.

Dataset	Rank list of NO. symptom
TCM	75, 8, 73, 52, 36, 50, 22, 54, 40, 31, 13, 26, 30, 42, 23, 74, 71, 6, 49, 27, 7, 25, 78, 11, 20, 35, 4, 60, 34, 65, 10, 72, 33, 32, 59, 63, 9, 3, 67, 61, 57, 17, 18, 66, 64, 43, 5, 45, 76, 19, 38, 77, 16, 24, 2,28, 14, 44, 62, 56, 70, 55, 1, 68, 53, 29, 21, 12, 37, 47, 39, 58, 15, 69, 48, 46, 51, 41
WM	17, 27, 26, 30, 13, 20, 18, 15, 11, 29, 16, 14, 12, 10, 7, 35, 33, 24, 22, 31, 5, 28, 34, 25, 19, 23, 4, 9, 32, 8, 3, 6, 1, 2, 21
Comprehensive	95, 71, 102, 108, 92, 78, 107, 101, 73, 7, 97, 40, 27, 8, 82, 22, 85, 75, 31, 23, 74, 109, 103, 42, 30, 5, 10, 35, 106, 50, 6, 52, 65, 11, 57, 20, 89, 18, 13, 81, 113, 111, 79, 77, 36, 54, 9, 104, 67, 60, 44, 25, 72, 64, 83, 16, 3, 59, 24, 32, 21, 49, 26, 55, 4, 63, 33, 43, 88, 99, 84, 66, 28, 68, 17, 45, 80, 34, 38, 70, 14, 94, 76, 37, 51, 62, 110, 100, 86, 112, 61, 48, 87, 1, 2, 90, 39, 91, 53, 41, 96, 56, 19, 47, 69, 46, 15, 58, 12, 93, 105, 29, 98

We selected features with high F-scores and then applied SVM for training/prediction. The procedure was as follows [34].

(1) Calculate F-score of every feature.

(2) Pick possible thresholds as cutoffs for F-scores.

(3) For each threshold, complete the following:

  (a) drop features with F-scores below this threshold,

  (b) randomly split the training data into X_{train} and X_{valid},

  (c) let X_{train} be the new training data. Use the SVM procedure to obtain a predictor; use the predictor to predict X_{valid},

  (d) repeat the steps above five times and then calculate the average validation error.

(4) Choose the threshold with the lowest average validation error.

(5) Drop features with F-scores below the selected threshold. Then apply the SVM procedure.

Finally, the features with efficient prediction power were selected.

Compared with SVM feature selection, we also designed Markov blanket feature selection which was firstly proposed by Koller and Sahami in 1996 [35]. A Markov blanket of a target attribute T renders it statistically independent from all the remaining attributes. That is, given the values of the attributes in the Markov blanket, the probability distribution of T is completely determined, and knowledge of any other variable(s) becomes superfluous [36]. Based on their work, several algorithms were proposed to find the optimal feature subset. Cui et al. [37] proposed an approximate feature selection algorithm based on the Markov blanket. They used Chi-Square tests and P values to scale the independence between

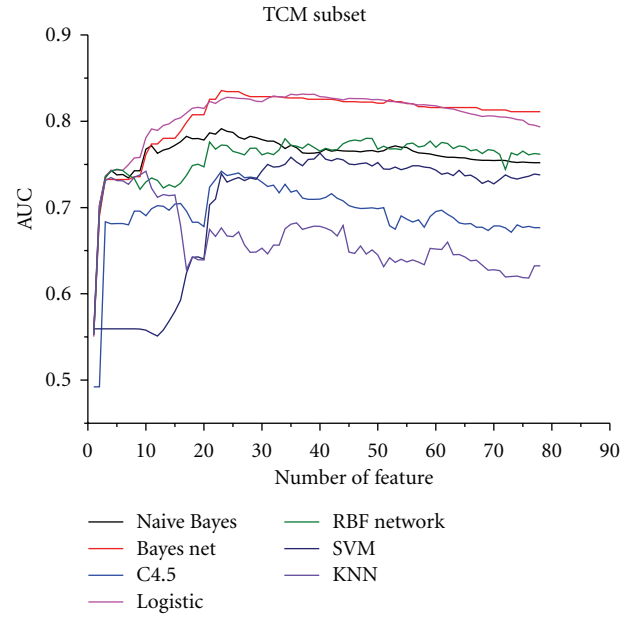

Figure 2: Relationship between AUC and symptom number in the TCM subset.

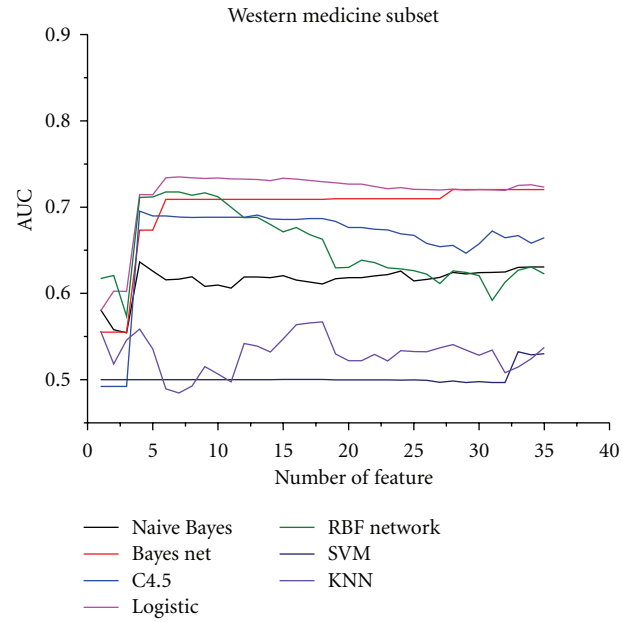

Figure 3: Relationship between AUC and symptom number in the western medicine subset.

features. For computational simplicity, they constrained the size of the Markov blanket to 1. Fi was declared a Markov blanket of fj when fi had a high correlation with class C and fj was more independent with class C given fi. Zhu et al. [38] proposedan information gain based on the Markov blanket feature selection algorithm: MBEGA. They defined fi to be a Markov blanket of fj on the condition that fi gives more information to class than fj, and fj gives more information to fi than to class C. Compared with MBEGA, MBFS is more

Table 3: Results of syndrome prediction based on Bayesian network.

Syndrome	Index			
	Weighted precision	Weighted recall	Weighted *F*-Measure	Weighted AUC
Blood stasis	0.763	0.761	0.762	0.811
Phlegm turbidity	0.740	0.746	0.742	0.791
Qi deficiency	0.750	0.747	0.748	0.766
Yin deficiency	0.656	0.663	0.640	0.589
Yang deficiency	0.926	0.926	0.926	0.946
Kidney deficiency	0.735	0.728	0.731	0.766

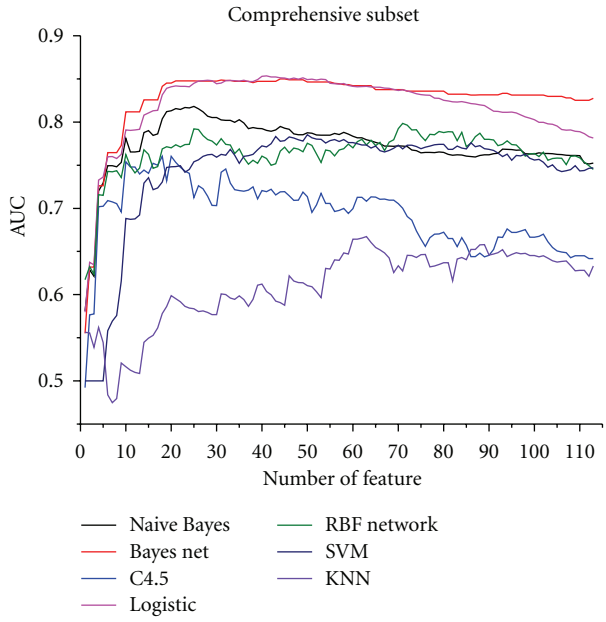

Figure 4: Relationship between AUC and symptom number in the comprehensive subset.

in line with the idea of Markov blanket and has a more comprehensive and profound base of information theory.

2.3.2. Syndrome Prediction. Syndrome prediction is important for doctors. In this study we presented a Bayesian network framework to construct a high-confidence syndrome predictor by integrating a comprehensive list of mingling symptoms. In fact, it is a classification that is a basic task in data analysis and pattern recognition that requires construction of a classifier, that is, a function that assigns a class label to instances described by a set of features [39].

Bayesian network, which is one of the most effective classification method for graphically representing and processing feature interdependencies, represents a joint probability distribution over a dataset [39, 40]. Bayesian network is directed acyclic graphs (DAG) that allow for efficient and effective representation of joint probability distributions. In this paper, we constructed a Bayesian network structure to simulate the data modelbased on 897 cases. The nodes in

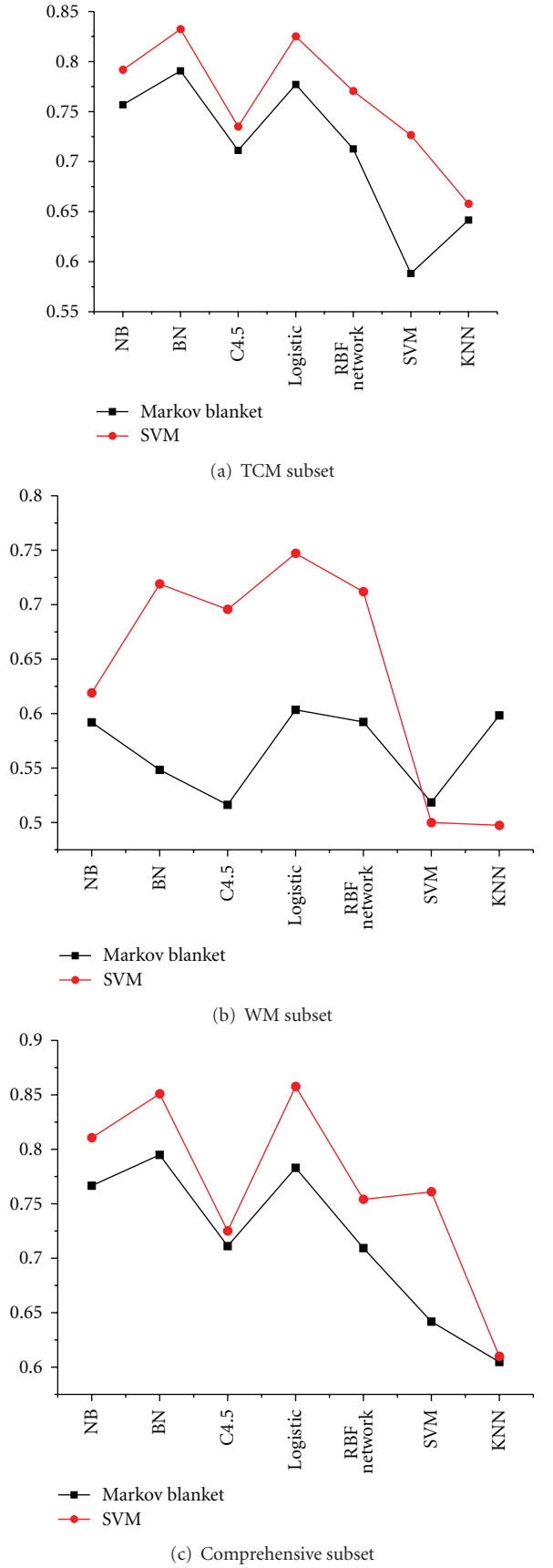

Figure 5: Comparative results of weighted AUC by using SVM and Markov blanket methods.

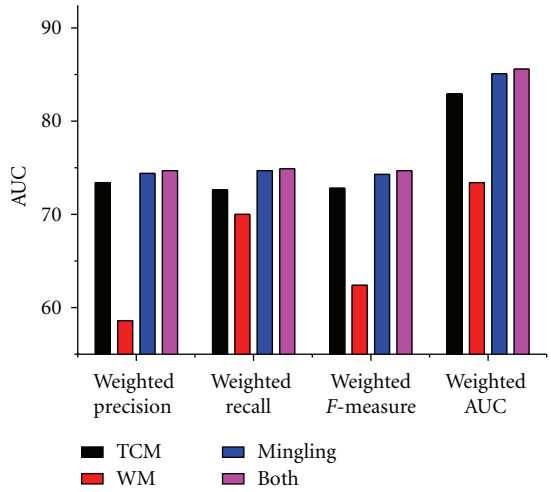

Figure 6: Comparative results of syndrome prediction using the Bayesian network classifier.

the network were predetermined, one for each symptom or syndrome. The network structures are learned by searching through the space of possible sets of edges, estimating the conditional probability stables for each set, and computing the log-likelihood of the resulting network based on the data as a measure of the network's quality [41].

The differences in Bayesian network was focused on the way in which they search through the space of nodes. In the process of searching, there are two steps: model evaluating and model optimization. There are many model evaluating methods such as Akaike Information Criterion (AIC), Minimum Description Length (MDL), and Cross-Validation Likelihood (CVL). In this paper, we adopted a simple estimator [42], as a fellow formula:

$$P(x_i = k \mid pa(x_i) = j) = \frac{N_{ijk} + N'_{ijk}}{N_{ij} + N'_{ij}}, \tag{4}$$

where N_{ijk} is 0.5 by default and sets the other.

For model optimization, we adopted K2 that one simple and very fast learning algorithm starts a given ordering of the features. Then it processes each node in turn and greedily considers adding edges from previously processed nodes to the current one. In each step it adds the edge that maximizes the network's score. When there is no further improvement, attention turns to the next node [41]. K2 uses the posteriori probability for estimating the structure of network:

$$P(D \mid B_s) = \prod_{i=1}^{n} P(x_i, pa(x_i)). \tag{5}$$

3. Results and Discussion

3.1. Symptoms Selection Based on Mingling Syndromes. Symptoms are selected to reduce the dimension of symptoms in predicting syndromes of CAD and to find the most related symptom subsets to improve the precision of syndrome prediction. In this experiment, datasets were grouped into three subsets: the TCM subset, the western subset, and the comprehensive subset. Every case was labeled with asthenia, sthenia or mingling syndrome. We collected 78 TCM symptoms in the TCM subset, 35 lab-measured indexes in the western medicine subset, and 113 mingling symptoms in the comprehensive subset. We quantitatively assessed the relatedness of each feature for syndrome prediction by SVM feature selection on the basis of tenfold cross-validation tests. By means of SVM feature selection, symptom ranking results of three subset sare shown in Table 2.

The performance of symptom selection was estimated by the classifier. In this experiment, we adopted seven classifiers: Naïve Bayes, Bayesian network, C4.5, Logistic, RBF Network, SMOSVM, and KNN. These seven classifiers are implemented in Weka [43, 44]. And parameters of classifiers are important in the processing of data mining. In our work, default parameters of software Weka are used. In general, the accuracy of the classifier is used to assess effectiveness of classification. However, in our dataset, the distribution of the three classes was not uniform. Consequently we adopted an integrative index to estimate the selected symptom subset. An ROC index was used for our experiment because it is insensitive to changes in class distribution and the ROC curves will not change if the proportion of positive to negative instances changes in the dataset [45–47]. The ROC curve is two two-dimensional graphs in which the true positives rate is plotted on the y-axis and the false positives rate is plotted on the x-axis. An ROC graph depicts relative tradeoffs between benefits and costs [45]. To compare classifiers, we may want to reduce ROC performance to a single scalar value representing expected performance. A common method is to calculate the area under the ROC curve (AUC) [45]. Multiclass problems are estimated by measuring AUC of every class, then summing the weighted AUC [45]:

$$\mathrm{AUC}_c = \sum_{c_i \in C} \mathrm{AUC}(c_i) \times p(c_i), \tag{6}$$

where $\mathrm{AUC}(c_i)$ is the AUC of class c_i, $p(c_i)$ is the distribution of class c_i.

The relationships between the AUC and symptom number in TCM subset are shown in Figure 2; Figure 3 is the western medicine subset; Figure 4 in the comprehensive subset. The horizontal coordinate is the weighted AUC with 1 as the highest value; the vertical coordinate represents the number of the feature.

Compared with SVM feature selection, we also constructed the Markov blanket method, which considered the performance in the field of feature selection. After Markov blanket feature selection, we observed 28 symptoms in the TCM subset, 10 in the western medicine subset, and 35 in the comprehensive subset. We selected the top 25, 10, and 35 symptoms from the ranked list of three subsets. These results are shown in Figure 5. Results show that SVM feature selection has better performance than the Markov blanket feature selection from Figure 5.

In all results, the optimum feature subset is essential to predict syndromes of CAD. From Figures 2, 3, and 4, the classification performance is optimum when 25 symptoms

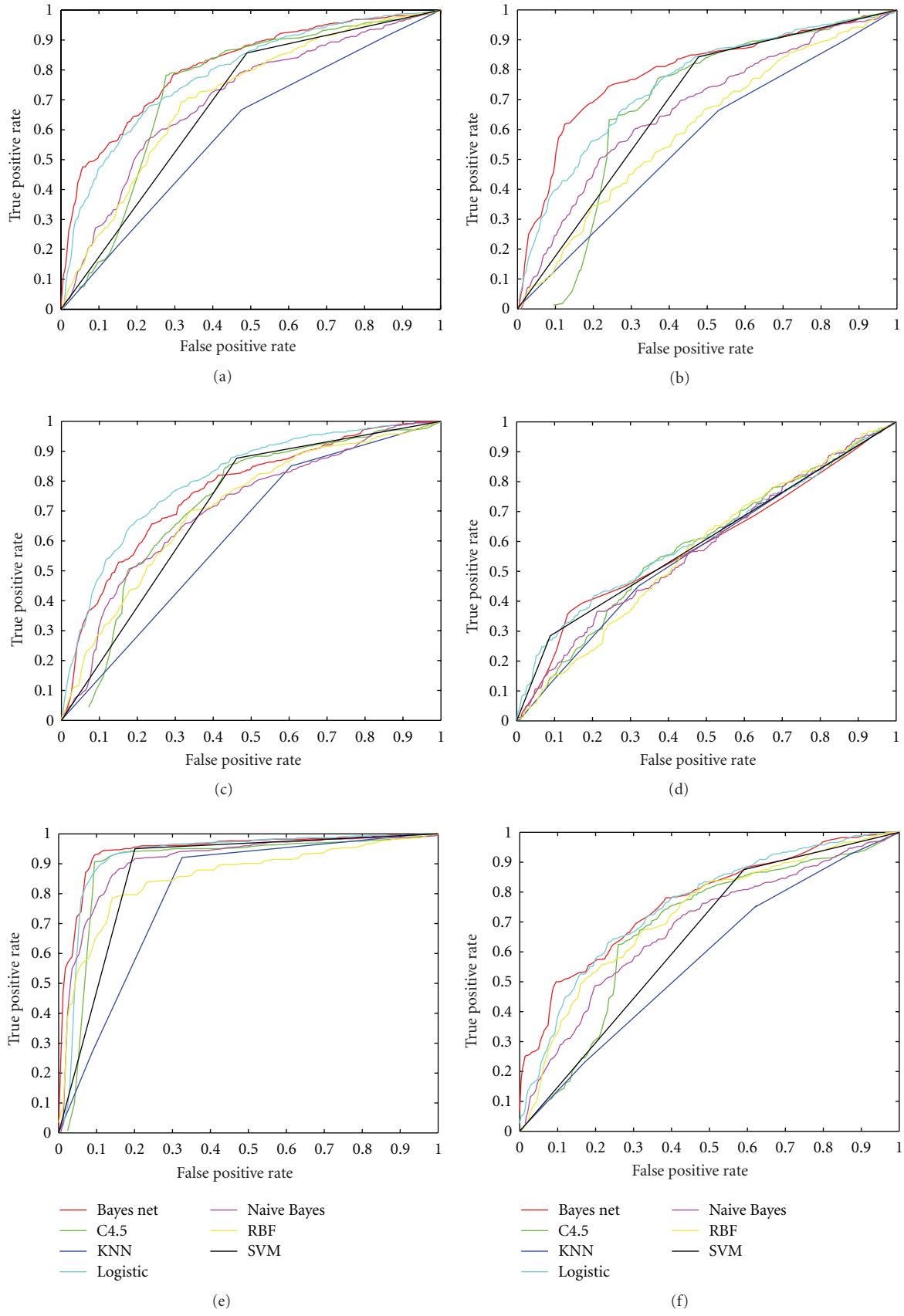

Figure 7: Comparative results of syndrome prediction with five classifiers.

are selected from the TCM subset, 10 symptoms from the western subset, and 35 symptoms from the comprehensive subset. In the comprehensive subset, some critical symptoms (both TCM and western medicine) were filter. Therefore, we constructed a new subset selected from the optimum TCM and the western medicine subsets. We built four syndrome prediction models by using the Bayesian network classifier for the above four subsets-based on tenfold cross-validation test. Results are shown in Figure 6, which shows that the new constructing symptom subset performed better than the others. Lastly, we adopted the new constructing symptom subset as the featured set for predicting syndromes of CAD.

3.2. Results of Predicting Syndromes. All 35 symptoms above were collected for predicting syndromes of CAD. According with the foundational theory of TCM, sthenia can be divided into Qi stagnation, blood stasis, cold coagulation, phlegm turbidity, heat accumulation, water retention, and dampness pathogen, while asthenia can be divided into Qi deficiency, blood deficiency, Yin deficiency, Yang deficiency, heart deficiency, liver deficiency, spleen deficiency, kidney deficiency, and lung deficiency. In this paper, we constructed syndrome prediction models of Qi stagnation, blood stasis, cold coagulation, phlegm turbidity, heat accumulation, water retention, and dampness pathogen. On the dataset with the optimum symptoms, a prediction model of the Bayesian network was built as described in Section 2. Results are shown in Table 3, where the weighted precision is $\sum_{c_i \in C} \text{precision}(c_i) \times p(c_i)$, the weighted recall is $\sum_{c_i \in C} \text{recall}(c_i) \times p(c_i)$, the weighted F-Measure is $\sum_{c_i \in C} f\text{measure}(c_i) \times p(c_i)$, and the weighted AUC is $\sum_{c_i \in C} \text{AUC}(c_i) \times p(c_i)$.

We extensively compare the Bayesian network predictor with the following four methods: C4.5, Logistic, Naïve Bayes, and RBF network. And these five methods are implemented by Weka. Default parameters are exploited to predict syndromes. ROC curve analyses were used for estimating the performance of five classifiers. Comparative results are shown in Figure 7.

Figure 7 shows that the Bayesian network predictor achieved better performance than the others. Overall, these comparisons further demonstrate the feasibility and effectiveness of the Bayesian network classification approach for predicting syndromes of CAD.

4. Conclusion

In this paper, we attempted to predict patient syndromes according to our constructed predicting model based on the related symptoms separately in TCM and western medicine. Instead of using all of the symptoms in diagnosis, SVM feature selection can be used to select 35 of the 113 symptoms by assessing the predictive power of syndrome prediction. The prediction process implemented by feature selection techniques achieved more successful forecasting performance. In addition, they reduced the dimensions of the dataset so that the complexity of the syndrome predictor was decreased. The 35 symptoms subset was significant to diagnosis in clinical practice. Syndrome prediction processes of CAD based on the Bayesian network wasemployed to construct the prediction models of six syndromes for CAD in TCM. It resulted in better performance than four classifiers by means of ROC curve analyses without affecting the distribution of classes. We can conclude that our methods may be used for predicting the syndromes of CAD. Further research is under way addressing doctors' experience and knowledge related to constructing a Bayesian network structure.

Authors' Contribution

P. Lu, J. Chen, and H. Zhao contributed equally to this work.

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Review Article

Atherosclerosis: An Integrative East-West Medicine Perspective

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Recent understanding of atherosclerosis and coronary heart disease has shifted the focus from lumen stenosis to vulnerable plaque, from lipid deposit to inflammatory reaction, and from vulnerable plaque to vulnerable patient. This has led to a new direction of treatment consisting of intervening the inflammatory reaction, stabilizing the vulnerable plaque, inhibiting thrombosis after plaque rupture, and treating the vulnerable patient instead of treating lumen stenosis. This seems to mirror the traditional Chinese medicine (TCM) focus on prevention and on the vulnerable patient with treatment matched to the pattern dysfunction and dysregulation using the Chinese herbal medicine multitargeted approach. Given the convergence of both the East and the West conceptualization of atherosclerosis, it is hopeful that the integrative East-West approach will facilitate early detection and more effective treatment of the vulnerable patients with coronary heart disease.

1. Introduction

Atherosclerosis (AS) is the most common type of arteriosclerosis. It mainly involves the large and middle muscular arteries, especially aorta, coronary and cerebral arteries, which often leads to serious outcomes such as sudden cardiac death, unstable angina pectoris, acute myocardial infarction, stroke, and intermittent claudication due to vessel obliteration or plaque rupture and subsequent thrombosis. In the beginning of the 21st century, we are facing serious challenges of cardiovascular disease (CVD). Although it is becoming less lethal, CVD prevalence is incessantly increasing, and it is still the most common cause of death. How to prevent AS and reduce the incidence and mortality of CVD have been one of the most important health-related issues all the time.

However, biomedicine is at its limits nowadays when confronting degenerative diseases, stress-related diseases, and most chronic diseases. It lacks reference to the self-healing capacity of the human mind and body and focuses on parts rather than the whole, treatment rather than prevention, the suffering disease rather than the diseased person. Confronted with these problems, more and more far-sighted Western scholars began to lay their eyes on traditional Chinese medicine (TCM) [1–3]. Drugs with Chinese herbal medicines as raw materials are increasingly favored by people all over the world for their unique advantages in preventing and curing diseases, rehabilitation, and health care. The benefit of TCM in CVD was also demonstrated in several multicenter clinical trials in recent years [4–7]. More importantly, the unique theory of TCM might also have some implications for the renewal of thinking in fighting against CVD [8]. Therefore, we reviewed traditional understanding and shifted concepts on AS pathophysiology along the track of previous studies and read these transitions taking full advantage of TCM theory together with our experimental and clinical studies in recent years, so as to provide an integrative East-West medicine perspective for future AS prevention and treatment.

2. Updated Concept of Atherosclerosis

2.1. From Emphasizing "Luminal Stenosis" to Highlighting "Vulnerable Plaques". With the deep understanding and active control of AS risk factors, dramatic advances have been made in primary prevention of chronic cardiovascular diseases since 1990s. However, there is still lack of effective measure to prevent acute cardiovascular events (ACEs), which cause 20 million deaths worldwide per year. Most of the victims die suddenly without any prior symptoms.

The previous studies focused on the severity of coronary stenosis, taking coronary heart disease (CHD) as an

example of AS, and highlighted detection of severe luminal stenosis and subsequent treatment of percutaneous coronary intervention (PCI). The development or improvement of coronary stenosis is also regarded as an important indicator to evaluate the state of illness or therapeutic effect. However, angiographic studies on patients before myocardial infarction showed that the majority of subsequent events involved sites with less than 70% obstruction. It indicated that the severity of stenosis was not the main cause of ACEs [9].

In 1989, Muller and his colleagues used the word "vulnerable" to describe rupture-prone plaques, with characteristics of a large lipid pool, a thin cap, and macrophage-dense inflammation on or beneath its surface [10], as the underlying cause of most clinical coronary events. More and more studies suggested that ACEs were triggered by thrombosis associated with rupture of vulnerable atherosclerotic plaques [11]. The change of plaque from its stable state to an unstable one was not related to the plaque size, quantity, or position or the severity of stenosis. Although PCI improves significant stenosis, it cannot influence the biological course of vulnerable plaque, thus the problem of "unstable" plaque is still unresolved.

In recent years, many clinical trials showed that statins could reduce ACEs significantly yet only improve the luminal size slightly [12]. Experimental researches have proved that statins have potential effects on stability of AS plaques [13]. Stenting (including drug-eluting stents) reduces restenosis and repeated intervention, but does not reduce mortality or myocardial infarction [14]. Therefore, it is necessary for us to reevaluate the benefits of active medicinal treatment and invasive PCI treatment in chronic myocardial ischemia. Based on in-depth understanding of AS pathogenesis, the vascular pathophysiological research has turned to new direction of stabilizing vulnerable plaque and inhibiting thrombosis after plaques rupture. The secondary prevention of CHD also focused on intervention of vulnerable plaque instead of treating luminal stenosis of coronary artery [15, 16].

2.2. From Predominant Theory of "Lipid Deposit" to General Acknowledgment of "Inflammatory Reaction" Theory. "Lipids deposit" theory of AS has been put forward for over 100 years based on the causal relation between hyperlipidemia and AS [17]. This theory holds that lipids deposition on the artery wall leads to the AS plaques and has played a very important role in AS pathogenesis for a long period.

In recent years, some researches indicated that AS had the basic manifestation of inflammation: degeneration, exudation, and proliferation. The cell-cell interaction is similar to other chronic inflammation diseases such as rheumatoid arthritis, chronic pancreatitis, and hepatic cirrhosis. With continuous detection of inflammatory cells and mediators, AS was no longer regarded as a simple disease of lipid deposition on vessel wall but also an advancing inflammatory reaction. Recent advances in basic science have established a fundamental role for inflammation in mediating all stages of this diseases from initiation through progression and, ultimately, the thrombotic complications of AS.

In 1999, based on his famous "injury reaction" theory, Ross declared that AS is one of the inflammatory disease [18]. AS is a process of active inflammatory reaction inside the vessel wall rather than a process of passive lipid deposit onto the vessel wall. This theory initiates a new epoch of AS treatment and it leads to deep understanding of cardiovascular diseases: inflammation fuels the development and progression of atherosclerosis as well as causes certain plaques to rupture and subsequent thrombosis, leading to such atherosclerotic complications as heart attack and stroke. High-sensitivity C-reactive protein (hs-CRP) and other blood inflammatory markers may be useful in the estimation of prognosis, risk level in AS patients, and even be a potential target of AS treatment and prevention [19]. Despite regulating blood lipids metabolism, statins should be recommended for their anti-inflammation and other protective effects on cardiovascular diseases. Aspirin can not only inhibit platelet aggregation but also prevent the malfunction of endothelial cells through its anti-inflammation effects [16]. Anti-inflammation has been one of the most important issues of AS research and several strategies that intervene with inflammation reaction are under study.

2.3. New Concept from "Vulnerable Plaque" to "Vulnerable Patient". Plaque rupture is the most common type of plaque complication, accounting for nearly 70% of fatal acute myocardial infarctions and/or sudden coronary deaths. Vulnerable plaque is the main, but not the unique, cause for ACEs. The position of plaque rupture, the size and amount of plaques, coronary spasm, hypercoagulable state, collateral circulation, and the degree of myocardial damage should also be considered. In 2003, an article named "From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies" was published on *Circulation* written by over fifty of the most famous cardiovascular experts of the world [20, 21]. The new concept of "vulnerable plaque" to "vulnerable patients" has led in a new direction to the prevention of ACEs.

The term "vulnerable patient" is proposed to define subjects susceptible to an acute coronary syndrome or sudden cardiac death based on plaque, blood, or myocardial vulnerability (1-year risk $\geq 5\%$). Extensive efforts are needed to quantify an individual's risk of an event according to each component of vulnerability (plaque, blood, and myocardium). Such a comprehensive risk-stratification tool capable of predicting acute coronary syndromes as well as sudden cardiac death would be very useful for preventive cardiology. The new concept of "vulnerable plaque" to "vulnerable patients" stresses evaluating patients as a whole and thus further optimizes overall assessment of cardiovascular risks, and prevents ACEs by early intervention of vulnerable patients.

3. An Integrative East-West Medicine Perspective for Future AS Management

The transitions in understanding AS, from local plaques to entire coronary tree and patient as a whole, from passive

lipid deposit process to an active inflammatory reaction and cell interaction process, innovate strategies of prevention and treatment for AS and CHD from coronary stenosis-targeted invasive PCI treatment to vulnerable patient-targeted comprehensive assessment, early-detection and preventive medication strategies, happen to mirror "holism concept" "living in harmony with the environment" "preventive treatment of disease" and "treatment based on syndrome differentiation or pattern diagnosis" advocated by TCM. They can also help us fully understand the two different medical systems, Western medicine (WM) and TCM, as well as make the best of the advantages of both of them.

The previous researches have shown that Chinese medicines of activating blood circulation (ABC) could treat AS by multiple ways such as lowering blood lipid, inhibiting platelet adhesion and aggregation, and improving blood viscosity and inhibiting SMC proliferation. In 2003, based on AS models of ApoE-deficient mice, we studied the effects of six ABC herbs (Radix Salviae Miltiorrhizae, Radix Paeoniae Rubra, Rhizoma Chuanxiong, Radix Notoginseng, Semen Persicae, Wine steamed Radix, and Rhizoma Rhei) and a compound preparation (consisting of Chuanxingol and Paeoniflorin) on stabilizing AS plaque and their potential mechanisms. The results indicated that most ABC herbs showed multiple effects on different links of AS, such as regulating blood lipids, influencing collagen metabolism, and anti-inflammatory reaction, thus had potential effect on stabilizing AS plaque [22, 23]. Although the final effect of ABC herbs on stabilizing plaque was slightly less than that of simvastatin, they showed better effects on certain links such as increasing high-density lipoprotein cholesterol (HDL-C), which exhibited the superiorities of Chinese medicine in overall regulation by influencing multiple targets [8]. The superior effect of the compound preparation to either herbal extractive component [24] indicated the synergetic effect based on TCM compatibility theory. Therefore, Chinese herbal medicines, especially compound prescriptions, warrant further investigation and might be an complementary or alternative therapy to statins in stabilizing vulnerable plaque through a synergistic and multitargeted effect.

The new concept of "vulnerable patient" also provides TCM with new opportunity in detecting high-risk CHD patients and further reducing ACEs by early intervention. Under the guidance of TCM holism concept and thought of treatment based on syndrome differentiation, we conducted a multicenter cohort study, enrolling stable CHD patients and documenting one-year follow-up cardiovascular endpoint events. Prognosis-related factors, including past medical history, symptoms, body signs, biochemical indicators, and tongue manifestations, were identified to establish an integrative risk-assessment system for detecting high-risk CHD patients [25–27]. A large-scale randomized controlled trial aiming at early intervening high-risk CHD patients based on this integrative risk assessment system is about to start soon. Given the convergence of both the East and the West conceptualization of AS, it is hopeful that this integrative East-West strategy will facilitate early detection and more effective treatment for the vulnerable patients with CHD and other AS-related diseases.

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Review Article

Sulfotanshinone Sodium Injection for Unstable Angina Pectoris: A Systematic Review of Randomized Controlled Trials

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Objective. To assess the effect of sulfotanshinone sodium injection for unstable angina. *Methods*. We searched for published and unpublished studies up to June 2011. We included randomized controlled trials that confoundedly addressed the effect of sulfotanshinone sodium injection in the treatment of unstable angina. *Results*. Twenty-five studies involving 2,377 people were included. There was no evidence that sulfotanshinone sodium alone had better or worse effects to routine western medicine treatments in improving clinical symptoms (RR 1.00, 95% CI 0.90 to 1.11) and ECG (RR 0.97, 95% CI 0.87 to 1.09). However, there was evidence that sulfotanshinone sodium combined with western medications was a better treatment option than western medications alone in improving clinical symptoms (RR 1.28, 95% CI 1.23 to 1.3), ECG (RR 1.26, 95% CI 1.18 to 1.35), C-reaction protein (mean difference 2.10, 95% CI 1.63 to 2.58), and IL-6 (mean difference −3.85, 95% CI −4.10 to −3.60). There was no difference between sulfotanshinone sodium plus western medications and western medications alone affecting mortality (RR 0.50, 95% CI 0.02 to 12.13). *Conclusion*. Compared with western medications alone, sulfotanshinone sodium combined with western medications may provide more benefits for patients with unstable angina. Further large-scale high-quality trials are warranted.

1. Introduction

Coronary artery disease is the leading cause of death in the United States [1]. Early hospital care for unstable angina includes anti-ischemic therapies, antiplatelet therapies, and anticoagulant/antithrombotic therapies and may also consider an early invasive strategy [2]. Thrombolytic agents are usually more frequently used for more severe conditions [3–5].

Danshen, *also known as Salvia miltiorrhiza Bge*, is a hemorheologic agent that may have protective effect in patients with unstable angina [6] and has been used for cardiovascular disorders for hundreds of years in China and now is widely used in other countries as well.

Danshen consists of a mixture of compounds, among which Tanshinone IIA (TIIA) represents the most biologically active ingredient [7]. TIIA, also known as Danshen ketone, Tanshinon II, Tanshinone B, is a diterpenoid naphthoquinone extracted and isolatedderivative from Danshen. Animal and cellular studies have shown various potential benefits of the agent, including (1) neuroprotective effect in cerebral ischemia and reperfusion [8], (2) antioxidant potential to prevent oxidation of low-density lipoproteins [9], (3) ability of rescuing PC-12 cells from hypoxia [10], (4) reducing cellular damage by free radicals [11], (5) protecting mitochondrial membrane from ischemia-reperfusion injury and lipid peroxidation [12], (6) decreasing PHB expression in oxidative stress-injured myocardial cells hence protecting

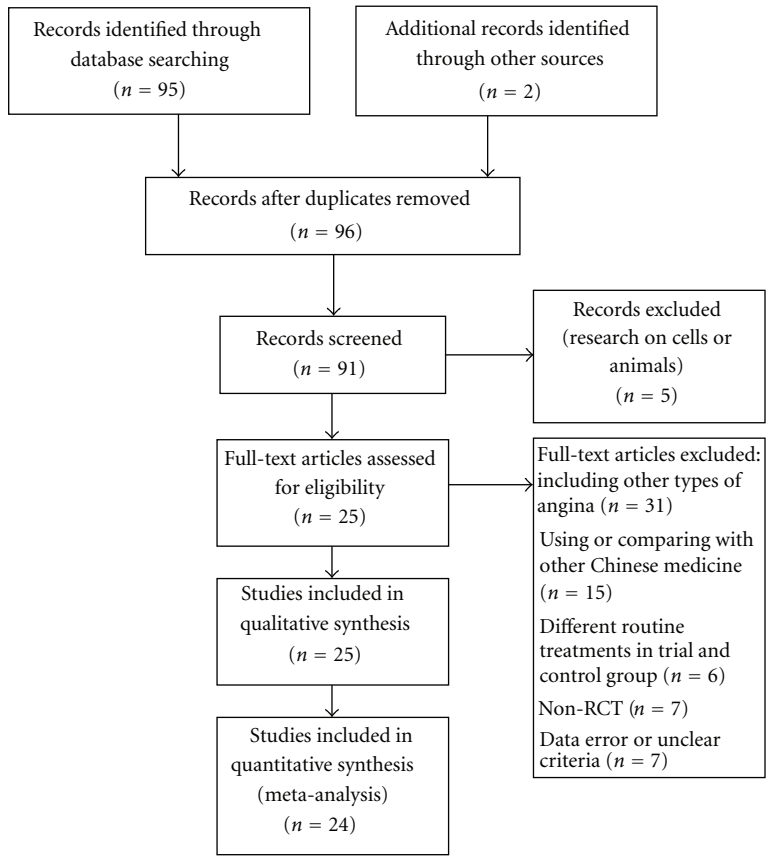

Figure 1: PRISMA flow chart of literature retrieval and selection.

Study or subgroup	SS		Routine			Risk ratio	Risk ratio
	Events	Total	Events	Total	Weight	M-H, fixed, 95% CI	M-H, fixed, 95% CI
Zhao 2007	37	39	37	39	100%	1 [0.90, 1.11]	
Total (95% CI)		39		39	100%	1 [0.90, 1.11]	
Heterogeneity: not applicable							
Test for overall effect: $Z = 0$ ($P = 1$)							

Figure 2: SS versus Isosorbide, outcome: clinical symptom improvement.

the myocardial cells [13], (7) protecting cardiomyocytes against oxidative stress-mediated apoptosis [14], and (8) cardioprotective in the context of diabetic cardiomyopathy through kinin B2 receptor-Akt-GSK-3β-dependent pathway [15]. Human studies also have demonstrated cardioprotective effects of TIIA, including reduction of myocardial infarct size and decrease of myocardial consumption of oxygen [16].

Until now, the clinically available TIIA agent, which is approved by State Food and Drug Administration of China, only includes sulfotanshinone sodium (SS) injection (i.e., sodium tanshinone IIA sulfonate injection) manufactured by two companies. TIIA is extracted from the raw herb Danshen and then chemically derivatized into water-soluble SS for the preparation of injection. Upon the administration of SS injection, SS transforms back into the bioactive ingredient TIIA in vivo [17]. The dosage for administration of SS injection is 40–80 mg per day. SS injection is given diluted at the point of treatment in 20 mL 25% glucose injection for intramuscular administration or in 250–500 mL 5% glucose injection for intravenous administration. It is widely used in the Chinese hospitals for unstable angina [18].

However, the effects of SS injection on unstable angina have not been well established. In this study, we evaluated the effect of SS through a rigorous systematic review and meta-analysis of randomized trials.

2. Methods

2.1. Eligibility Criteria. We included randomized controlled trials that compared SS with placebo or active agents in patients with unstable angina defined as new onset (≤2

Table 1: Randomized controlled trials of SS injection for unstable angina pectoris.

Study	Method	N (M : F)	Mean age	Interventions	Outcomes
Zhao 2007 [19]	RCT, not blinded Duration: 2 W	78 (54 : 24)	62.8	(1) Isosorbide mononitrate 40 mg (2) SS 40 mg	(1) clinical symptom improvement, (2) ECG, (3) frequency, duration and intervals of angina attacks
Yan et al. 2009 [20]	RCT, not blinded Duration: 4 W	94 (53 : 41)	52	(1) Routine (Aspirin 300 mg–100 mg qd, Enoxaparin, Elantan 50 mg, Betaloc 100 mg) (2) Routine + SS 60 mg	(1) clinical symptom improvement, (2) ECG, (3) FIB, (4) D-dimer
Wang and Hou 2010 [21]	RCT, not blinded Duration: 2 W	100 (65 : 35)	62	(1) Routine (Aspirin, Nitrates, Calcium antagonists, Ozagrel) (2) Routine + SS 40 mg	(1) clinical symptom improvement, (2) ECG
Yang et al. 2010 [13, 22]	RCT, not blinded Duration: 1 W	64 (35 : 39)	59	(1) Routine (Aspirin 100 mg qd, Isosorbide mononitrate 20 mg bid, Metoprolol 25 mg bid) (2) Routine + SS 60 mg	(1) clinical symptom improvement, (2) ECG, (3) C-reaction protein, (4)IL-6, (5) plasma viscosity, (6) FIB
Ge et al. 2010 [23]	RCT, not blinded Duration: 15 D	60 (39 : 21)	58	(1) Routine (Nitrates, Betaloc, anticoagulant and antiplatelet aggregation medication, ACEI, Statins) (2) Routine + SS 60 mg	(1) clinical symptom improvement, (2) ECG, (3) TC, TG, LDL-C, HDL-C
Ge and Zhu 2009 [24]	RCT, not blinded Duration: 2 W	48 (32 : 16)	40–80Range	(1) Routine (Aspirin, Betaloc, ACEI, Calcium antagonists, Isosorbide mononitrate, antiplatelet agents, Trimetazidine) (2) Routine + SS 50 mg	(1) clinical symptom improvement.
Hu et al. 2009 [25]	RCT, not blinded Duration: 2 W	148	60	(1) Routine (Statins, ARB, ACEI, Nitrates, Aspirin, LMWH, Betaloc) (2) Routine + SS 40 mg	(1) clinical symptom improvement, (2) ECG.
Pei and Chen 2009 [26]	RCT, not blinded Duration: 2 W	71 (48 : 23)	65	(1) Routine (Aspirin, Clopidogrel, LMWH, Nitrates, Betaloc, Statins, nondihydropyridine calcium antagonists) (2) Routine + SS 40 mg	(1) clinical symptom improvement, (2) plasma viscosity, (3) blood viscosity at high/low shear stress, (4) hematocrit.
Zuo and Hou 2009 [27]	RCT, not blinded Duration: 2 W	83 (58 : 25)	72	(1) Routine (Aspirin, Betaloc, Nitrates, Statins, LMWH) (2) Routine + SS 40 mg	(1) clinical symptom improvement, (2) length of angina from attacking to alleviating, (3) length of angina from attacking to vanishing, (4) times of myocardial ischemia onset.
Song 2008 [28]	RCT, not blinded Duration: 2 W	105	72	(1) Routine (Aspirin, Simvastatin, Betaloc, Nitrates, Diltiazem, ARB, ACEI) (2) Routine + SS 60 mg	(1) clinical symptom improvement.
Xu and Su 2008 [29]	RCT, not blinded Duration: 1 W	74 (40 : 30)	45–78Range	(1) Routine (Fluvastatin, Aspirin, Betaloc, LMWH) (2) Routine + SS 80 mg	(1) C-reaction protein, (2) IL-6, (3) P-selectin, (4) PAI-1
Huang et al. 2008 [30]	RCT, not blinded Duration: 2 W	220 (140 : 80)	62	(1) Routine (LMWH, Betaloc, Isosorbide mononitrate, calcium antagonists, Statins, Aspirin) (2) Routine + SS 60 mg	(1) clinical symptom improvement, (2) ECG, (3) plasma/whole blood viscosity, (4) systolic/diastolic blood pressure, (5) heart rate, (6) hematocrit, (7) Platelet aggregation, (8) FIB.

Table 1: Continued.

Study	Method	N (M : F)	Mean age	Interventions	Outcomes
Li et al. 2008 [31]	RCT, not blinded Duration: 2 W	125 (80 : 45)	62.41	(1) Routine (ACEI, vasodilator, antiplatelet agents, anticoagulants) (2) Routine + SS 60 mg	(1) NO, (2) FMD, (3) ET.
Hua et al. 2007 [32]	RCT, not blinded Duration: 2 W	112 (69 : 43)	60	(1) Routine (Aspirin, LMWH, Betaloc, Nitroglycerin, ACEI, Isosorbide mononitrate) (2) Routine + SS 80 mg	(1) clinical symptom improvement, (2) ECG, (3) plasma viscosity, (4) whole blood viscosity, (5) erythrocyte aggregation, (6) morality, (7) FIB.
Wang et al. 2007 [33]	RCT, not blinded Duration: 2 W	50 (28 : 22)	48.5	(1) Routine (Betaloc, Isosorbide mononitrate, Diltiazem, Aspirin) (2) Routine + SS 50 mg	(1) clinical symptom improvement, (2) ECG, (3) D-dimer, (4) C-reaction protein, (5) plasma viscosity, (6) erythrocyte aggregation, (7) hematocrit.
Ma et al. 2007 [34]	RCT, not blinded Duration: 2 W	59 (37 : 22)	62.7	(1) Routine (Betaloc, Aspirin, ACEI, Isosorbide mononitrate, calcium antagonists, anticoagulants,) (2) Routine + SS 40 mg	(1) clinical symptom improvement, (2) ECG, (3) morality.
X. G. Zhang and Y. M. Zhang 2006 [35]	RCT, not blinded Duration: 4 W	60 (33 : 27)	62	(1) Routine (antiplatelet agents, Nitrates, Betaloc, ACEI, Diuretics) (2) Routine + SS 60 mg	(1) clinical symptom improvement, (2) ECG, (3) systolic/diastolic blood pressure, (4) heart rate, (5) frequency and duration of angina attacks, (6) Premature ventricular contractions in 24 hours.
Zhang et al. 2006 [36]	RCT, single blinded Duration: 2 W	52	—	(1) Routine (Nitrates, Betaloc, Aspirin) (2) Routine + SS 80 mg	(1) clinical symptom improvement, (2) ECG.
Liu and Yang 2010 [37]	RCT, not blinded Duration: 2 W	100 (61 : 49)	65	(1) Routine (LMWH 6000 U q12h, Nitrates, Simvastatin 20 mg qn, Betaloc, ACEI, Aspirin) (2) Routine + SS 50 mg	(1) clinical symptom improvement.
Yang and Cai 2009 [38]	RCT, not blinded Duration: 4 W	64 (32 : 32)	49.5	(1) Routine (Captopril 25 mg qd, Betaloc 25 mg bid, Isosorbide mononitrate 40 rag qd, Aspirin 0.1 g pd, Simvastatin 25 rag, qn) (2) Routine + SS 60 mg	(1) clinical symptom improvement, (2) ECG, (3) frequency and duration of angina attacks.
Bai and Ding 2007 [39]	RCT, not blinded Duration: 4 W	80 (42 : 38)	61	(1) Routine (Nitrates, Betaloc, ACEI, antiplatelet agents, LMWH) (2) Routine + SS 80 mg	(1) clinical symptom improvement, (2) ECG.
Fang and Wang 2007 [40]	RCT, not blinded Duration: 10 D	120 (54 : 66)	74	(1) Routine (LMWH 5000 U, Nitroglycerin 10 mg) (2) Routine + SS 60 mg	(1) clinical symptom improvement.
Jiang 2004 [41]	RCT, not blinded Duration: 3 W	156 (82 : 74)	60.9	(1) Routine (ACEI, Betaloc) (2) Routine + SS 40 mg	(1) clinical symptom improvement, (2) C-reaction protein, (3) times of angina attacks daily.
Qi and Qu 2008 [42]	RCT, not blinded Duration: 2 W	68 (38 : 30)	63	(1) Routine (Nitrates, Aspirin) (2) Routine + SS 60 mg	(1) frequency of angina attacks, (2) duration of angina attacks, (3) TC, TG, HDL, LDL.

Table 1: Continued.

Study	Method	N (M : F)	Mean age	Interventions	Outcomes
Han and Wang 2011 [43]	RCT, not blinded Duration: 2W	186 (94 : 92)	55	(1) Routine (nitroglycerin 20 mg) (2) Routine + SS 60 mg	(1) clinical symptom improvement.

RCT: randomized clinical trial; F: female; M: male; W: week(s); D: day(s); 1: control group; 2: trial group; SS: SS; LMWH: Low molecular weight heparin; ACEI: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blocker; TC: total cholesterol; TG: Triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein; NO: nitric oxide; FMD: flow-mediated dilation; ET: endothelin; FIB: fibrinogen; qd: once per day; qn: once per night; bid: twice per day; q12 h: once every 12 hours.

months) exertional angina of at least Canadian Cardiovascular Society Classification (CCSC) class III in severity, significant recent increase in frequency and severity of angina, or angina at rest.

The eligible comparisons include

(i) SS injection versus any current western medications,

(ii) SS injection plus any current western medications for unstable angina versus western medications alone,

(iii) SS injection versus placebo.

Our prespecified primary outcome is all-cause mortality, and secondary outcomes include resolution of angina, ECG improvement, inflammatory factors (such as C-reaction protein and IL-6), and adverse events. The improvement of clinical symptoms is measured as the reduction in chest pain and shortness of breath or the frequency, severity, and length of acute angina attacks. "Very effective" includes that there is no angina attack, chest pain disappears, ST segment depression is back to normal, or the depression of ST segment reduces >0.1 mV; "effective" includes that times of angina attacks reduce by >2/3 or the length, frequency, and severity of angina attacks and chest pain significantly reduce, the depression of ST segment reduces <0.1 mV but >0.05 mV; "ineffective" includes that there is no change or very little change in chest pain and shortness of breath, or the frequency, severity and length of acute angina attacks, the depression of ST segment reduces <0.05 mV. For the systematic review, the outcomes of both "very effective" and "effective" were considered successful treatments.

2.2. Search Strategy. We searched the Cochrane Library (Issue 7, 2011), Chinese Cochrane Centre Controlled Trials Register (to June 2011), Medline (1995 to June 2011), EMBASE (1995 to June 2011), CNKI database (1979 to June 2011), Wanfang Data (1998 to June 2011), and VIP Information (1985 to June 2011) using the following key words: unstable angina, angina, SS, tanshinone IIA, and sodium tanshinone IIA sulfonate, as well as the brand names of the agent. We also searched databases of ongoing trials, including Current Controlled Trials and the UK National Research Register.

We also searched Chinese medicine journals not indexed in the electronic databases. We screened the reference lists of relevant trials and identified reviews. We contacted experts in this field and relevant pharmaceutical companies for additional references or unpublished studies. We restricted the language of publications to English and Chinese.

2.3. Data Collection. Two reviewers (Qiu and Yang) independently screened the titles, abstracts, and key words of each searched article for potentially eligible studies. Reviewers then screened full texts for final eligibility. The full-text articles were retrieved for further assessment if the information given suggests that the study: (1) included patients with unstable angina, (2) compared SS injection with western medication in the presence or absence of cointerventions in both groups, (3) assessed one or more relevant clinical outcome measure such as morality, clinical symptoms, or electrocardiogram (ECG), (4) had clearly outlined criteria for successful treatment and treatment success was not measured in terms of illness severity scores or the intensity of individual symptoms, and (5) used random allocation.

Reviewers independently extracted data from eligible studies, using pilot-tested data extraction forms. Reviewers extracted the following data: age and number of participants in the SS group and the control group, male-female ratio in each group, diagnosis criterion, treatment dosage and duration, side effects, and symptoms that improved after treatments. Important missing data were obtained by contacting article authors whenever possible.

We excluded studies if they (1) included nonunstable angina, used or compared with other Chinese Medicine, (2) used different routine western medications in the trial group and control group, (3) were not randomized trials, (4) had unclear criteria of symptom improvement or data error, (5) were duplicated, or (6) were not conducted on human subjects.

2.4. Risk of Bias. Two reviewers (Qiu and Yang) independently assessed the risk of bias for each trial according to the Cochrane Handbook for Systematic Reviews of Interventions version 5.1.0 [44]. The items included the random sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting, and other potential threats to validity. Summary assessments of the risk of bias for important outcomes within and across studies was made. Based on Cochrane handbook [44], a study is considered at low risk of bias if there is low risk of bias for all key domains within a study; it is considered to be unclear risk of bias if unclear risk of bias is for one or more key domains within studies at unclear risk of bias across studies; it is considered to be high risk of bias if high risk of bias for

Table 2: Assessment of risk of bias in included studies.

Study	Random sequence generation	Allocation concealment	Blinding	Incomplete outcome data	Selective reporting	Free of other bias	Summary assessments
Zhao 2007 [19]	U	U	H	U	U	H	H
Yan et al. [20] 2009	U	U	H	U	U	H	H
Wang and Hou 2010 [21]	U	U	H	U	U	H	H
Yang et al. 2010 [22]	U	U	H	U	U	H	H
Ge et al. 2010 [23]	U	U	H	U	U	H	H
Ge and Zhu 2009 [24]	U	U	H	H	U	H	H
Hu et al. 2009 [25]	U	U	H	U	U	H	H
Pei and Chen 2009 [26]	U	U	H	U	U	H	H
Zuo and Hou 2009 [27]	U	U	H	U	U	H	H
Song 2008 [28]	U	U	H	H	U	H	H
Xu 2008 [29]	U	U	H	L	U	H	H
Huang et al. 2008 [30]	U	U	H	U	U	H	H
Li et al. 2008 [31]	U	U	H	U	U	H	H
Hua et al. 2007 [32]	L	L	H	U	U	H	H
Wang et al. 2007 [33]	U	U	H	U	U	H	H
Ma et al. 2007[34]	U	U	H	U	U	H	H
X. G. Zhang and Y. M. Zhang 2006 [35]	U	U	H	U	U	H	H
Zhang et al. 2006 [36]	U	U	H	H	U	H	H
Liu and Yang 2010 [37]	U	U	H	H	U	H	H
Yang and Cai 2009 [38]	U	U	H	U	U	H	H
Bai and Ding 2007 [39]	U	U	H	H	U	H	H
Fang and Wang 2007 [40]	U	U	H	H	U	H	H
Jiang 2004 [41]	U	U	H	U	U	H	H
Qi and Qu 2008 [42]	U	U	H	U	U	H	H
Han and Wang 2011 [43]	U	U	H	H	U	H	H

L: low risk of bias, U: unclear, H: high risk of bias.

Study or subgroup	SS Events	SS Total	Routine Events	Routine Total	Weight	Risk ratio M-H, fixed, 95% CI
Zhao 2007	36	39	37	39	100%	0.97 [0.87, 1.09]
Total (95% CI)		39		39	100%	0.97 [0.87, 1.09]
Heterogeneity: not applicable						
Test for overall effect: $Z = 0.46$ ($P = 0.64$)						

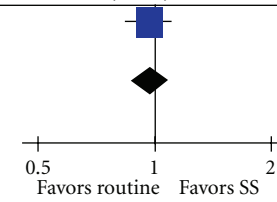

Figure 3: SS versus Isosorbide, outcome: ECG.

Study or subgroup	SS + routine Events	SS + routine Total	Routine Events	Routine Total	Weight	Risk ratio M-H, fixed, 95% CI
Bai and Ding 2007	34	40	24	40	3.3%	1.42 [1.07, 1.88]
Fang and wang 2007	49	60	31	60	4.3%	1.58 [1.20, 2.08]
Ge and Zhu 2009	22	24	17	24	2.4%	1.29 [0.97, 1.72]
Ge 2010 et al.	27	30	20	30	2.8%	1.35 [1.02, 1.79]
Han and wang 2011	95	96	79	90	11.4%	1.13 [1.04, 1.22]
Hu 2009 et al.	70	74	60	74	8.4%	1.17 [1.03, 1.32]
Hua 2007 et al.	51	56	41	56	5.7%	1.24 [1.04, 1.49]
Huang 2008 et al.	100	110	76	110	10.6%	1.32 [1.15, 1.51]
Jiang 2004	70	80	41	76	5.9%	1.62 [1.30, 2.03]
Liu and Yang 2010	45	50	35	50	4.9%	1.29 [1.05, 1.58]
Ma 2007 et al.	26	30	22	29	3.1%	1.14 [0.89, 1.46]
Pei and Chen 2009	29	36	23	35	3.3%	1.23 [0.92, 1.64]
Song 2008	50	53	42	52	5.9%	1.17 [1.01, 1.35]
Wang 2007 et al.	22	25	14	25	2.0%	1.57 [1.08, 2.29]
Wang and Hou 2010	46	50	40	50	5.6%	1.15 [0.98, 1.35]
Yang and Cai 2009	28	32	20	32	2.8%	1.40 [1.04, 1.89]
Yang 2010 et al.	30	32	23	32	3.2%	1.30 [1.03, 1.65]
Yan 2009 et al.	42	46	34	48	4.6%	1.29 [1.05, 1.58]
Zhang 2006 et al.	25	26	22	26	3.1%	1.14 [0.95, 1.36]
X.G. Zhang and Y.M. Zhang 2006	26	30	19	30	2.6%	1.37 [1.01, 1.86]
Zuo and Hou 2009	39	42	30	41	4.2%	1.27 [1.04, 1.56]
Total (95% CI)		1022		1010	100.0%	1.28 [1.23, 1.34]
Total events	926		713			
Heterogeneity: $\chi^2 = 27.31$, df = 20 ($P = 0.13$); $I^2 = 27\%$						
Test for overall effect: $Z = 11.13$ ($P < 0.00001$)						

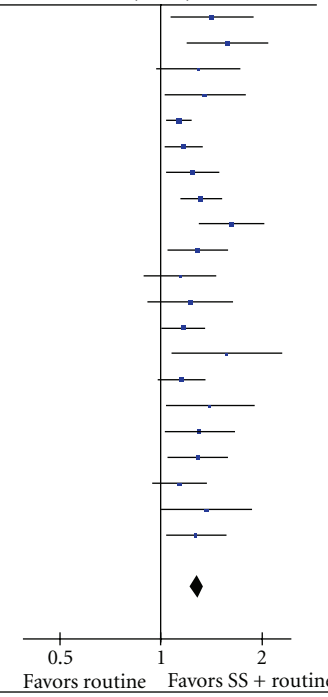

Figure 4: SS + routine therapy versus routine therapy, outcome: clinical symptom improvement.

one or more key domains within a study is sufficient to affect the interpretation of results across the studies. Disagreements were resolved by discussion and by adjudicated by a third reviewer (Jiang) when necessary.

2.5. Data Analysis. Our comparisons included SS versus western medication and SS plus routine therapy versus routine therapy. We reported risk ratio (RR) and 95% confidence intervals (CI) for the pooled binary data, and mean differences (MD) for continuous data. The test of homogeneity was used with a significance level of 0.1. We also used the I-square statistic to assess the heterogeneity. Publication bias was assessed by the funnel plot. We performed sensitivity analysis by using different statistical methods (fixed-effect and random-effects models) for combining data to explore the influence of study quality on effect size.

3. Results

A total of twenty-five trials [19–43], involving 2,377 participants with unstable angina defined as new onset (≤2 months) exertional angina of at least Canadian Cardiovascular Society Classification (CCSC) class III in severity, significant recent increase in frequency and severity of angina, or angina at rest, proved eligible (Figure 1).

All 25 trials were conducted in China. The treatment duration ranged from 1 to 4 weeks, and the dose from 40 to 80 mg per day. One trial [34] compared SS injection versus isosorbide mononitrate. The other 24 trials compared SS injection plus western medications versus western medications alone. There were no placebo controlled studies. SS injection is given diluted at the point of treatment in 20 mL 25% glucose injection for intramuscular administration

Study or subgroup	SS + routine Events	SS + routine Total	Routine Events	Routine Total	Weight	Risk ratio M-H, fixed, 95% CI
Bai and Ding 2007	28	40	15	40	4%	1.87 [1.19, 2.92]
Ge 2010 et al.	25	30	19	30	5%	1.32 [0.96, 1.80]
Hu 2009 et al.	65	74	57	74	15%	1.14 [0.98, 1.33]
Hua 2007 et al.	49	56	40	56	10.6%	1.23 [1.01, 1.49]
Huang 2008 et al.	100	110	81	110	21.4%	1.23 [1.09, 1.40]
Ma 2007 et al.	25	30	19	29	5.1%	1.27 [0.93, 1.73]
Wang 2007 et al.	21	25	13	25	3.4%	1.62 [1.07, 2.44]
Wang and Hou 2010	41	50	38	50	10%	1.08 [0.88, 1.32]
Yang and Cai 2009	27	32	25	32	6.6%	1.08 [0.85, 1.37]
Yang 2010 et al.	29	32	19	32	5%	1.53 [1.12, 2.08]
Yan 2009 et al.	24	46	25	48	6.5%	1.00 [0.68, 1.48]
Zhang 2006 et al.	23	26	17	26	4.5%	1.35 [0.99, 1.85]
X.G. Zhang and Y.M. Zhang 2006	20	30	11	30	2.9%	1.82 [1.07, 3.10]
Total (95% CI)		581		582	100%	1.26 [1.18, 1.35]
Total events	477		379			
Heterogeneity: $\chi^2 = 14.99$, df = 12 ($P = 0.24$); $I^2 = 20\%$						
Test for overall effect: $Z = 6.57$ ($P < 0.00001$)						

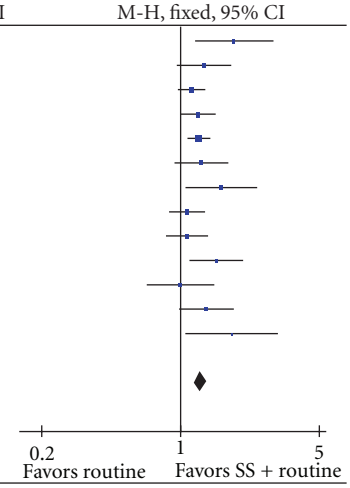

Figure 5: SS + routine therapy versus routine therapy, outcome: ECG.

Study or subgroup	SS + routine Mean	SS + routine SD	SS + routine Total	Routine Mean	Routine SD	Routine Total	Weight	Mean difference IV, fixed, 95% CI
Jiang 2004	11.0	9.14	80	8.5	9.35	76	2.7%	2.50 [−0.40, 5.40]
Wang 2007 et al.	3.11	3.38	25	0.35	3.25	25	6.8%	2.76 [0.92, 4.60]
Xu and Su 2008	7.43	1.05	37	5.07	2.27	37	35.4%	2.36 [1.55, 3.17]
Yang 2010 et al.	7.62	1.23	32	5.78	1.4	32	55.1%	1.84 [1.19, 2.49]
Total (95% CI)			174			170	100%	2.1 [1.63, 2.58]
Heterogeneity: $\chi^2 = 1.59$, df = 3 ($P = 0.66$); $I^2 = 0\%$								
Test for overall effect: $Z = 8.61$ ($P < 0.00001$)								

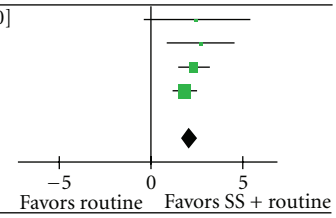

Figure 6: SS + routine therapy versus routine therapy, outcome: C-reaction Protein.

or in 250–500 mL 5% glucose injection for intravenous administration. The dosage and administration are not clearly described in every study (Table 1).

Two trials [33, 35] reported data on mortality. Most trials reported improvement of clinical symptoms and ECG.

All studies were at high risk of bias (Table 2). One trial [33] described the method of randomization in detail, and the method was also appropriate. All the other studies did not report information on the allocation concealment. One trial [37] mentioned it is a single-blinded study, and none were double blinded. Loss to followup was recorded in none of the studies. No studies conducted intention-to-treat analysis.

3.1. SS versus Western Medications. One trial [34] compared SS alone versus western medicine. There were no significant differences in improvement of clinical symptoms (RR 1.00, 95% CI 0.90 to 1.11, Figure 2) and improvement in ECG (RR 0.97, 95% CI 0.87 to 1.09, Figure 3).

3.2. SS + Western Medications versus Western Medications. Two trials [33, 35] comparing SS plus western medications versus western medications reported only one sudden death in the western medication group [33] (RR 0.50; 95% CI 0.02 to 12.13).

Sodium plus western medications achieved statistically significant improvement of clinical symptoms than western medications alone (RR 1.28, 95% CI 1.23 to 1.34, Figure 4), and improvement of ECG (RR 1.26, 95% CI 1.18 to 1.35, Figure 5), C-reaction protein (mean difference 2.10, 95% CI 1.63 to 2.58, Figure 6), and IL-6 (mean difference −3.85, 95% CI −4.10 to −3.60, Figure 7).

Of the 25 trials, 7 reported adverse events. In the routine treatment group, adverse reactions included headache, dizziness, facial flushing, fatigue, and bruises at injection site. Totally 32 cases were reported. In the SS + routine treatment group, adverse reactions included facial flushing, dizziness, bruises, tension or swell at injection site, blood in sputum, and gum bleeding. Totally 13 cases were reported. No severe adverse events were found and no treatment was stopped because of adverse events.

Because the funnel plot seems symmetric, the possibility that the result of this review might be misled by publication bias is likely to be little (Figure 8).

Study or subgroup	SS + routine			Routine			Weight	Mean difference	Mean difference
	Mean	SD	Total	Mean	SD	Total		IV, fixed, 95% CI	IV, fixed, 95% CI
Xu and Su 2008	5.25	0.22	37	9.1	0.88	37	74.2%	−3.85 [−4.14, −3.56]	
Yang 2010 et al.	5.45	0.94	32	9.31	1.08	32	25.8%	−3.86 [−4.36, −3.36]	
Total (95% CI)			69			69	100%	−3.85 [−4.1, −3.6]	

Heterogeneity: $\chi^2 = 0$, df = 1 ($P = 0.97$); $I^2 = 0\%$
Test for overall effect: $Z = 29.99$ ($P < 0.00001$)

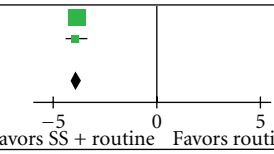

Figure 7: SS + routine therapy versus routine therapy, outcome: IL-6.

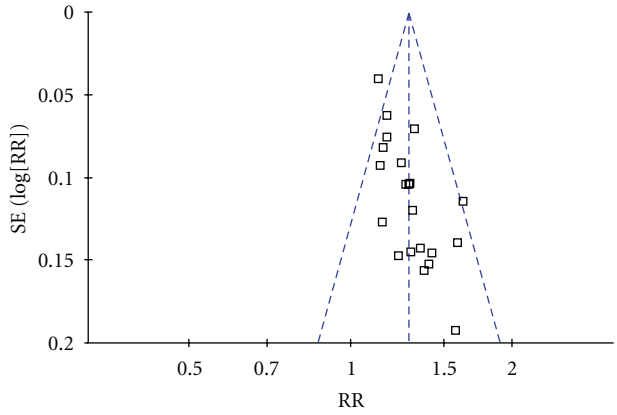

Figure 8: Funnel plot of comparison: SS plus routine therapy versus routine therapy, outcome: clinical symptom improvement. (Each dot represents one study. All the dots are conforming to a triangular form, meaning that publication bias is low).

4. Discussion

SS injection appeared an effective and safe treatment option for unstable angina pectoris. The present results showed that SS plus routine therapies appear to be more effective than western medications alone.

However, trials are at high risk of bias, making the findings less compelling. Except for one trial, none of the other trials reported the method of randomization. Although all trials claimed randomization, they failed to provide enough information to judge whether the randomization procedures had been carried out properly. No multicenter, large-scale RCTs were identified. No dropouts and withdrawals were described. No placebo control was used and none of the trials were of double blind. Routine therapy varied from trial to trial. The dosage and administration of control and trial therapies are not clearly described in every study.

The main outcomes from the included 25 trials were the improvement of clinical symptoms and ECG. The primary outcome measure was reported in only two trials. There is lack of data from RCTs on clinically relevant outcomes from long-term followup such as mortality and health-related quality of life.

18 out of 25 trials referred to observation of side effects. There were less side events in the SS injection group. None of the events were severe and no patients dropped out because of the side effects. SS injection appears to be relatively safe.

We have conducted comprehensive searches. However, only trials published in English and Chinese were indentified. Unpublished studies were found but none of them met the inclusion criteria. Since all of the trials were of small size with positive results and were conducted China, geographic biases may be induced.

The poor evidence does not allow any conclusion regarding the effectiveness of SS, and none of the included trials were ideally suited to investigate the effectiveness of SS in treating unstable angina. While SS is a widely used therapy for unstable angina in China, the results of the present review suggest that high-quality controlled trials are required for assessment.

5. Conclusions

Compared with western medications alone, SS combined with western medications was of more benefits for patients with unstable angina with fewer side effects. However, the methodological concerns, such as allocation concealment, lack of blinding, lack of information on the hazards of treatment, and the risk of publication bias, make it difficult to determine the role of SS injection in management of unstable angina.

Considering the strength of the evidence, more rigorously designed, randomized double-blind placebo-controlled trials are required for assessing the effects of SS injection before SS injection can be recommended routinely. Some aspects should be specially considered, including methodological improvement (such as details on the methods of randomization and the allocation concealment, blinding and placebo control, dropouts and withdrawals), adverse reactions, and reporting clinically outcomes from

long-term followup such as mortality and health-related quality life.

Disclosure

No grants or funding were provided for the performance of this study.

Conflict of Interests

There is no conflict of interests with any financial organization regarding what is discussed in the paper.

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Review Article

Natural Product Nitric Oxide Chemistry: New Activity of Old Medicines

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The use of complementary and alternative medicine (CAM) as a therapy and preventative care measure for cardiovascular diseases (CVD) may prove to be beneficial when used in conjunction with or in place of conventional medicine. However, the lack of understanding of a mechanism of action of many CAMs limits their use and acceptance in western medicine. We have recently recognized and characterized specific nitric oxide (NO) activity of select alternative and herbal medicines that may account for many of their reported health benefits. The ability of certain CAM to restore NO homeostasis both through enhancing endothelial production of NO and by providing a system for reducing nitrate and nitrite to NO as a compensatory pathway for repleting NO bioavailability may prove to be a safe and cost-effective strategy for combating CVD. We will review the current state of science behind NO activity of herbal medicines and their effects on CVD.

1. Introduction

Complementary and alternative medicine (CAM) is the term for medical products and practices that are not a part of standard care. There is generally a lack of understanding of their mechanisms of action and/or the active compounds. Rigorous, well-designed clinical trials for many CAM therapies are often lacking; therefore, the safety and effectiveness of many of these types of therapies are uncertain and as a result are not recognized as mainstream therapy. However, Traditional Chinese Medicine (TCM) is a form of CAM that remains the primary form of medicine throughout a large portion of Asia and Asian communities in the rest of world with a long history of safety and efficacy in a number of different diseases. In fact, one could argue that TCM is the earliest form of CAM. Defining CAM here in the USA is difficult because the field is very broad and constantly changing. The National Institutes of Health now has a dedicated Center for Complementary and Alternative Medicine (NCCAM) due to the growing popularity of such approaches to ensure safety and promote rigorous clinical trials to demonstrate efficacy. NCCAM defines CAM as a group of diverse medical and health care systems, practices, and products that are not generally considered part of conventional medicine, as practiced by medical doctors and their allied health professionals. The boundaries between CAM and conventional medicine are not absolute, and specific CAM practices may, over time, become widely accepted. Complementary medicine refers to use of alternative treatments together with conventional medicine, such as using acupuncture or herbal medicines in addition to usual care to help with disease management. Most use of CAM by Americans is complementary. Alternative medicine refers to use of CAM in place of conventional medicine. Integrative medicine combines treatments from conventional medicine and CAM for which there is some high-quality evidence of safety and effectiveness. It is also called integrated medicine. Since many CAM-based therapies are derived from TCM, we will provide a brief historical account behind the theory of TCM, and how this has now been integrated into CAM approaches here in the USA.

TCM has long been used as a major health care system in China and many other countries in Asia. Here in the USA, it

is typically referred to as CAM. TCM has its origin in ancient Taoist philosophy, which views a person as an energy system in which the body, mind, and spirit are unified into one when in harmony and are disrupted in disease. TCM practice treats the patient as a whole, not as a part, and it emphasizes a holistic approach that attempts to bring the mind, body, and spirit into harmony. TCM theory is extremely complex and originated thousands of years ago through meticulous observation of nature, the cosmos, and the human body. In TCM theory, imbalance between yin and yang is a summation of all kinds of basic disease and disorders. There is a growing and sustained interest in CAM and TCM fueled by a combination of factors including recognition of the benefits, dissatisfaction with and ineffectiveness of traditional Western medicines, increasing commitment to holistic care, skepticism regarding adverse side effects of drugs, and increasing evidence for the personalized nature of various combinations of herbs for specific disorders [1]. The use of TCM is increasing in Western nations, mostly among people of Southeast Asian origin. Patients who use TCM in Western countries report that the main reason for using it is that TCM is a "more natural" and potentially safer alternative in the treatment of chronic illness than pharmaceutical drugs or surgery [1]. Of the approximately 500 herbs that are in use today, 50 or so are very commonly used alone or in combination. Rather than being prescribed individually, single herbs are combined into formulas that are designed to adapt to the specific needs of individual patients. A herbal formula can contain from 3 to 25 herbs. Each herb has one or more of the four flavors/functions and one of five "temperatures" "氣" (pronounced "chi") (hot, warm, neutral, cool, cold). Herbal formulations work to balance the body from the inside out. Traditional herbal medicines include herbs, herbal materials, herbal preparations, and processed herbal products that contain parts of plants or other plant materials as active ingredients, which assist with strengthening the vital energies (chi), blood, and fluids internally. They are typically administered as tablets, pills, elixirs, soups, liquid extracts, and teas and broadly classified as dietary supplements here in the USA. There are a number of published reports on the association of TCM and NO-related effects [2, 3]. Recently, there are increased interests in the role for NO system in regulation of cardiovascular function by TCM used commonly for cardiovascular disorders. Recognizing and understanding NO activity of TCM may help explain centuries of treatment efficacy in a number of diseases and highlight new treatment options for conditions of NO insufficiency.

2. Current Global Markets for CAM

A 2002 survey of US adults 18 years and older conducted by the National Center for Health Statistics (CDC) and the NCCAM indicated that 74.6% had used some form of CAM, 62.1% had done so within the preceding 12 months, 54.9% used CAM in conjunction with conventional medicine, and 14.8% sought care from a licensed or certified practitioner suggesting that most individuals who use CAM prefer to treat themselves [4]. The industry of alternative and complementary medicines broadly classified as dietary supplements is expected to be $250 billion by 2016 worldwide. In 2004, a survey of nearly 1,400 US hospitals found that more than one in four offered alternative and complementary therapies such as acupuncture, homeopathy, and massage therapy. Herbal treatments are the most popular form of traditional medicine or CAM and are highly lucrative in the international marketplace. Annual revenues in Western Europe reached $5 billion in 2003-2004. In China, sales of products totaled $14 billion in 2005. Herbal medicine revenue in Brazil was $160 million in 2007, according to WHO Fact Sheet no. 134, 2008. Therefore, this industry and type of health care can no longer be ignored. In order to better understand the mechanism of action and the safety profile of herbal remedies, more research and resources are needed. Identifying physiological systems or molecular targets that may be affected by TCM will help propel the field and industry forward and create a better safety and efficacy profile of certain CAMs.

3. Production and Regulation of NO in the Cardiovascular System

Appropriate levels of NO production are critical in tissue blood perfusion and protection of cardiovascular tissues against ischemia and infarction. NO is endogenously generated from the amino acid L-arginine and molecular oxygen in reactions catalyzed by a family of enzymes called NO synthases (NOS). There are three mammalian NOS isoforms: neuronal (nNOS), endothelial (eNOS), and inducible (iNOS). They share 50–60% homology at the amino acid level [5]. Under physiological conditions, the dominant NOS isoform in the vasculature is eNOS, which rather than being a constitutive enzyme as was first suggested is dynamically regulated at the transcriptional, posttranscriptional, and posttranslational levels [6]. Endothelial dysfunction arises from downregulation of eNOS expression and activity and uncoupling of NOS generating free radicals [7]. We now recognize and appreciate that the endothelial production of NO declines progressively with age and can be further reduced by poor diet and lifestyle [8–10]. This becomes the basis for endothelial dysfunction and the etiology of a number of CVD-related symptoms. Restoring endogenous production of NO or providing an exogenous source of NO would be an attractive therapeutic option if this intervention could slow down the progression of endothelial dysfunction and reduce the risk of CVD.

Recently, an alternative pathway for NO generation was discovered, wherein the inorganic anions nitrate and nitrite, most often considered inert end products from NO oxidation, can be reduced back to NO and other bioactive nitrogen oxide species. This nitrate-nitrite-nitric oxide pathway is regulated differently than the classic L-arginine-nitric oxide synthase pathway as shown in Figure 1, and it is greatly enhanced during hypoxia and acidosis. Nitrite and nitrate have now moved to the forefront of NO biology [11] with the discovery that it represents a major storage form of NO in both blood and tissues [12]. Dietary nitrite and nitrate have been shown

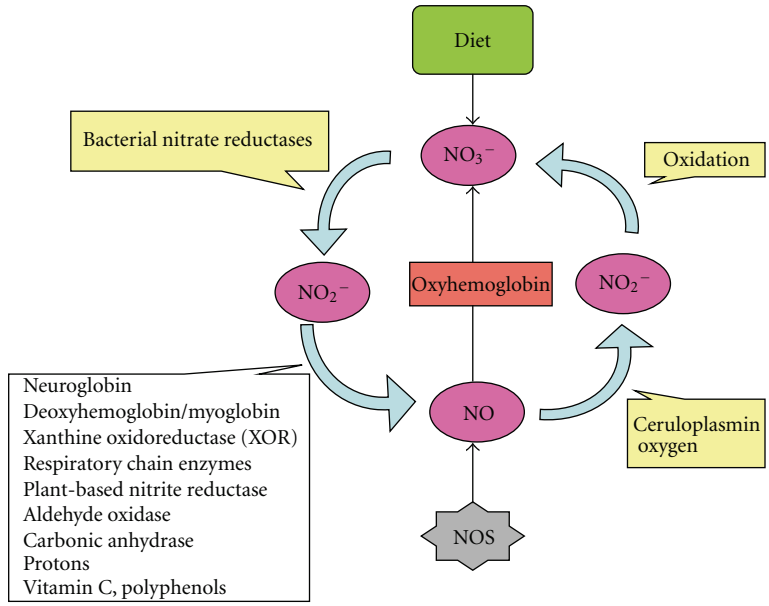

Figure 1: A schematic presentation of a mammalian nitric oxide (NO) cycle. NO is generated by nitric oxide synthases (NOS) in most cells of the body and participates in regulation of numerous physiologic functions. The bioactivity of nitric oxide is partly regulated by its rapid oxidation to nitrite (NO_2^-) or, in the presence of oxyhemoglobin, to nitrate (NO_3^-). Nitrate is the predominant nitric oxide oxidation product in the circulation. In our bodies, nitrate can undergo reduction to nitrite, and this process is strongly dependent on oral commensal bacteria. In blood and tissues, nitrite can be further reduced to nitric oxide and other bioactive nitrogen oxides. There are several enzymatic and nonenzymatic routes that can catalyze this reduction, most of which are greatly enhanced under hypoxic conditions. This mammalian nitrogen cycle can be fueled by the diet because vegetables contain high amounts of inorganic nitrate.

to protect from tissue injury and restore NO homeostasis in eNOS /− mice [13, 14]. Orally administered nitrite also attenuates cardiac allograft rejection in rats [15]. Nitrate is the primary anion present in green leafy vegetables and has been hypothesized to account for some of the health benefits of vegetables [16]. There are a number of published studies to support this hypothesis via its reduction to nitrite [17, 18]. The nitrate-nitrite-nitric oxide pathway is boosted by dietary intake of nitrate. Dietary nitrate supplementation has been shown to reduce diastolic blood pressure [19], inhibit platelet aggregation, and protect the heart from ischemia-reperfusion injury [20]. This pathway may also explain the effects of certain ethnic diets. Dietary nitrate in Japanese food was shown to lower blood pressure in healthy volunteers [21]. It is well established that diets rich in fruit and vegetables (e.g., the Mediterranean diet) protect against development of cardiovascular disease [22–24] and these data may provide evidence as to why.

The first metabolic activation step for this pathway requires commensal bacteria to reduce nitrate to nitrite [25]. There are a number of endogenous systems in mammals capable of reducing nitrite to NO although most are very inefficient and inhibited by oxygen [26]. For this system to proceed, there must be enough substrate for reduction and the necessary bacteria and reductive machinery to reduce nitrate all the way down to NO. Dietary and enzymatic sources of nitrate are a potentially large source of nitrite and ultimately NO in the human body. The nitrite converted from nitrate by oral bacteria disproportionates with formation of NO after entering the acidic environment of the stomach, helping to reduce gastrointestinal tract infection, increase mucous barrier thickness and increase gastric blood flow [27]. In addition to the simple protonation of nitrite in the stomach, there are several enzymatic pathways for conversion of systemic nitrite to NO and other bioactive nitrogen species. Hemoglobin, myoglobin, neuroglobin, xanthine oxidoreductase, aldehyde oxidase, carbonic anhydrase, and mitochondrial enzymes have all been identified with having a role in nitrite bioactivation [28] (Figure 1). However, all of these pathways are grossly inefficient along the physiological oxygen gradient with oxygen being a potent inhibitor of nitrite reduction [26, 29]. Therefore, for this system to generate enough bioactive NO, there must be sufficient nitrate and nitrite available for this inefficient reduction. A consequence of endothelial dysfunction is reduced blood and tissue levels of nitrite available for reduction [14, 30], further comprising this alternative NO pathway. Since most of the TCMs are herbal extracts, we initially thought they may be good sources of nitrite and nitrate. Also antioxidants and polyphenols have been shown to effectively reduce nitrite to NO [31]. This combination of nitrite, nitrate and polyphenols could provide then a system for repleting NO homeostasis. We recently tested and confirmed this hypothesis [32] and find that select herbal extracts contain high amounts of nitrate and also the capacity to reduce nitrite to NO. If our current paradigm is true, then restoration of NO homeostasis may be a primary target for treating and preventing CVD. A short review of this paradigm follows.

4. NO Insufficiency Is the Root Cause of CVD

CVD is a group of disorders including congestive heart failure (CHF) and ischemic heart disease that are becoming the leading cause of morbidity and mortality in the world [33, 34]. CVD can result from a quantitative or functional NO deficiency that can limit NO-dependent signal transduction pathways to the detriment of normal cellular function. NO formation by endothelial NO synthase (eNOS) plays an important role in the regulation of vasomotor tone in the pulmonary and systemic vascular beds [35–37]. However, NO generation by eNOS may be rapidly depleted in ischemic conditions, since eNOS is dependent upon the availability of oxygen [38, 39]. Loss of endogenous NO activity has a number of detrimental actions, most notably, vasoconstriction, increased activity and adherence of platelets, and accumulation of inflammatory cells at sites of endothelial damage. Endothelial damage is associated with most forms of CVD. NO possesses a number of physiological properties that make it a potent cardioprotective-signaling molecule [40]. First, NO is a potent vasodilator [41], which allows for regulation of blood flow and essential perfusion of tissue as needed. Secondly, NO reversibly inhibits mitochondrial respiration [42]. The inhibition of mitochondrial respiration during an ischemia-reperfusion event such as heart attack or stroke counterintuitively leads to a decrease in mitochondrial-driven injury by extending the zone of adequate tissue cellular oxygenation away from vessels [43]. Thirdly, NO is a potent inhibitor of neutrophil adherence to vascular endothelium [44]. Neutrophil adherence is an important event initiating further leukocyte activation and superoxide radical generation, which in turn leads to injury to the endothelium and perivascular myocardium in the ischemic heart [45]. Fourth, NO also prevents platelet aggregation [46], which together with the antineutrophil actions of NO attenuates capillary plugging [47]. Finally NO inhibits apoptosis directly or indirectly by inhibiting caspase-3-like activation via a cGMP-dependent mechanisms and/or through protein S-nitrosylation [48, 49]. Without sufficient NO production, the body loses its ability to regulate and control normal vascular function. Therefore, strategies designed to enhance NO production and reduce reactive oxygen species production will likely limit injury and improve recovery from CVD or perhaps even prevent onset and progression of CVD.

5. Therapeutic Effects and Nitric Oxide Bioactivity of TCM

Traditional herbal medicines used for thousands of years in Asia and other regions have been proven effective in certain cardiovascular disorders. Some of the herbal medicines have profound NO bioactivity primarily due to the nitrate-nitrite-NO reduction pathway [32]. They contain very large amounts of nitrate/nitrite in the extracts given to patients [32]. The described benefits of these ancient medications may be attributed to their inherent nitrate/nitrite content combined with their robust nitrate/nitrite reductase activity to generate NO independent of the L-arginine-NO pathway [14, 26, 32, 50]. The first use of nitrate for treatment of patients with symptoms that appears to be angina was described in an 8th century Chinese manuscript uncovered at the Buddhist grotto of Dunhuang [51]. The patients were instructed to take Xiao Shi Xiong Huang San, hold it under the tongue for a time, and then swallow the saliva. The significance of the instructions is that under the tongue, even in a healthy mouth, nitrate-reducing bacteria convert some of the nitrate into nitrite. Therefore, if the patient follows the physician's instructions fully, he or she will be taking nitrite, known to be effective in alleviating pain resulting from angina. Chinese physicians in traditional medicine have more recently tested the therapeutic effects of Xiao Shi Xiong Huang San (the Nitrum and Realgar Powder), one of the Dunhuang prescriptions, on angina pectoris caused by coronary heart disease. Compared to nitroglycerin, Xiao Shi Xiong Huang San showed much higher efficacy and improvement in a clinical trial of 61 patients [52]. Recently, the research results demonstrate that certain TCM increased NO release in rat vascular endothelial cells under hypoxia [53]. Purified ginsenoside Rg1 enhanced the production of NO from IFN-gamma-activated-macrophage cells RAW 264-7 [54]. Mu-Fang-Ji-Tang (TJ-36), a traditional Chinese herbal medicine, is made from four natural traditional Chinese drugs: Panax ginseng radix, Cinnamomum cassia, Sinomenum acutum, and Gypsum fibrosum, which is said to have been used for more than 1800 years to treat heart failure in China. Recent studies have shown that Mu-Fang-Ji-Tang (TJ-36) had protective effects against myocardial injury in a murine model of congestive heart failure induced by viral myocarditis [55]. Our studies have provided convincing evidence that many of these TCM contain significant amounts of nitrate and nitrite and also active nitrite reductase activity in certain herbs known to have protective or therapeutic benefits to patients with CVD (Table 1), such as the Danshen Root (radix salviae miltiorrhizae), Sanchi (radix notoginseng), and Hongshen (radix ginseng) [32]. These herbs, with specific indications for cardiovascular disease can generate NO from nitrite, and relax blood vessels. The therapeutic benefits of these herbal medicines are providing an alternative source of NO to patients that may be unable to make NO from L-arginine owing to endothelial dysfunction. There is an endogenous nitrite reductase activity in animal tissues, such as the liver and aorta, but this inherent biological capacity is low (around 1 pmoL/mg protein). The reductase activity in some of these herbal medicines may exceed that detected in the animal tissues [32]. It is estimated that the increased reductase activity may occur by orders of magnitude, almost 1000 times higher than endogenous production of NO. This would equate to 300 nmoles per day of NO from a single herbal preparation. The average NO production in the human body (70 kg) is 1.68 mmoL NO per day (based on an NO production rate of 1 μmoL/kg/h). By supplying the exogenous nitrate/nitrite and reductase activities, herbal medicines offer an alternative therapeutic strategy to combat or treat any condition related to NO insufficiency including heart disease and hypertension. Maintaining NO homeostasis requires the repletion of nitrite and nitrate through which the ability to generate NO can be restored to compensate

Table 1: The measurements of nitrite, nitrate, nitroso, nitrite reductase activity in several TCM herbs commonly used for CAD.

Chinese name	English name	Latin name	Indication	Nitrite (ng/g)	Nitrate (mg/g)	Nitroso (nmoL/g)	NO production (pmol/mg)
DanShen	Danahen Root	Radix Salviae Miltiorrhizae	CAD	330	12000	120	7
GuaLou	Snakegourd Fruit	Fructus Trichosanthis	CAD, acute MI, Hyperlipidemia	260	278	120	46
XieBai	Longstamen Onion Bulb	Bulbus Allii Macrostemi	CAD, acute MI, Hyperlipidemia	150	530	842	134
SanChi	Sanchi	Radix Notoginseng	CAD	210	2069	73	13
RuXiang	Frankincense	Resina Olibani	Hypertension	980	61	3210	72
ChiShao	Red Peony Root	Radix Paeonia Rubra	CAD	120	37	450	255
HongSheng	Ginseng	Radix Ginseng	Heart failure, CAD	300	243	76	360
BingPiang	Borneol	Borneolum Syntheticum	Increase other herb's function for CAD or brain disease	120	2.99	6	45
TianRanBingPiang	Borneol	Cinnamomum	Increase other herb's function for CAD or brain disease	160	2.39	0	875

for the inability of the endothelium to convert L-arginine to NO in coronary heart disease. This concept has recently been tested through the development of a rationally designed dietary supplement with natural products selected for their NO activity based on their nitrite, nitrate content, and an oxygen independent nitrite reductase. In a double-blinded placebo-controlled study in patients over the age of 40 with at least 3 cardiovascular risk factors, this type of technology was found to significantly restore plasma levels of nitrite and nitrate, reduce triglycerides by 27%, and modestly reduce blood pressure and C-reactive protein thereby modifying the cardiovascular risk profile of patients after only 30 days [56].

Recent clinical studies have provided new evidence on Danshen, a commonly used herbal medicine for CVD in China, which may have a similar efficacy to the known NO donor nitroglycerin [57–59]. The extract of *Salviae Miltiorrhiae*, or Danshen in Chinese, contains large amounts of nitrate [32]. The demonstration of beneficial effects of this herb on ischemic diseases offers an alternative avenue for the management of angina pectoris, myocardial infarction, or stroke [60, 61]. Danshen-related Chinese herbal medicines have been widely used for treatment of coronary heart disease in the East, and a clinical trial is on the way in the United States. Danshen is a routine herbal medication for acute angina pectoris. In addition, it may be effective for dyslipidemia, blood hyperviscosity syndrome, peripheral angiopathy (superficial thrombophlebitis, venous thrombosis, allergic arteriolitis), diabetes mellitus, and cirrhosis and is also used for altitude sickness. Experimental studies have shown that Danshen dilates coronary arteries, increases coronary blood flow, and scavenges free radicals in ischemic diseases, reducing cellular damage from ischemia and improving heart functions, remarkably similar to known effects of NO and nitrite [59]. However, the nitrate/nitrite reductase activity in Danshen is relatively low. Often, Danshen is mixed with other herbal products. One of which is an extract of cinnamon or borneol. Borneol is consumed excessively in China and Southeast Asian countries, particularly in a combined formula for preventing cardiovascular disease. Borneol exerts a concentration-dependent inhibitory effect on venous thrombosis [62]. The antithrombotic activity of borneol contributes to its action in combined formula for preventing cardiovascular diseases. Our recent studies have shown that although the natural form of borneol itself contains very little nitrite and nitrate, it displays a potent nitrite reductase activity [32] that when used in combination with nitrite and nitrate-rich DanShen provides the system for generating NO. Another herbal medicine made from the root of *Radix ginseng* may also have synergistic effects with Danshen. Ginseng contains modest amounts of nitrate but has stronger reductase activity [32]. Therefore, this may explain the mechanism behind the theory of synergy of specific combination of herbs.

6. Effects of TCM on L-Arginine/eNOS/NO Signaling Pathway

Some TCM may regulate NO production by exerting regulatory effects on the L-arginine/eNOS/NO signaling pathway. Puerarin is a major active ingredient extracted from the traditional Chinese medicine Ge-gen (Radix Puerariae, RP). It has long been used to treat cardiovascular diseases including coronary artery diseases (CAD), arrhythmia, and hypertension. Recent studies have shown that puerarin increases serum nitrite concentrations in rats with myocardial ischemia through the induction of protein expression and activation of eNOS and through Akt/PKB phosphorylation [63]. Tongxinluo (TXL), a mixture of traditional Chinese medicines, can attenuate the no-reflow and ischemia-reperfusion injury in an infarct animal model [64, 65]. TXL is composed of *Radix ginseng, Buthus martensii, Hirudo, Eupolyphaga seu steleophaga, Scolopendra subspinipes, Periostracum cicadae, Radix paeoniae rubra, Semen ziziphi spinosae, Lignum dalbergiae odoriferae, Lignum santali albi,* and *Borneolum syntheticum*. Owing to its efficacy and minimal adverse effects, TXL has been widely used in China to treat patients

with acute coronary syndrome. In a 90-minute ischemia and 3-hour reperfusion model, miniature pigs were randomly assigned to treatment with TXL (gavaged 1 hour prior to ischemia); TXL plus H-89 (protein kinase-A inhibitor intravenously infused before ischemia); or TXL plus N(omega)-nitro-L-arginine (L-NNA; an eNOS inhibitor, intravenously administered prior to ischemia). The results of this study demonstrate that TXL treatment can decrease creatine kinase elevation, improve coronary flow, and reduce infarct size. The effects of TXL may be partially abolished by H-89 or completely reversed by L-NNA. In addition, TXL treatment can elevate the kinase activity and expression, evidenced by expression of Thr198 phosphorated-PKA, Ser1179 phosphorated-eNOS (p-eNOS), and Ser635 p-eNOS in the ischemic myocardium. Addition of H-89 diminishes the TXL activities. Thus, pretreatment with a single low loading dose of TXL 1 hour before ischemia reduces the myocardial no-reflow phenomenon and ischemia-reperfusion injury by upregulating the phosphorylation of eNOS at Ser1179 and Ser635, and this effect is partially mediated by the PKA pathway [64]. Recent studies also have shown that Salvianolic acid B (Sal B) and Tanshinone IIA (Tan IIA) are two of the major components in Danshen, have cardioprotective effects in an in vivo myocardial infarction model of C57 mice, have vasodilator action in an ex vivo microartery system through the endothelial nitric oxide synthase (eNOS)/nitric oxide pathway, and are involved in the regulation of the L-arginine/eNOS/NO pathways in human umbilical vein endothelial cells (HUVECs). Both Sal B and Tan IIA inhibited cardiac hypertrophy and infarction sizes and improved cardiac function at 4 weeks after induction of infarction. Furthermore, an eNOS inhibitor (L-NAME) obliterated the observed effects. Sal B and Tan IIA mediated vasodilatation in mice coronaries ex vivo, the effect of which was decreased with either L-NAME or PI3K inhibitor (LY294002). In addition, Sal B and Tan IIA-induced vasodilatation was observed ex vivo in the microvessels of eNOS−/− mice. Sal B and Tan IIA also stimulated eNOS phosphorylation in a concentration- and time-dependent manner in the HUVEC culture, which was diminished by LY294002. In addition, Sal B and Tan IIA were found to stimulate the phosphorylation of AMPK (Thr(172)) and Akt (Ser(473)), while compound C significantly decreased the phosphorylation of Akt (Ser(473)) mediated by both. Finally, Sal B and Tan IIA were found to induce [(3)H]-L-arginine uptake and increase the CAT-1 and CAT-2B mRNA levels in HUVEC culture [66]. It appears that select TCM herbs alone and in combination can restore NO homeostasis both through an endothelium dependent manner by activating the L-arginine pathway and through an endothelium-independent manner via the nitrate-nitrite-NO pathway that may overcome endothelial dysfunction.

7. Effects of CAM on Oxidative Stress and Preservation of NO Activity

In several chronic diseases, free radicals are by-products of abnormal body metabolism and are important factors for late complications and secondary disease especially those related to NO and endothelial dysfunction [67]. There is increasing evidence that in certain pathologic states the increased production and/or ineffective scavenging of reactive oxygen species (ROS) may play a critical role. Medicinal plants are a source for a wide variety of natural antioxidants [68] and my exhibit their effects via several proposed mechanisms, including inhibition of the activities of cyclooxygenase-2 (COX-2) and nuclear factor-Kappa B (NF-κB), inhibition of angiogenesis, and activation of Nrf2-mediated antioxidant signaling [69], all known to interface with the NO pathway. Dang Gui has been shown to hold anti-inflammatory properties and antioxidant activities, especially when used concurrently with other herbs [70]. Dietz and colleagues have reported that the major lipophilic constituent of Dang Gui, Z-ligustilide, reduces oxidative stress through upregulation of antioxidant enzymes such as NQO1, an Nrf2 pathway gene [71]. Also, *A. sinensis* can protect against oxidant injury through elevated glutathione synthesis whose rate limiting step is regulated by the Nrf2-regulated gene γ-GCS [72]. Of the different components in Dan Shen, tanshinone IIA has been identified with the most potent antioxidant activity and cytotoxic properties by inducing apoptosis and differentiation in various human cancer cell lines [73]. Tanshinone IIA also displays antioxidant protection against reactive oxygen species (ROS) induced oxidative stress through stress-activated kinases JNKs and p38 MAPK and by an increase in scavenging of oxygen free radicals [74]. Drinking tea is a tradition that began in ancient China over 5000 years ago. Green tea from the leaves of *Camellia sinensis* is the second most consumed beverage in the world, after water. It is a well-studied herb for its effectiveness in chemoprevention, cancer therapy, and other benefits to overall health. The main components of green tea are polyphenols, known as catechins, which account for 30–42% of the solid weight in green tea leaves. The most abundant polyphenol in green tea is (−)-epigallocatechin-3-gallate (EGCG), which is also the most commonly studied green tea component [75]. EGCG contributes to 10–50% of the total polyphenol contents in green tea and appears to possess the strongest antioxidant activity, about 25–100 fold more potent than vitamins C and E [75]. Reducing oxidative stress and promoting endogenous antioxidant enzymatic defense systems very likely provides an additional benefit of traditional medicines. These antioxidants defenses will enhance endogenous NO production by keeping essential cofactors such as BH4 from becoming oxidized and also preserve and prolong NO activity once it is produced by preventing its scavenging by oxygen radicals.

8. Conclusion

Taken together, the NO signaling pathway plays an important role in the therapeutic effect of many CAMs used for cardiovascular disease (Figure 2). The NO donor-like herbs may serve as an alternative source of nitrate and nitrite or other NO-donors. They contain high activities of nitrite reductase that converts the inorganic anion into NO, which, in turn,

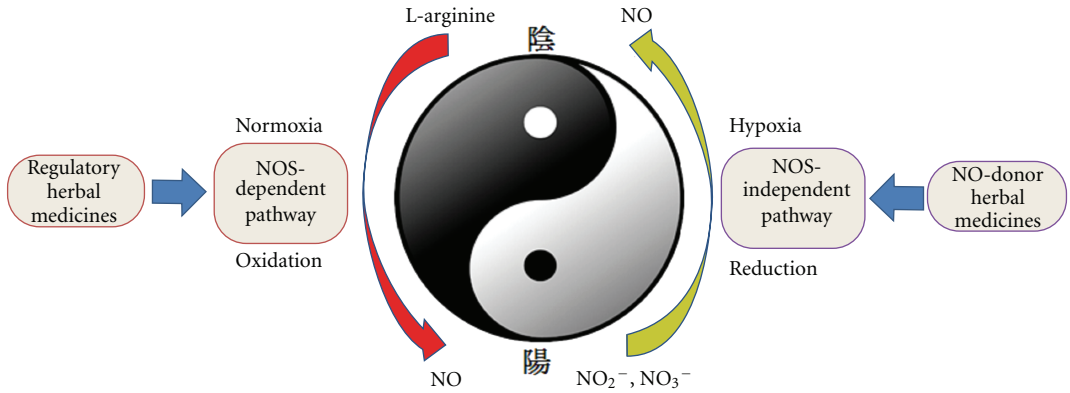

Figure 2: Potential mechanisms of TCM on the regulation of NO Signaling pathway TCM herbs alone and in combination can help restore NO homeostasis in the CVD and maintain "Yin-Yang" balance through reduction of Nitrate/nitrite and oxidation of L-arginine.

relaxes blood vessels and prevents thrombosis. There are also some TCM which contain regulatory factors that influence the expression and activity of eNOS (Figure 2) and may interact with endogenous factors that modulate L-arginine/eNOS/NO signaling pathway. Modern research is providing more evidence to understand specific activity of CAMs that will hopefully provide a mechanistic understanding of their clinical efficacy and allow for better combination of different herbs. However, currently the use of CAM is influenced by legal restrictions, with shifts towards increasing regulation and formal recognition of CAM as means to treat or prevent disease. Further investigation and research are warranted in terms of the functional mechanisms, biosafety, and large-scale clinical trials to firmly establish the efficacy of many of these approaches in the prevention and treatment of illnesses.

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Review Article

Traditional Chinese Herbal Products for Coronary Heart Disease: An Overview of Cochrane Reviews

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Objective. The aim of this overview was to evaluate and summarize Cochrane reviews of traditional Chinese herbal products (TCHPs) as the treatment for coronary heart disease (CHD). *Methods*. We searched the Cochrane Database that was concerned with the effectiveness of TCHPs for CHD. We also searched the Cochrane Central Register of Controlled Trials. Reviews and primary studies of TCHP as the treatment of any type of CHD were included. Data were extracted according to predefined inclusion criteria by two independent reviewers. *Results*. Six Cochrane reviews were included. They related to a wide range of TCHPs for different types of CHD. Four reviews were concerned with angina pectoris (unstable or stable), one review was concerned with heart failure, and for acute myocardial infarction. No reviews concluded that TCHPs were definitely effective for CHD because of the weak evidence. Eight primary studies were TCHPs from CHD. These studies also maybe result in bias, but better than before. *Conclusion*. Several Cochrane reviews of TCHPs for the treatment of different types of CHD have recently been published. None of these reviews got definite conclusion favoring the effectiveness of TCHPs due to the weak evidence. With the improved quality of the new registered RCTs. The potential role of TCHPs in treating CHD is anticipated to be detected.

1. Introduction

Coronary heart disease (CHD) is one of the most dangerous threats to human health, manifested by different clinical types such as angina pectoris, myocardial infarction, heart failure, cardiac arrhythmia, and so forth. Although treated with intensive medication or revascularization therapy, uncontrolled angina and recurrent acute cardiovascular events are still the major problems confronting modern medicine. Traditional Chinese medicine (TCM) has a history of thousands of years and has made great contributions to the health and well-being of the people and to the maintenance and growth of the population [1]. Currently, more than 90% of the urban and rural Chinese population has sought for TCM in their lifetimes [2]. TCM has been studied extensively and seems to be safe and effective in treating CHD [3, 4]. Recently, the potential benefit of integrative Western and Chinese medicine regimen has also been indicated in a large-scale registry study in China [5]. Cochrane reviews are regarded as the highest standard of evidence [6]. They adopt transparent and comprehensive methods of finding all of the relevant evidence. Their quality and reliability are generally higher than any other systematic review because they employ a predefined, rigorous, and explicit methodology. Cochrane reviews are also reviewed and published in advance. Therefore, conclusion made from the overview of Cochrane reviews is more credible. Some Cochrane systematic reviews of traditional Chinese herbal products (TCHPs) for CHD have been conducted in recent years. These reviews provide preliminary evidence of TCHPs benefits to certain CHD patient populations, which call for a comprehensive evaluation on the effectiveness of TCHPs in CHD patients. This overview aims to evaluate and summarize all Cochrane reviews of TCHP as a treatment of CHD critically.

2. Methods

We searched the titles and abstracts of all reviews in September 2011 of the Cochrane Database of Systematic Review.

Table 1: Cochrane Reviews of TCHP for CHD.

First author	TCHP	Control group	Condition	Number of RCTs	Participants	Conclusion
Wu et al. [8]	Danshen as part of decoction	Different basic treatment	Acute myocardial infarction	6	2368	B
Wang et al. [9]	Puerarin	Different basic treatment	Unstable angina pectoris	20	1240	A
Zheng et al. [10]	Different forms of Shengmai	Different basic treatment	Heart failure	6	440	A
Duan et al. [11]	Suxiao jiuxin wan	Isosorbide dinitrate or nitroglycerin or other TCHP	Angina pectoris	15	1776	A
Wu et al. [12]	Tongxinluo	Different basic treatment	Unstable angina pectoris	18	1413	A
Zhuo et al. [13]	Different herbal products	Isosorbide dinitrate or other TCHP	Stable angina	3	216	B

Notes: RCT: randomized clinical trial.
A: TCHP may be or appears to be effective.
B: The evidence is insufficient, reliable conclusions could not be drawn.

The search terms were "Herb* and medic* and heart" and "Herb* and medic* and cardiac" and "Herb* and medic* and circulation" and "Chinese and heart" and "Chinese and cardiac" and "Chinese and circulation." We read the title and abstract of each retrieved review in order to confirm that the review was relevant. Articles were included if they related to any type of TCHP as a treatment of CHD. Data were extracted according to predefined inclusion criteria by two independent reviewers (Qiu Y. and Xu H.). Disagreements were resolved by discussion between the authors.

We also searched the Cochrane Central Register of Controlled Trials (CENTRAL) in The Cochrane Library Issue 4 of 4, Oct 2011. Studies of TCHP as the treatment of any type of CHD were included. Studies without results were excluded. The methodological quality was assessed using the Cochrane Collaboration risk of bias criteria with 6 domains [7]: (1) random, (2) blinding of participants, doctor, and outcome assessors, (3) allocation concealment, (4) incomplete outcome data, (5) free of the suggestion of selective outcome reporting, and (6) informed consents. Discrepancies were resolved by consensus through discussion between the two reviewers.

3. Results

Six articles met our inclusion criteria (Table 1) [8–13]. The Cochrane reviews included were published between 2006 and 2011. The studies in these reviews mainly originated from China. They included between 3 and 18 primary studies. Four reviews were concerned with angina pectoris (unstable or stable) [9, 11–13], one review was concerned with heart failure [10] (heart failure was primary caused by CHD), and one review was concerned with acute myocardial infarction [8].

Four Cochrane reviews concluded positively that TCHP may be or appears to be effective. Two reviews showed that the evidence is too weak to make conclusion. No reviews made definite conclusion. All reviews indicated that high-quality trials are required to assess the efficacy and safety of TCHP for CHD and the finding should be interpreted with care because of the very low methodological quality of studies and potential publication bias.

There are 69 studies in the six reviews. Two studies were reported from 1981 to 1985; one study was reported from 1986 to 1990; three studies were reported from 1991 to 1995; twenty-six studies were reported from 1996 to 2000; thirty-five studies were reported from 2001 to 2005; only two studies were reported from 2006 to 2011. Therefore, the most likely reason for the weak evidence of TCHP for CHD is the previous poor methodology.

The randomized clinical trials (RCTs) contained in four Cochrane reviews [8–10, 12] were mainly on the basis of conventional western medicine. But the basic treatment is not unchangeable. The RCTslisted in two Cochrane reviews [11, 13] directly contrasted one TCHP with western medicine or other TCHP. Two Cochrane reviews [8, 13] summarized different TCHP for CHD. The TCHP mentioned in these RCTs were injection (e.g., Shengmai Injection, Puerarin), oral Chinese patent medicine (e.g., Yi Xin Mai, Bao Xin Bao, Li Nao Xin, Shengmai Oral Liquid, Suxiao Jiuxin Wan, Tong Xin Luo), or Chinese herbal decoction. Four Cochrane reviews [9–12] summarized single TCHP for CHD.

In order to assess the status of the quality of the studies of TCHP, we also searched the CENTRAL in The Cochrane Library Issue 4 of 4 Oct 2011. Eight studies were included (Table 2) [14–21]. These studies primary originated from China. These studies were all making an explicit statement that the participants were randomly assigned to different groups, but two were not describing the details. Only four RCTS adopted the application of blinding: one did not report details [18] and three reported that the participants and doctors were blind [14, 19, 21]. One of the trials adopted allocation concealment [14]. Trials with inadequate blinding and inadequate allocation concealment may result in limited evidence. Six trials did well in the incomplete outcome data adequately addressed [14–16, 18, 19, 21]. Only one trial did well in the free of the suggestion of selective outcome reporting [18]. Not every trial made explicit statement that the participants signed the informed consents [16, 17, 19]. These RCTs had more participants

Table 2: Registered Studies of TCHP for CHD in the Cochrane Central Register of Controlled Trials.

First author	TCHP	Condition	Participants	Random	Blinding of participants, personnel, or outcome assessors	Allocation concealment	Incomplete outcome data adequately addressed	Free of the suggestion of selective outcome reporting	Informed consents	Conclusion
Chu et al. [14]	Xuefu Zhuyu capsule	Unstable anginal patients after percutaneous coronary intervention	90	Yes but no details	Participants and doctor	Yes	Yes	Unclear	Yes	A+
Li et al. [15]	Specific TCHP	Myocardial perfusion in AMI patients after revascularization	80	Yes	Not mentioned	Not mentioned	Yes	Unclear	Yes	A+
Li et al. [16]	Specific TCHP	Ventricular wall motion in AMI patients after revascularization	80	Yes	Not mentioned	Not mentioned	Yes	Unclear	Unclear	A
Hu et al. [17]	Shenfu injection	Heart function in patients with chronic heart failure	63	Yes but no details	Not mentioned	Not mentioned	Unclear	Unclear	No	A+
Tam et al. [18]	Salvia miltiorrhiza and Pueraria lobata	Vascular function and structure in coronary patients	100	Yes	Double-blind but no details	Not mentioned	Yes	Yes	Yes	A+
Qiu et al. [19]	Specific TCHP	The clinical symptoms and quality of life of the AMI patients undergoing PCI	35	Yes	Participants and doctor	Not mentioned	Yes	No	Unclear	A+
Fan et al. [20]	Qihong decoction	Rehabilitation of patients after coronary artery bypass	72	Yes	Not mentioned	Not mentioned	No	No	No	A+
Wang et al. [21]	Shenshao tablet	The quality of life for CHD patients with UA	66	Yes	Participants and doctor	Not mentioned	Yes	Unclear	Yes	A+

Notes: A+: TCHP has definitely effect.
A: TCHP may be or appears to be effective.

than usual RCTs. They usually have 60 to 100 participants [15–18, 20, 21]; only 1 RCT has 35 participants [19] and 1 RCT has 859 participants [14]. These shortcomings highlight the importance of following CONSORT procedures in the future studies [22]. Anyway, the quality of primary studies was better than before, and we still need further progress.

4. Discussion

The current Cochrane reviews indicated the potential benefit of TCHP in treating CHD, but none of them drew a definite conclusion because of the poor quality of primary studies. Although Cochrane reviews have the reputation for being more transparent and rigorous than other systematic reviews, the conclusion needs further discussion. The RCTs listed in two reviews [8, 13] were not the same TCHP. The treatments in the control groups, and the durations of the RCTs were also varied. In addition, different TCHP applys to different syndrome according to TCM theory. All of these reviews did not involve this question.

Therefore, four reviews [9–12] about single TCHP are more persuasive. They all made the conclusion of "A," indicating the TCHP may be or appears to be effective. The other two reviews made the conclusion of "B." One review about "Danshen for acute myocardial infarction" concerned with the herb Danshen, but Danshen was not the only part of the treatment. Thus the heterogeneity of included RCTs cannot be ignored. The other review of "herbal products for stable angina" is concerned with three different TCHPs comparing with isosorbide dinitrate [13]. It also made the conclusion of "B," indicating the evidence is insufficient and reliable conclusions could not be drawn.

In conclusion, although some Cochrane reviews have shown the potential benefit of TCHP in treating CHD, more evidence from high-quality trials is needed to support the clinical use of TCHP. However, well-designed randomized clinical trials of TCHP with rigorous methodology are in progress or have been completed at several institutions around the world [6]. We hope that the effectiveness and safety of TCHP can be confirmed in the near future.

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Research Article

Ethanolic Extract of *Vitis thunbergii* Exhibits Lipid Lowering Properties via Modulation of the AMPK-ACC Pathway in Hypercholesterolemic Rabbits

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Vitis thunbergii (VT) is a wild grape that has been shown to provide various cardioprotective effects. The present study was designed to examine whether a VT extract could reduce serum lipid levels and prevent atherogenesis in a hypercholesterolemic rabbit model. At the end of an 8-week study, our results showed that a VT extract supplement markedly suppressed the serum levels of cholesterol and low-density lipoprotein, reduced lipid accumulation in liver tissues, and limited aortic fatty streaks. Our findings suggest that the VT extract activated AMPK (5′-adenosine monophosphate-activated protein kinase) with subsequent inhibition of the activation of ACC (acetyl-CoA carboxylase). Our results suggest that this VT extract could be further developed as a potential lipid-lowering agent and as a natural health food to prevent atherogenesis.

1. Introduction

The treatment and prevention of cardiovascular diseases, particularly atherosclerosis, remains a critical research topic for modern medicine. Atherosclerosis is a slow progressive disease and is the most common cardiovascular disease among Western societies, where it remains the leading cause of both illness and death. Atherosclerosis may be initially caused by damage to the inner layers (endothelium) of the arteries from several risk factors, which include hypercholesterolemia (or hyperlipidemia), hypertension, diabetes, and cigarette smoking. Lipid-lowering agents, which include statins and fibrates, reduce the blood levels of fats such as cholesterol and triglycerides and have become some of the most common and effective prescribed drugs for the treatment of atherosclerosis. Nevertheless, the clinically adverse side effect of myotoxicity has been associated with the use of lipid-lowering drugs, and this condition may eventually lead to renal failure and death in the worst cases [1]. Accordingly, developing new lipid-lowering agents or natural supplements is an important issue that warrants further study.

AMPK (5′-adenosine monophosphate-activated protein kinase) is a known physiological cellular senor for energy homeostasis. This enzyme is composed of three parts (α, β, and γ subunits) that bind together to make a functional kinase. The energy-sensing capability of AMPK can be attributed to its ability to detect and react to the changes in the ratio of AMP/ATP molecules [2]. Under conditions of fasting or an insufficient energy supply, activated AMPK regulates several intracellular metabolic systems to either generate energy or reduce energy depletion. These metabolic changes include increases in the cellular uptake of glucose through inhibiting gluconeogenesis [3, 4] and the upregulation of the glucose transporter type 4 (GLUT4) [4, 5], the acceleration of lipid catabolism *via* the suppression of acetyl-CoA carboxylase (ACC) [6], inhibition of cholesterol synthesis via depressed activity of HMG-CoA reductase (HMGCR) [7], and decreased fatty acid *de novo* biosynthesis via suppression of fatty acid synthase (FAS) [8]. Accordingly, AMPK protein has the potential to be a therapeutic drug target for the treatment of hyperlipidemia or atherosclerosis.

Vitis thunbergii (VT) is a wild grape that is native to Taiwan and East Asia. It is widely used in medicinal wines and beverages. Several natural components isolated from *V. thunbergii* have been shown to provide various beneficial pharmacological effects, including antiinflammation [9, 10],

antioxidation [11], antitumor [12], antiplatelet aggregation [11], antimicrobial [13], and neuroprotective activities [14, 15]. Although some of the isolated compounds from *V. thunbergii* have cardioprotective effects, their effects on lipid levels and atherosclerosis are unknown. Therefore, the purpose of the present study was to evaluate whether *V. thunbergii* could reduce serum lipid levels and prevent atherosclerosis formation. This study also investigated whether the molecular mechanisms underlying the lipid-lowering effects of *V. thunbergii* are associated with AMPK pathway activation.

2. Materials and Methods

2.1. Materials. *V. thunbergii* was kindly provided and plant origin identified by Dr. Tzer-Kaun Hu (Department of Agronomy, National Chung Hsing University, Taiwan). Anti-phospho-ACC (#3661) and anti-phospho-AMPKα (#2531s) were obtained from Cell signaling Technology, Inc. (Beverly, MA, USA). The primary antibodies against β-actin (#ab6276) and HMGCR (#07-457) were purchased from Abcam (Cambridge, MA, USA) and Millipore/Upstate (Bedford, MA, USA), respectively. Horseradish peroxidase-labeled secondary antibodies against mouse IgG (#sc2005) and rabbit IgG (#sc2004) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other reagents were purchased from Sigma-Aldrich (Louis, MO, USA).

2.2. Preparation of V. thunbergii Extract. *V. thunbergii* was homogenized to a powder. Subsequently, the homogenized material (about 1 kg) was soaked in 10 L of 50% ethanol solution (extractive solvent) at 80°C for 1 hr. The solid residue of the extracted herbs was filtered and discarded through a Buchner funnel lined with Whatman filter paper. The filtrate was concentrated to a paste by distillation under reduced pressure. The concentrated *V. thunbergii* extract was further diluted with deionized water for all of the subsequent experiments.

2.3. Experimental Model. Twenty-four male New Zealand White rabbits (average of six-week-old) were purchased from Lu-Hop ranch (Changhua, Taiwan). The animals were housed in individual cages with free access to water and maintained on a 12 hr light/dark photocycle. All animal care followed the institutional animal ethical guidelines of China Medical University. After 1 week of adaptation, the rabbits were randomly divided into four groups, which were fed the following daily treatment diets for 8 weeks: a regular diet (Control group; FwuSow Ind., Taiwan), 0.5% (w/w) cholesterol diet alone (CHOL group), 0.5% (w/w) cholesterol diet with 0.01% (w/w) lovastatin supplement (LOVA group; YungShin Pharm. Ind., Taiwan), and 0.5% (w/w) cholesterol diet with a 7% (w/w) *V. thunbergii* extract supplement (VT group). The daily feeding amount for each rabbit was 50 g/kg body weight per day. At the beginning and end of the 8-week study, the rabbits were anesthetized by an intramuscular injection of Zoletil 50 (1 mL/kg) (Virbac Ltd., France), and blood samples were harvested. Finally, the aortas (from aortic arch to the bifurcation of the iliac arteries) and whole livers were collected from the rabbits after they were sacrificed for further histopathological and western blot analyses.

2.4. Blood Chemistry Analysis. The animals were fasted overnight before blood drawing. The blood was collected from the marginal ear veins of rabbits into BD Vacutainer EDTA Blood Collection Tubes. Plasma was separated by centrifugation at 3,000 rpm at 4°C for 10 min. Measurements for changes in blood chemistry parameters included serum levels of low-density lipoprotein (LDL), cholesterol (Chol), triglycerides (TG), glutamate oxaloacetate transaminase (GOT), and glutamate pyruvate transaminase (GPT) (ZhenXing Co., Ltd, Taiwan).

2.5. Cryosectioning of Liver Tissues. The rabbit liver tissues were perfused with normal saline and fixed in 10% (v/v) formalin-neutralized solution (J.T. Baker, Inc., USA) for 24 hr. Afterward, the tissues were embedded in Tissue-Tek OCT Compound (#4583; Sakura Finetek Inc., USA). Embedded tissues were cut into 10 μm thick slices and stained with Sudan IV and hematoxylin (Merck, USA). Briefly, the slices were washed with pure water for 1 min to remove the OCT compound, washed with 50% (v/v) ethanol for 30 sec, and then stained with 2% (w/v) Sudan IV for 1 hr. After further washing with 50% (v/v) ethanol and pure water for 2 min, the slices were counterstained with hematoxylin. Photographs were acquired using a microscope equipped with a 10-fold magnification objective and quantified on an Alpha Imager 2200 documentation system (Alpha Innotech, USA). The manifestation of fatty liver progression was presented as the percentage of the area of oil droplets to the total liver tissues (cells).

2.6. Aortic Fatty Streak Staining. The aortas were opened longitudinally to expose the intimal surface and rinsed gently with normal saline. Aortas were incubated in 2% (w/v) Sudan IV, rinsed with several concentrations (100%, 90%, 80%, 70%, and 60%) of ethanol for 1 min, and then rinsed with pure water. The photographs were acquired using a digital camera (Nikon D80, Japan) and quantified on an Alpha Imager 2200 documentation system (Alpha Innotech, USA). The progression of the fatty streak lesions was presented as the percentage of the stained area to the total area.

2.7. Western Blot. Proteins extracted from the frozen liver tissues were subjected to SDS-PAGE under reducing conditions on 10% acrylamide gels and transferred to polyvinylidene fluoride (PVDF) membranes by electroblotting. After blockade of nonspecific binding sites, membranes were incubated with primary antibodies (1 : 1,000 dilution), followed by horseradish peroxidase-conjugated secondary antibodies (1 : 2,000 dilution). Protein expression was visualized using SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, USA), and the luminescence signal was acquired and analyzed with a Fujifilm LAS-4000 system (Japan). The amounts of p-AMPKα, p-ACC, and HMGCR

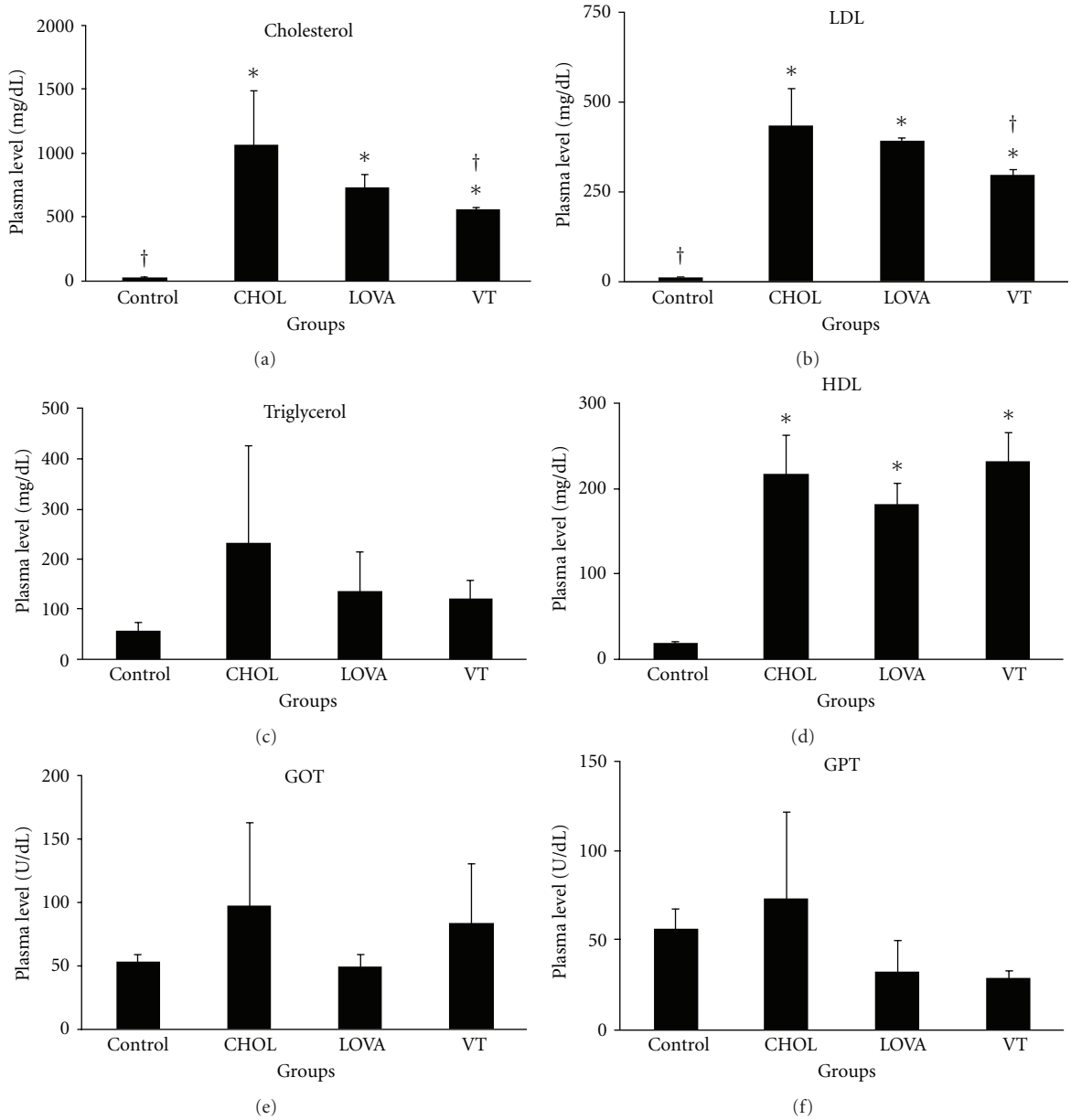

Figure 1: Blood chemistry parameters were measured in the hypercholesterolemic rabbit model after the 8-week study. Control group: regular diet; CHOL group: 0.5% (w/w) cholesterol diet alone; LOVA group: 0.5% (w/w) cholesterol diet with 0.01% (w/w) lovastatin; VT group: 0.5% (w/w) cholesterol diet with 7% (w/w) *V. thunbergii* extract. * and † indicate a $P < 0.05$ as compared with the control group and CHOL group, respectively.

were expressed relative to the amount of β-actin (loading control).

2.8. Statistical Analysis. All values are expressed as mean ± standard deviation (SD). Data were compared with a one-way analysis of variance (ANOVA) to evaluate differences among multiple groups. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Regulatory Effect of VT Extract on Serum Lipids. The blood chemistry parameters were examined to evaluate whether the VT extract could reduce serum lipids (Figure 1). Our data revealed that the plasma level of cholesterol, LDL, triglycerides, HDL, GOT, and GPT did not significantly vary among the different groups prior to study (data not shown). At the end of the 8-week study, our results showed that a 0.5% (w/w) cholesterol diet markedly stimulated plasma levels of cholesterol, LDL, and HDL (Figures 1(a), 1(b), and 1(d)) and slightly increased triglyceride levels without statistical significance (Figure 1(c)). Under the same conditions, a 7% (w/w) VT extract significantly reversed a 0.5% (w/w) cholesterol diet-induced accumulation of serum lipids, which was similar to the effect in the LOVA group (Figures 1(a) and 1(b)). Besides, the serum LDL/HDL ratio

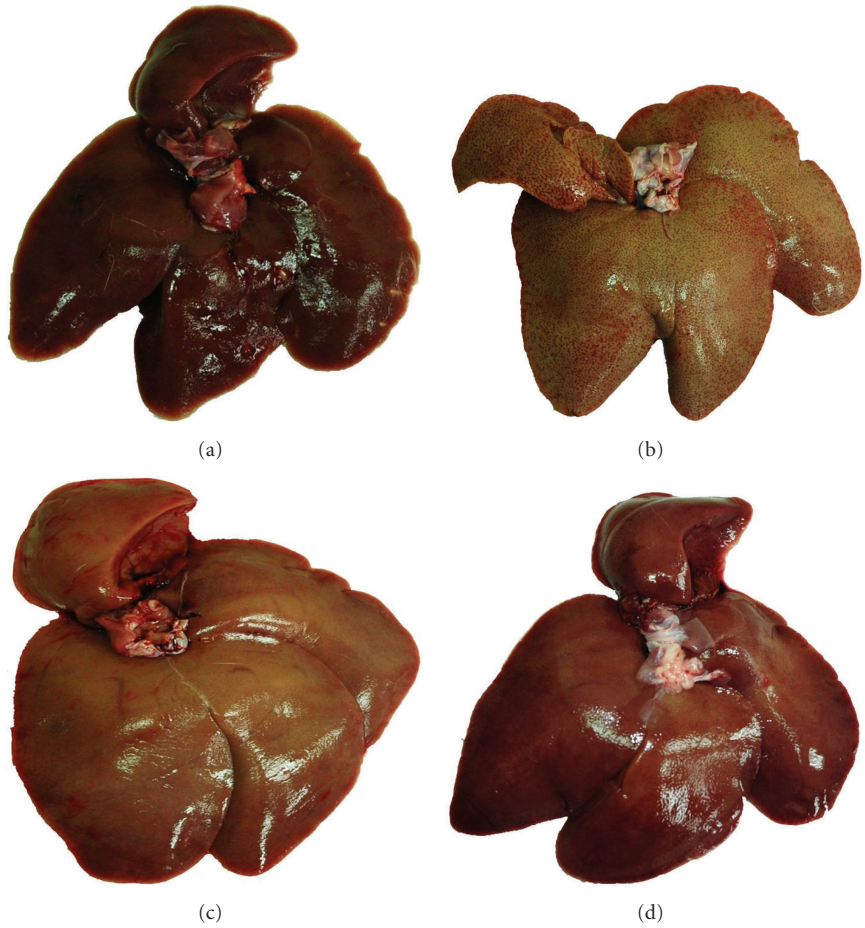

Figure 2: Photographs of liver appearance in the hypercholesterolemic rabbit model after the 8-week study. (a) Control group; (b) CHOL group; (c) LOVA group; (d) VT group.

was increased by 0.5% (w/w) cholesterol diet (ratio = 1.97) as compared with control group (ratio = 0.36), which can be reversed by 7% (v/v) VT supplement (ratio = 1.26). Furthermore, the experimental data also demonstrated that a 7% (w/w) VT extract supplement did not cause obvious liver damage or toxicity, which was determined by examining plasma levels of GOT and GPT at the end of the 8-week study (Figures 1(e) and 1(f)).

3.2. Regulatory Effect of VT Extract on Fatty Liver Disease and Lipid Accumulation. After the 8-week study, a histopathological analysis of liver cryosections was performed in order to determine whether the VT extract could prevent the formation of fatty liver (Figure 2) and lipid accumulation within liver tissues (Figure 3). Our data demonstrated that a 0.5% (w/w) cholesterol diet could induce phenomena resembling fatty liver (Figure 2(b)) and a 7% (w/w) VT extract reduced the severity of cholesterol diet-induced fatty liver (Figure 2(d)). Similarly, a 0.5% (w/w) cholesterol diet also increased lipid (or oil droplets) accumulation within the liver tissues (Figures 3(b) and 3(e)), and this effect was markedly reversed by a 7% (w/w) VT extract supplement (Figures 3(d) and 3(e)), which was similar to the results of the LOVA group (Figures 3(c) and 3(e)).

3.3. Regulatory Effect of VT Extract on Atherosclerosis Formation. Sudan IV staining of the fatty streak lesions within the aorta was used to estimate whether the VT extract could reduce the formation of atherosclerosis plaques after the 8-week study (Figure 4). The present study showed that a 0.5% (w/w) cholesterol diet could dramatically increase aortic fatty streak lesions as compared with the control group (Figures 4(a), 4(b), and 4(e)). In addition, a 7% (w/w) VT extract markedly reduced the intensity of fatty streaks on the aorta intima as compared with the CHOL group (Figures 4(b), 4(d), and 4(e)), which was the same result as the LOVA group (Figures 4(b), 4(c), and 4(e)).

3.4. Regulatory Effect of VT Extract on Lipid Metabolism-Associated Proteins. Lipid metabolism-associated proteins such as AMPK, ACC, and HMGCR were examined to determine the molecular mechanisms underlying these VT extract-induced lipid-lowering effects (Figure 5(a)). Experimental data revealed that the 8-week treatment with the 0.5% (w/w) cholesterol diet significantly decreased the protein expression of phospho-AMPK (Figure 5(b)), phospho-ACC (Figure 5(c)), and HMGCR (Figure 5(d)). The addition of a 7% (w/w) VT extract clearly returned the expression of these

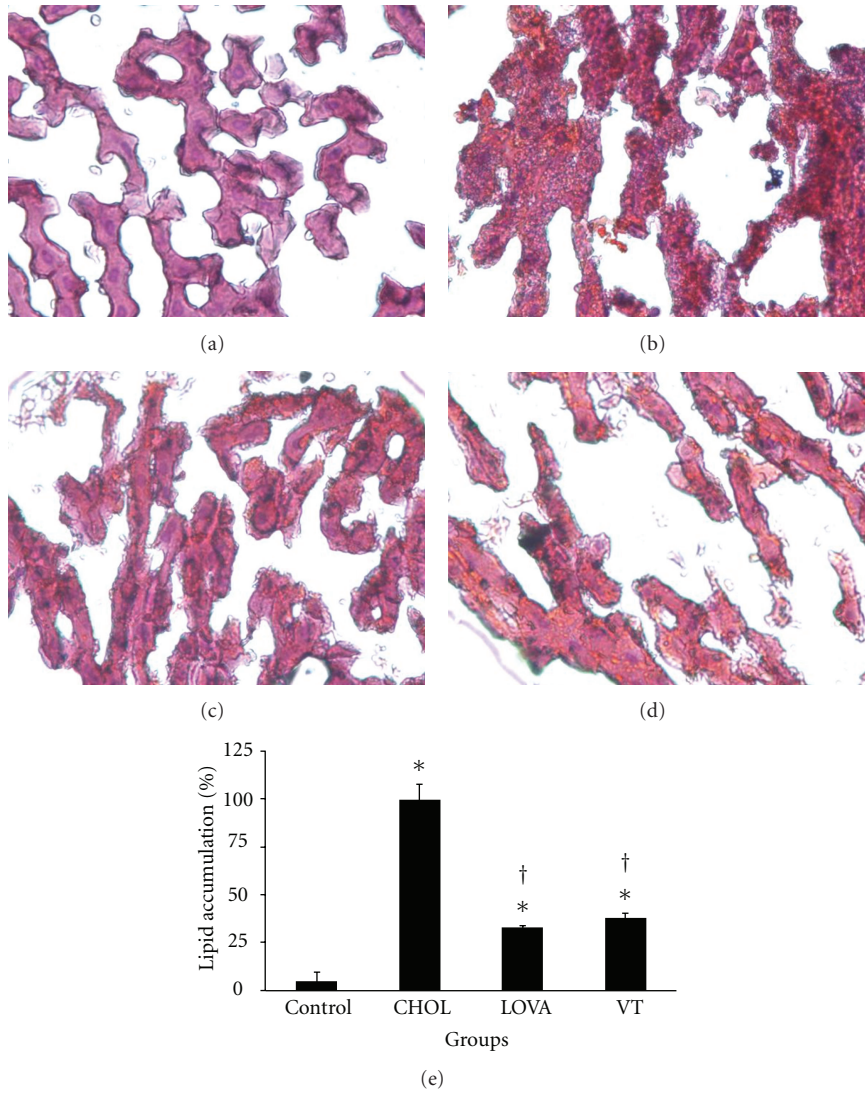

Figure 3: Histopathochemical examination of liver tissues in the hypercholesterolemic rabbit model after the 8-week study. (a) Control group; (b) CHOL group; (c) LOVA group; (d) VT group; (e) relative lipid accumulation within liver tissues among different groups. * and † indicate a $P < 0.05$ as compared with the control group and CHOL group, respectively.

proteins to near basal levels as compared with the CHOL group, which was the same result as the LOVA group.

4. Discussion

Cardiovascular diseases such as coronary heart disease and myocardial infarction are complications of atherosclerosis. In addition, abnormalities in lipid metabolism and coagulation are major contributors to the pathology of atherosclerosis. *V. thunbergii* has been demonstrated to provide many cardiac benefits that may be modulated through some of its identified components, which include the following compounds: β-sitosterol, β-sitosterol-3-O-β-D-glucoside, ampelopsin C, betulinic acid, botulin, caffeic acid, friedelin, heyneanol A, l-dotriacontanol, lupeol, luteolin-7-O-glucoside, miyabenol A, narcissin, oligostilbenes, piceatannol, quercetin-3-O-galactoside, quercitrin, rutin, stigmasterol, triacontanoic acid, vanillic acid, viniferin, viniferal, vitisin A, vitisin C, and vitisinols A-D [11–13, 16, 17]. Of these components, ampelopsin C, miyabenol A, viniferin, viniferal, vitisin A, vitisin C, and vitisinols A-D belong to the class of resveratrol derivatives. Resveratrol is a polyphenolic antioxidant that is found in grape skin and is strongly related to the cardioprotective effect of red wine [18], which is a potent atheroprotective compound [19, 20]. Growing evidences suggest that resveratrol protects the cardiovascular system in numerous ways, including antiapoptotic [21, 22], antiplatelet [11, 23, 24], antioxidative [25–27], and antiinflammatory effects [28–30], as well as regulating lipid metabolism [20, 31].

The regulation of lipid metabolism by resveratrol is related to the modulation of lipid turnover, inhibition of eicosanoid production, and prevention of low-density lipoprotein oxidation [31]. Several *in vivo* studies have revealed that resveratrol can reduce plasma levels of LDL, cholesterol,

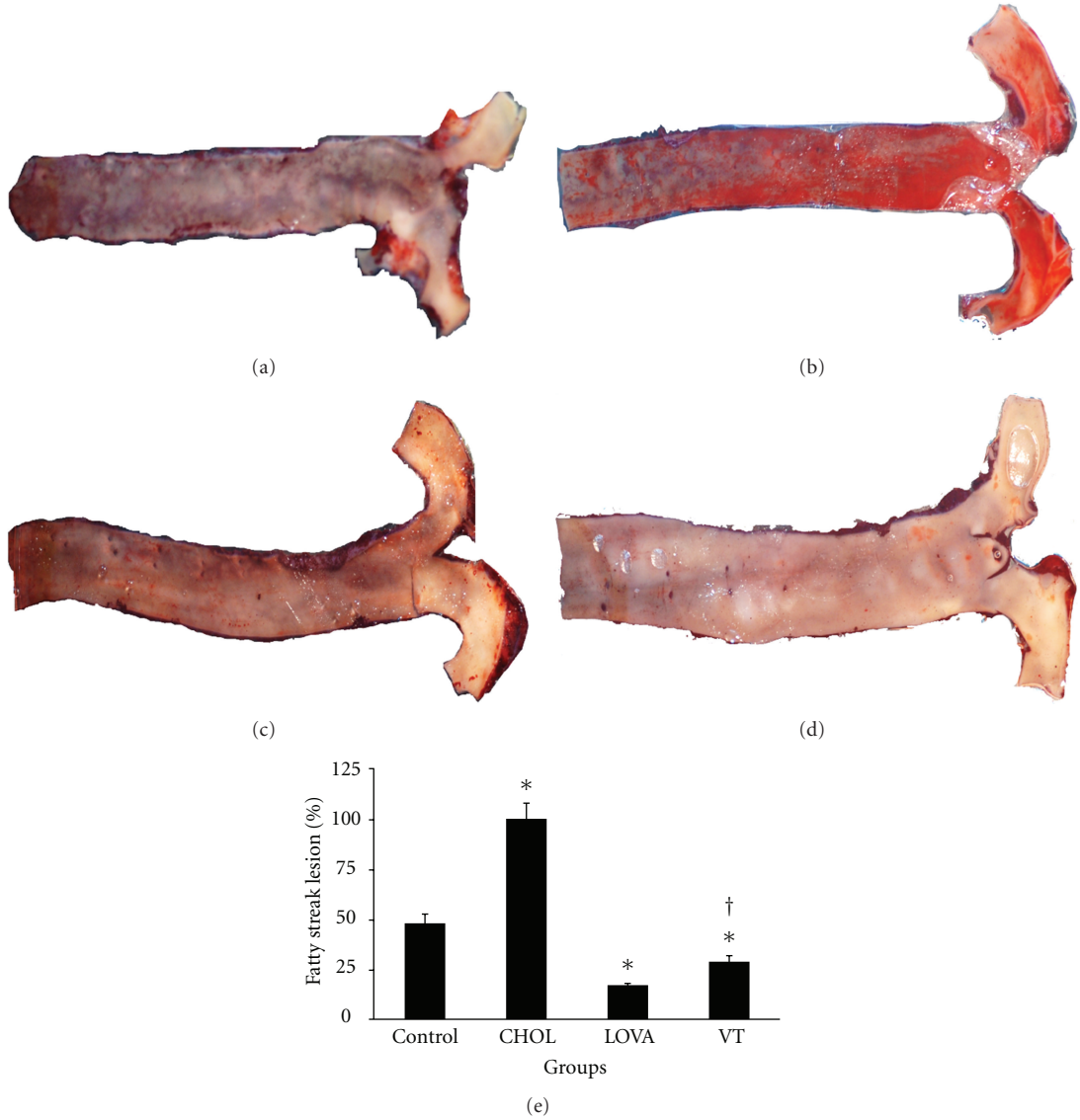

Figure 4: Histopathochemical examination of aortic fatty streak lesions in the hypercholesterolemic rabbit model after the 8-week study. (a) Control group; (b) CHOL group; (c) LOVA group; (d) VT group; (e) relative area of fatty streak lesion on aortic intima among different groups. * and † indicate a $P < 0.05$ as compared with the control group and CHOL group, respectively.

and triglycerides in high cholesterol diet-induced hypercholesterolemia in rodent models [32–34]. Similarly, long-term resveratrol administration can also decrease plasma levels of triglycerides, total cholesterol, and free fatty acids and hepatic total lipids in obese Zucker rats [35]. Ahn et al. [36] demonstrated that a supplementary diet with resveratrol can reduce atherosclerotic lesions and found that it decreased the levels of total hepatic lipids and triglycerides as well as their accumulation. In addition, mRNA expression and enzymatic activity of hepatic HMG-CoA reductase can also be downregulated by treatment with resveratrol, which may result in decreased cholesterol synthesis [37, 38]. *In vitro* studies have also shown promising beneficial effects of resveratrol on lipid metabolism. In isolated normal rat hepatocytes, resveratrol reduced the synthesis of fatty acids and triglycerides, which suggests a possible mechanism underlying resveratrol's effects in reducing the levels of triglycerides and other lipoprotein in the circulation [39]. Goldberg et al. [40] indicated that resveratrol effectively reduced LDL production and modulated hepatic lipid metabolism via decreasing secretion of triglycerides and cholesterol esters in HepG2 cells. Our results indicated that the VT extract reduced the plasma levels of cholesterol and LDL (Figure 1), decreased lipid accumulation in liver tissues (Figures 2 and 3), and diminished aortic fatty streak lesions (Figure 4). These lipid-lowering effects of the VT extract may be partially explained by the activities of the resveratrol derivatives mentioned previously.

Epidemiological studies have consistently shown that serum HDL concentration is inversely correlated with the incidence of cardiovascular disease. However, it appears that the relationship between HDL and CVD risk is more

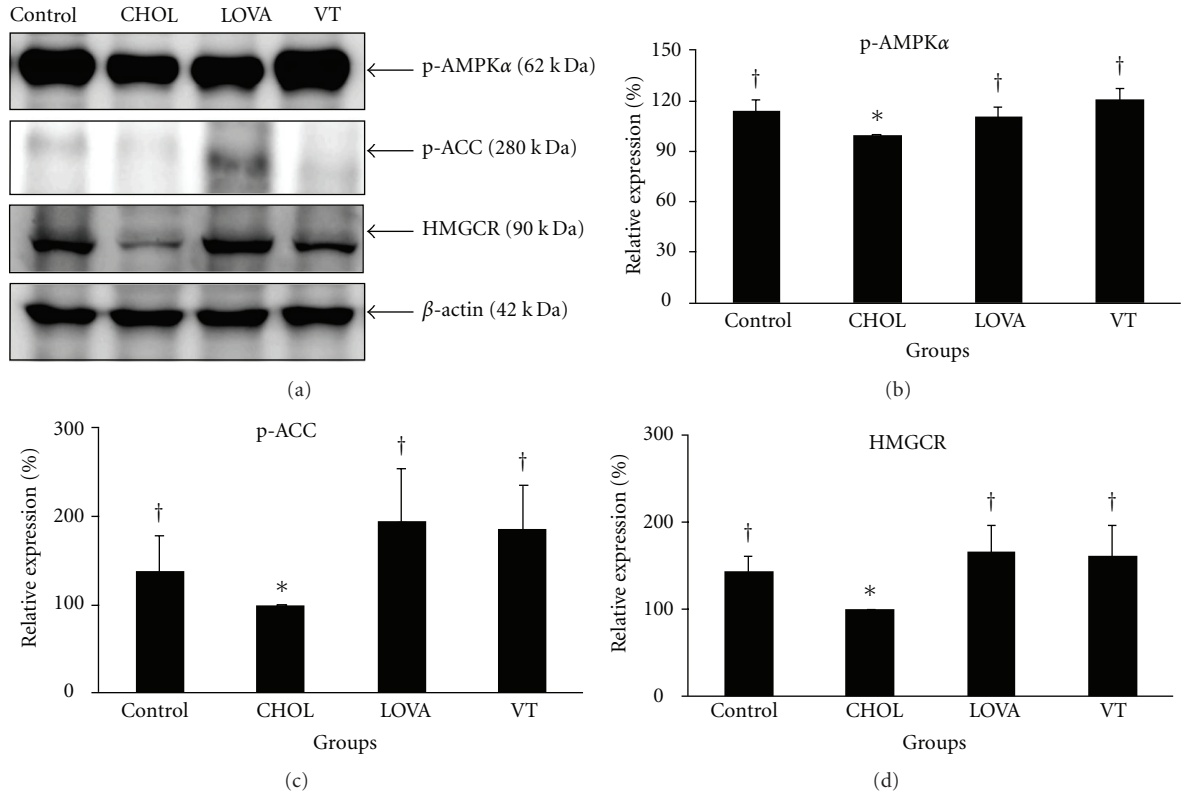

Figure 5: Protein expression of lipid metabolism associated molecules in the hypercholesterolemic rabbit model after the 8-week study. * and † indicate a $P < 0.05$ as compared with the control group and CHOL group, respectively.

complex and not just merely related to the serum HDL levels. Barter et al. [41] noted that torcetrapib can markedly increase the serum HDL, but the results of clinical trial showed to increase the risk of deaths and cardiac events in patients receiving torcetrapib. Several studies also showed that serum HDL levels are not always consistent with the incidence of CVD [42, 43]. In the present study, the results showed that high-cholesterol diet can markedly induce the serum levels of both LDL and HDL (Figures 1(b) and 1(d)), whose effects have be reported by others [44, 45]. Thereby, evaluating CVD risk on the basis of serum HDL concentration alone may be misleading, and the serum LDL/HDL ratio should also be evaluated together for CVD risk assessment. Our data showed that the high-cholesterol diet leads to a five-fold increase in the serum LDL/HDL ratio as compared with control group and VT supplement can reverse this parameter to support its lipid-lowering effect in the present study.

AMPK plays a critical role in the modulation of lipid metabolism by inhibiting the activation of ACC and HMGCR, which results in the acceleration of fatty acid oxidation and the suppression of cholesterol biosynthesis. Numerous studies have indicated that the stimulating activity of the AMPK pathway can effectively regulate lipid metabolism. Adiponectin is an adipocyte-derived adipokines and can protect against alcoholic fatty liver disease via the activation of the SIRT1- (sirtuin 1-) AMPK pathway [46]. Kusakabe et al. reported that leptin decreased liver and skeletal muscle triacylglycerol content, which was accompanied by an increase of AMPK activity in skeletal muscle [47]. Lin et al. found that theaflavins significantly reduced lipid accumulation, suppressed fatty acid synthesis, and stimulated fatty acid oxidation via stimulating AMPK and then inhibiting ACC activity [48]. Luteolin is another component identified from *V. thunbergii* that has been shown to reduce lipid accumulation in HepG2 cells. This effect may be partially mediated by that activation of the AMPK signaling pathway, which upregulates carnitine palmitoyl transferase 1 (CPT-1) and downregulates sterol regulatory element binding protein 1c (SREBP-1c) and FAS gene expression [49]. Metformin is an oral antidiabetic drug that has been demonstrated to lower the hepatic lipid content by activating AMPK, which may mediate the beneficial effects of this drug in hyperglycemia and insulin resistance [50, 51]. In addition, a number of studies have also demonstrated that resveratrol treatment can stimulate AMPK activity by regulating lipid metabolism [35, 52–54]. In the present study, the data showed that a VT extract could stimulate phosphorylation of AMPK and ACC (Figure 5). However, this effect may be partially mediated by some of the bioactive components of the VT extract, such as luteolin and the resveratrol derivatives mentioned previously. On the other hand, previous studies have demonstrated that activated AMPK will phosphorylate and then inactivate HMGCR [7]. Nevertheless, the total protein expression of HMGCR was upregulated by supplementation with the VT extract in the present study. HMGCR is regulated via a negative feedback mechanism that is mediated by sterols and

nonsterol metabolites derived from mevalonate. Moreover, this enzyme is suppressed by cholesterol in mammalian cells [55–57]. Therefore, the upregulation of HMGCR protein after an 8-week dietary supplement with the VT extract may have been triggered by the low levels of serum cholesterol (Figure 1).

5. Conclusions

Our experimental study revealed that a VT extract supplement can reduce serum LDL/HDL ratio as well as plasma levels of cholesterol and LDL, decrease lipid accumulation in tissues, and diminish aortic fatty streak lesions. Moreover, the lipid-lowering effects of the VT extract may have been partially mediated by the activation of AMPK, which was followed by the inhibition of the activation of ACC. These results suggest that *V. thunbergii* may have the potential to be developed as a lipid-lowering therapeutic agent or medicinal health food for the prevention or treatment of cardiovascular diseases such as atherosclerosis.

Acknowledgments

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Research Article

Qiliqiangxin Affects L Type Ca^{2+} Current in the Normal and Hypertrophied Rat Heart

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Qiliqiangxin capsule is newly developed Chinese patent drug and proved to be effective and safe for the treatment of patients with chronic heart failure. We compared the effects of different dose Qiliqiangxin on L type Ca^{2+} current ($I_{Ca\text{-}L}$) between normal and hypertrophied myocytes. A total of 40 healthy Sprague—Dawley rats were used in the study. The rats were randomly divided into two groups (control group and hypertrophy group). Cardiac hypertrophy was induced by pressure overload produced by partial ligation of the abdominal aorta. The control group was the sham-operated group. After 1 month, cardiac ventricular myocytes were isolated from the hearts of rats. Ventricular myocytes were exposed to 10 and 50 μmol/L Qiliqiangxin, and whole cell patch-clamp technique was used to study the effects of Qiliqiangxin on $I_{Ca\text{-}L}$. The current densities of $I_{Ca\text{-}L}$ were similar in control group (-12.70 ± 0.53 pA/pF, $n = 12$) and in hypertrophy group (-12.39 ± 0.62 pA/pF, $n = 10$). They were not statistically significant. 10 and 50 μmol/L Qiliqiangxin can decrease $I_{Ca\text{-}L}$ peak current 48.6% ± 16.8% and 59.0% ± 4.4% in control group. However, the peak current was only reduced 16.73% ± 8.03% by 50 μmol/L Qiliqiangxin in hypertrophied myocytes. The inhibited action of Qiliqiangxin on $I_{Ca\text{-}L}$ of hypertrophy group was lower than in control group. Qiliqiangxin affected L-type Ca^{2+} channel and blocked $I_{Ca\text{-}L}$, as well as affected cardiac function finally. Qiliqiangxin has diphasic action that is either class IV antiarrhythmic agent or the agent of effect cardiac function.

1. Introduction

The traditional Chinese medicines have proven the safety and efficiency of herbs in the management of some diseases since ancient times. Qiliqiangxin capsule is newly developed Chinese patent drug and proved to be effective and safe by phase 3 clinic trial for the treatment of patients with chronic heart failure. It includes over 11 ingredients such as Ginseng, Radix Astragali, Aconite Root, Salvia Miltiorrhiza, and Semen Lepidii Apetali [1, 2]. Previous studies showed Qiliqiangxin capsule can improve heart function and decrease serum level of TNF-α and relieve inflammatory cell infiltration of myocardium in rats with adriamycin induced cardiomyopathy [3]. Nevertheless, the excitation-contraction coupling in the cardiac myocyte is triggered by the influx of Ca^{2+} through L type Ca^{2+} channel and inducing Ca^{2+} release from the sarcoplasmic reticulum [4, 5]. Blocking Ca^{2+} channel and reducing Ca^{2+} overload will be of benefit in the progress of heart failure. Qiliqiangxin should affect L type Ca^{2+} channel and further alter cellular Ca^{2+} regulation as well as effect the heart function finally. We compared the effects of different dose Qiliqiangxin on L type Ca^{2+} current ($I_{Ca\text{-}L}$) between normal and hypertrophied myocytes and to comprehend the rational usage of Qiliqiangxin on hypertrophied myocytes in this study.

2. Material and Methods

2.1. Vegetal Material. Qiliqiangxin consists of Ginseng, Radix Astragali, Aconite Root, Salvia Miltiorrhiza, Semen

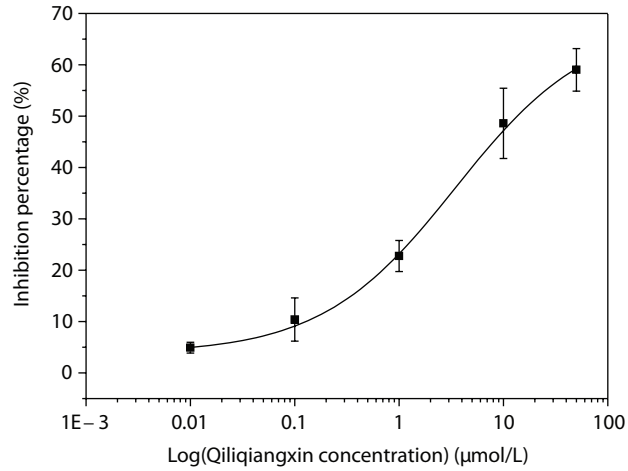

Figure 1: The concentration-dependent manner of Qiliqiangxin on $I_{Ca\text{-}L}$ in cardiac myocytes of rat (IC_{50} = 10.38 μmol/L).

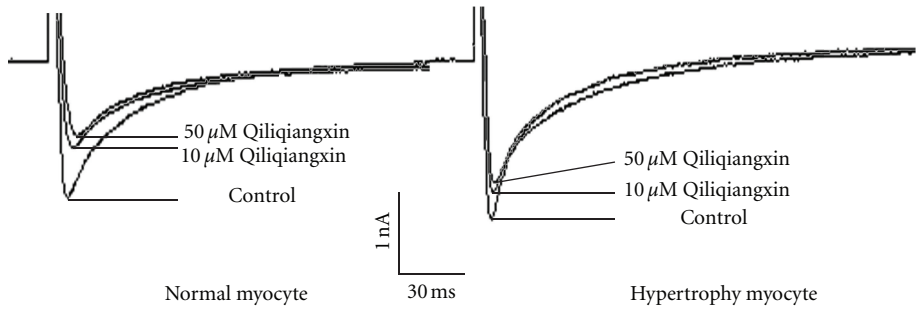

Figure 2: The effects of 10 and 50 μmol/L Qiliqiangxin on $I_{Ca\text{-}L}$ in normal and hypertrophied myocytes.

Lepidii Apetali, Cortex Periplocae Sepii Radicis, Rhizoma Alismatis, Carthamus Tinctorius, Polygonatum Odorati, Seasoned Orange Peel, and Rumulus Ginnamomi [3] (Yiling Pharmaceutical Corporation, Shijiazhuang, China). The drug powder was dissolved with sterile water at the concentration of 2.67 g/mL. 10 μmoL/l and 50 μmol/L Qiliqiangxin were prepared for the study.

2.2. Study Models. A total of 40 healthy Sprague-Dawley rats (9–11-week old, either sex, weight 210 to 300 g) were used in the study. All the rats used in the following experiments were subject to the Guiding Principles for the Care and Use of Laboratory Animals and the Recommendations from the Declaration of Tongji University. The rats were randomly divided into two groups (control group and hypertrophy group). Cardiac hypertrophy was induced by pressure overload produced by partial ligation of the abdominal aorta by using the method described by Anderson [6–8]. The control group was the sham-operated group; the aorta was dissected without application of the ligation. After operation, both groups were fed up with normal fodder and tap water in different cages for one month.

2.3. Cardiac Ventricular Myocytes Isolation. Cardiac ventricular myocytes were isolated from the hearts of rats using previous protocols [9]. Briefly, hearts were rapidly excised and cycloperfused with low calcium Tyrode's solution containing 0.08% Collagenase, 0.006% Protease, and then get single ventricular myocyte. The single ventricular myocyte selected for study is rod shaped, had clear striations and smooth and glossy surface.

2.4. Whole Cell Patch Clamp. We recorded Ca^{2+} current in a Na^{+}-free bath solution. To block outward K^{+} currents the bath contained (mM): 120 CsCL, 2 $CaCl_2$,10 TEA, 5 4-AP, 1 $MgCl_2$, 5 HEPES, 5 Glucose. PH = 7.4 (CsOH). The patch pipettes (borosilicate glass, 1.5–3 MΩ) were filled with the pipette solution (mM): 120 CsCl, 1 $CaCl_2$, 10 HEPES, 5 Mg-ATP, 10 EGTA. PH = 7.2 (CsOH). All recordings are at room temperature. The external solution was filled with 95% O_2 and 5% CO_2. Ca^{2+} currents were elicited by voltage steps from −90 to +55 mV. Compensated series resistance was 1.59 ± 0.20 MΩ. Cell capacitance averaged 26.9 ± 4.1 pF (n = 10 per group). To normalize for differences in total membrane area, current densities (in pA/pF) were calculated by dividing the total current by the membrane capacitance of the cell. Data were sampled at 10 kHz and filtered at 2 kHz by using an Axopatch 200A amplifier (Axon Instruments).

2.5. Statistical Analysis. pCLAMP 9.0 software was used for data acquisition and analysis values are presented as

Table 1: The measurement of rats basic characteristics.

	BW (g)	HW (mg)	LVW (mg)	HW/BW (mg/g)	LVW/BW (mg/g)
Control	237 ± 23	730 ± 26	507 ± 48	2.67 ± 0.10	2.01 ± 0.15
Hypertrophy	229 ± 18	810 ± 15*	672 ± 50*	3.43 ± 0.15*	2.63 ± 0.19*

Notation. $*P < 0.05$, compared to control group. BW: body weight, HW: heart weight, left ventricular weight: LVW.

means ± S.D. Statistical comparisons between the different amiodarone concentrations groups were obtained by ANOVA. Comparisons between control and hypertrophied myocytes group means were performed with Student's t-test. Differences with $P < 0.05$ were considered significant, completed by SPSS 11.5 Statistically package. Concentration-response relationships were fit to the Hill equation to determine the concentration of drug required for 50% inhibition (IC_{50}).

3. Results

3.1. Rats Characteristics. The rat hearts were significantly larger in hypertrophy group (810 ± 15 mg, $n = 22$) than in control group (730 ± 26 mg, $n = 18$). However, there was no difference in body weight between the two groups. Heart weight index (heart weight/body weight, HW/BW) and left ventricular weight index (left ventricular weight/body weight, LVW/BW) in hypertrophy group were greater than those in control group. They were statistically significant (Table 1).

3.2. Effects of Qiliqiangxin on $I_{Ca\text{-}L}$. The current densities of $I_{Ca\text{-}L}$ were similar in control group (−12.70 ± 0.53 pA/pF, $n = 12$) and in hypertrophy group (−12.39 ± 0.62 pA/pF, $n = 10$). They were not statistical significant. Qiliqiangxin obviously decrease $I_{Ca\text{-}L}$ of normal myocytes and represented a concentration-dependent manner. Its IC_{50} was 10.38 μmol/L (Figure 1). 10 and 50 μmol/L Qiliqiangxin can decreased $I_{Ca\text{-}L}$ peak current 48.6% ± 16.8% and 59.0% ± 4.4% in control group. Interestingly, $I_{Ca\text{-}L}$ represented insensitivity for Qiliqiangxin in hypertrophied myocytes. The peak current was only reduced 16.73% ± 8.03% by 50 umol/L Qiliqiangxin. Therefore, the inhibited action of Qiliqiangxin on $I_{Ca\text{-}L}$ of hypertrophy group was lower than in control group (Figure 2).

4. Discussion

Cardiac hypertrophy is associated with a significantly increased risk of cardiovascular morbidity and mortality that were frequently induced by electrical remodeling and arrhythmogenesis. The antiarrhythmic research was most based on normal myocytes, and whether they have same action on pathosis myocytes was unknown. As a result, means of treating hypertrophy-associated arrhythmias remain disappointingly ineffective. So far, there were four main classes of antiarrhythmic agents. Class IV agents are slow calcium channel blockers and decrease conduction through the AV node. They shorten the plateau of the action potential and reduce the contractility of the heart. Class IV agents may be inappropriate in cardiac hypertrophy treatment. Nevertheless, blocking Ca^{2+} channels and reducing Ca^{2+} overload will be of benefit in the progress of cardiac hypertrophy.

In pressure overload hypertrophy models, we found that the currents amplitude of $I_{Ca\text{-}L}$ on hypertrophied myocytes were higher than those in control. But the current densities were similar because of the swelling volume of hypertrophied myocytes. Acute application of Qiliqiangxin does inhibit $I_{Ca\text{-}L}$ in normal cardiac myocytes. IC_{50} was 10.38 μmol/L. 10 and 50 μmol/L Qiliqiangxin can, respectively, decreased 48.6% ± 16.8% and 59.0% ± 4.4% of the peak current of $I_{Ca\text{-}L}$ in control group. To compare with the hypertrophy group, $I_{Ca\text{-}L}$ showed different effects of Qiliqiangxin. Interestingly, the peak current was only reduced 16.73% ± 8.03% by 50 μ mol/L Qiliqiangxin. The inhibited action of Qiliqiangxin on $I_{Ca\text{-}L}$ of hypertrophy group was lower than in control group. In other words, $I_{Ca\text{-}L}$ represented more insensitivity for Qiliqiangxin in hypertrophied cardiac myocytes. Qiliqiangxin displayed the insensitiveness that may be facilitated for its utilization in cardiac hypertrophy and heart failure. Because it partly blocked $I_{Ca\text{-}L}$ and did not weaken myocardial contractility basically. 10 μmol/L Qiliqiangxin obviously decreased 48.6% ± 16.8% of the peak current of $I_{Ca\text{-}L}$ in normal cardiac myocytes, which made it reserve antiarrhythmic activity as class IV agents. That also signifies we should deal with difference between hypertrophied heart and normal heart when we use Qiliqiangxin in the clinic.

Qiliqiangxin includes over 11 ingredients. The mechanism of the antiarrhythmic action is complex and not completely understood. It is hard to prove which herb has mainly contributed to the effect on L type Ca^{2+} channel. Recently, ShenSongYangXin capsule, a traditional Chinese herb, has been reported to effectively block $I_{Ca\text{-}L}$ [10]. Zhao et al. had reported that Radix Astragali effectively protected against cardiac dysfunctional and morphological aberrations in experimental myocardial infarction [11]. Aconite Root was proved to have positive inotropic, positive chronotropic, vasodilation, and diuretic effects in the management of congestive heart failure [12]. Qiliqiangxin is composed of Radix Astragal, Aconite Root and parts of Shensong Yangxin which are the main active constituents of Qiliqiangxin. In the cardiac function, the excitation-contraction coupling of cardiac myocyte is triggered by Ca^{2+} influx through L-type Ca channels [4, 5]. Ca^{2+} influx activates calmodulin kinase that may activate transcription factors and cAMP response element binding protein (CREB). CREB promoted several cytokine secretions such as interleukin-10 (IL-10) [13, 14]. Inflammatory cytokines mainly derived from cardiac myocytes were involved in the progression of heart failure [15]. A proinflammatory cytokine (TNF-α) has been

linked to accelerate myocardial necrosis and deteriorated cardiac function. Serum level of TNF-α in patients with chronic heart failure increased and correlated with poor cardiac performance [16]. IL-10 and TNF-α induced left ventricular remodeling and dysfunction in the failing heart [17]. Qiliqiangxin may improve cardiac function of rats with MI through regulation the balance between TNF-α and IL-10 [3]. Blocking Ca^{2+} channels and reducing Ca^{2+} influx as well as weakening myocardial contractility will be the key point in the progress of cardiac hypertrophy and heart failure. The mechanism underlying the beneficial effects of Qiliqiangxin may involve the regulation of Ca^{2+} channel and reduce Ca^{2+} influx. Meantime, it influences several cytokine secretions indirectly. We concluded Qiliqiangxin affected L-type Ca^{2+} channel and blocked $I_{Ca\text{-}L}$, as well as affected cardiac function finally. Qiliqiangxin has diphasic action that is either class IV antiarrhythmic agent or the agent of effect cardiac function.

4.1. Study Limitations. This study was focused on the effect of Qiliqiangxin in $I_{Ca\text{-}L}$. Further studies should be on the regulation of Na^+ and K^+ channels.

Authors' Contribution

Y. Wei and X. Liu are cofirst authors.

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Research Article

Clinical and Epidemiological Investigation of TCM Syndromes of Patients with Coronary Heart Disease in China

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To compare the regional differences in TCM syndromes of patients with coronary heart disease (CHD) between North and South China. A total of 624 patients with a diagnosis of CHD, confirmed by coronary angiography, were included in the comparative analysis to determine the occurrence pattern, characteristics of TCM syndrome distribution, and differences in syndrome combinations and major syndrome types (deficiency or excess) between North and South China. The incidence of CHD tended to be higher in North China (54.6%) compared with that in South China (45.4%). The proportions of patients with a qi-deficiency syndrome (83.7%), turbid phlegm syndrome (68.9%), or blood stasis syndrome (91.5%) were generally higher in the South group, while the proportion of patients with a cold congelation syndrome (7.9%) was identified to be obviously higher in the North group ($P < 0.01$). Moreover, compared with that in the South group, the overall frequency of syndrome combinations tended to be lower in the North group ($P < 0.01$); and the most common types of TCM syndrome were excess syndrome (193, 56.6%) and primary deficiency and secondary excess syndrome (244, 86.2%) in the North and South groups, respectively ($P < 0.01$). A regional difference does exist in the TCM syndromes of patients with CHD between North and South China, indicating that the prevention and treatment of CHD in South China should not only focus on promoting blood circulation and removing blood stasis, but also include supplementing qi and eliminating phlegm.

1. Introduction

Coronary heart disease (CHD) is the most common form of heart disease and remains to be a persistent public health burden worldwide. An epidemiological study showed that there were about 1,300,000 new cases of CHD diagnosed in China each year [1]. Previous studies have documented the efficacy and safety of Xiongshao capsule in reducing restenosis after percutaneous coronary intervention (PCI) in CHD patients [2, 3], indicating that traditional Chinese medicine (TCM) has potential advantages in the treatment of CHD. As an essential element of TCM theories, syndrome differentiation is the basis for the treatment of all diseases, including CHD. Therefore, it is important to characterize the TCM syndromes of patients with CHD; however, previous studies have not addressed the potential regional differences in TCM syndromes of these patients. In our study, we conducted a comparative analysis of 624 patients with a CHD diagnosis confirmed by coronary angiography to determine the characteristics and differences in TCM syndrome distribution between North and South China.

2. Materials and Methods

2.1. Study Design. This is a prospective observational study about the regional differences in TCM syndromes of patients with CHD between North and South China. Hospitalized patients with a CHD diagnosis confirmed by coronary angiography in Beijing, Fuwai Cardiovascular Hospital and Guangdong Provincial Hospital of TCM from December 2007 to December 2010 were included in the study.

2.2. Inclusion/Exclusion Criteria. Male or female patients diagnosed as CHD and confirmed by coronary angiography were eligible for inclusion. The diagnosis of CHD was made according to the "nomenclature and criteria for diagnosis

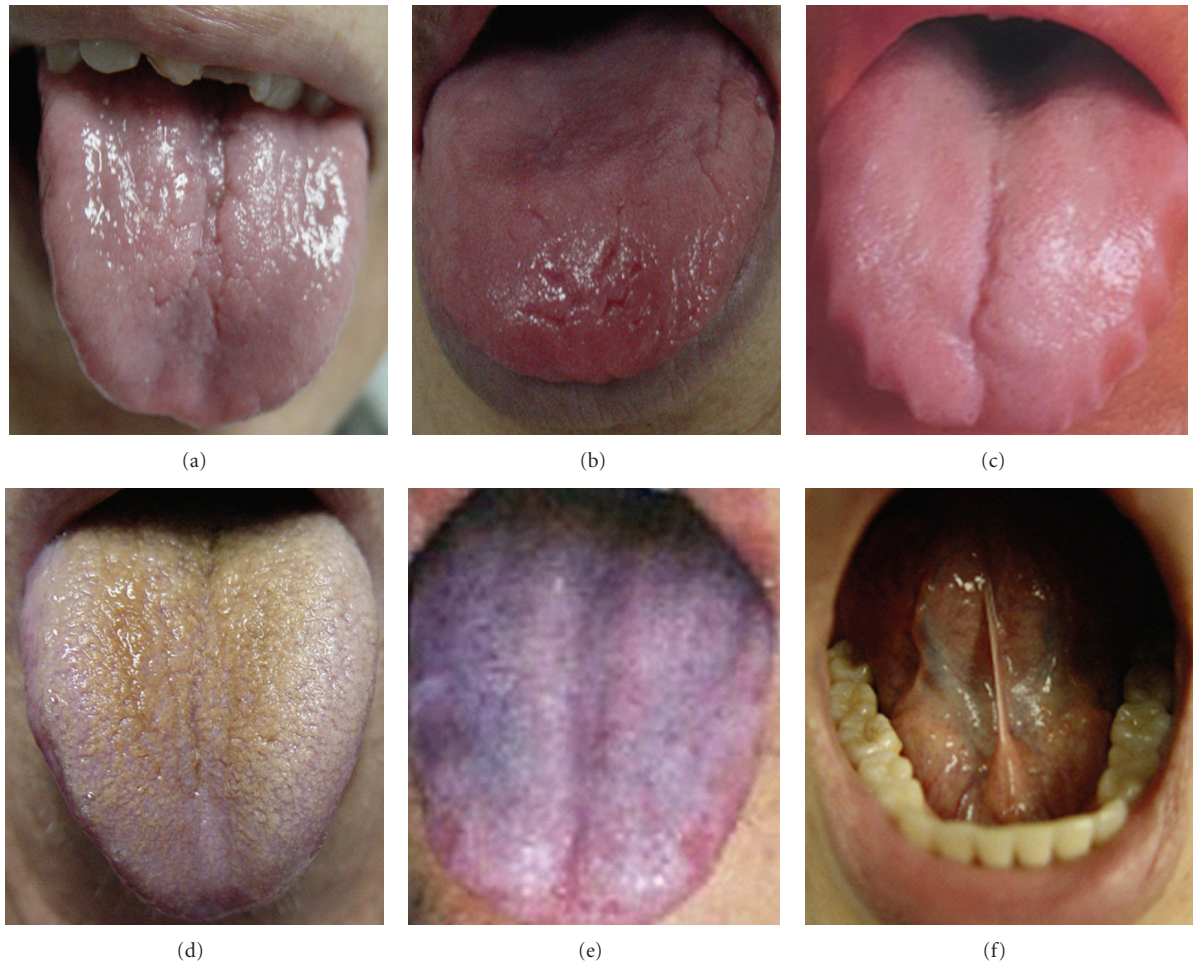

Figure 1: Typical tongue manifestations in the study. Notes: (a) common tongue manifestation of yang deficiency syndrome; (b) common tongue manifestation of yin deficiency syndrome; (c) common tongue manifestation of Qi deficiency syndrome; (d) common tongue manifestation of turbid phlegm syndrome; (e) and (f) common tongue manifestation of blood stasis syndrome.

of ischemic heart disease. Report of the Joint International Society and Federation of Cardiology/World Health Organization task force on standardization of clinical nomenclature" [4]. And the diagnostic criteria for TCM syndrome differentiation was based on the "criteria for TCM syndrome differentiation of patients with coronary heart disease" (revised in 1990), released by China Society of Integrated Traditional Chinese and Western Medicine [5].

All of the procedures of coronary angiography were performed by highly skilled physicians using Judkins approach. The findings of coronary angiography were interpreted by at least two experienced readers, and the final diagnosis of CHD is made according to the angiography report.

Patients complicated with other diseases that may interfere with the syndrome differentiation of this study, or those suffering from other serious diseases of major organs or infective diseases were exclude from the study. And those who cannot or not willing to complete the study; or those who had psychiatric disorders or intellectual dysfunctions were also excluded.

2.3. Syndrome Differentiation. The TCM syndrome differentiation was performed on the day of admission, which was based on the information gathered from inspection, auscultation, olfaction, inquiring, and palpation of the pulse. This study collected information on the tongue with a unified type of digital camera, and pictures shown in Figure 1 represented some typical tongue manifestations. Pulse manifestations and other diagnostic information were determined independently by two experienced cardiovascular physicians with attending doctor title to ensure objective evaluation. The 7 basic syndromes of patients with CHD included Qi-deficiency syndrome, yin-deficiency syndrome, yang-deficiency syndrome (including yang Qi vacuity desertion), Qi stagnation syndrome, blood stasis syndrome, cold congelation syndrome, and turbid phlegm syndrome (including phlegm heat).

2.4. Statistical Analysis. The statistical analysis of this study was performed by using SPSS18.0 software. An independent sample t-test was conducted for between group comparisons

Table 1: Provincial distribution of the 624 patients with CHD.

	Beijing	Hebei	Shanxi	Guangdong	Henan	Shandong	Heilongjiang	Neimenggu	Anhui	Others
N	119	70	56	259	18	18	15	14	11	44
Percentages (%)	19.1	11.2	9.0	41.5	2.9	2.9	2.4	2.2	1.8	7.1

Table 2: North-South distribution of the 624 patients with CHD.

	North China	South China
N	341	283
Percentages (%)	54.6	45.4

of quantitative data, which were represented as $\bar{x} \pm s$; the Wilcoxon-rank sum test was applied for nonnormal data and unequal variances. The qualitative data were analyzed by contingency table χ^2 test, and the significance level was set at $\alpha = 0.05$.

3. Results

3.1. General Characteristics. A total of 624 patients with a CHD diagnosis confirmed by coronary angiography were included in the study, 351 from Beijing Fuwai Cardiovascular Hospital and 273 from Guangdong Provincial Hospital of TCM. Of these patients, 472 (75.6%) were male and 152 (24.4%) were female, with an average age of 60.2 ± 11.8 years. The diagnosis of CHD could be classified into stable angina (109 and 17.4%), unstable angina (202 and 32.4%), and acute myocardial infarction (313 and 50.2%). The most common co-morbidities included hypertension (373 and 59.8%), diabetes (159 and 25.5%), hyperlipemia (229 and 36.7%), and cerebrovascular disease (44 and 7.1%).

3.2. Regional Distribution of CHD Patients. The division of North or South China was based on the provinces that patients come from (see Table 1), with the Tsinling Mountains—Huai River as the dividing line, north of the Tsinling Mountains—Huai River as North China while south of the Tsinling Mountains—Huai River as South China. It was shown that the incidence of CHD tended to be higher in North China compared with that in South China (see Table 2).

3.3. North-South Differences in TCM Syndromes. The presence or absence of each of the 7 TCM syndrome types, considered as a binary variable, was compared between the North group and the South group. Results showed that the proportions of patients with a Qi-deficiency syndrome, turbid phlegm syndrome, or blood stasis syndrome were generally higher in the South group, while the proportion of patients with a cold congelation syndrome was identified to be obviously higher in the North group ($P < 0.01$, see Table 3). No significant differences were noted between the two groups in terms of the proportions of patients with a yin-deficiency syndrome, yang-deficiency syndrome, or Qi stagnation syndrome ($P > 0.05$, see Table 3).

Moreover, the trends of syndrome combinations as well as the proportions of major syndrome types (deficiency or excess) were also compared between the two groups. Results revealed that, compared with that in the South group, the overall frequency of syndrome combinations tended to be lower in the North group ($P < 0.01$, see Table 4); the most common types of major TCM syndromes were excess syndrome (193, 56.6%) and primary deficiency and secondary excess syndrome (244, 86.2%) in the North and South groups, respectively ($P < 0.01$, see Table 4).

4. Discussion

The incidence of CHD varies substantially around the world. Chambless et al. [6] reported that in 18 (e.g., Finland and England, etc.) out of the 29 MONICA populations the incidence of acute myocardial infarction or possible coronary events was higher than 400/100,000, of which North Karelia, Finland had the highest rate (818/100,000) and Beijing, China had the lowest (79/100,000). The incidence of CHD not only varies across different countries, but also across different regions even in a same country. Wielgosz and Lynne [7] reported that the mortality of CHD in white Americans aged 35–74 years old varied among different regions and cities across the United States, of which the northeast region had the highest mortality followed by the middle-west and south regions, and the west region had the lowest rate with about 2.5-fold difference among them. The results of the Sino-MONICA project [8] conducted in 12 monitoring areas revealed that Beijing, Hebei, Neimenggu, Anshan Liaoning, Heilongjiang, and Xinjiang had a male incidence of CHD ≥50/100,000; Shenyang, Liaoning, and Jinlin had an incidence between 25/100,000–50/100,000; while Shanghai, Jiangsu, and other regions in South China all had an incidence lower than 15/100,000, indicating a generally higher incidence of CHD in North China compared with that in South China. In the 624 patients with a CHD diagnosis confirmed by coronary angiography included in our study, with the Tsinling Mountains—Huai River as the dividing line, the proportions of patients from North China and South China were 54.6% and 45.4%, respectively. The incidence trend of CHD identified in our study was generally consistent with that in the Sino-MONICA project.

The investigation of inspection, auscultation and olfaction, inquiring and palpation of the pulse manifestations have great significance to analyze Chinese medicine pathogenesis of coronary heart disease and its treatment based on syndrome differentiation [9]. Previous studies on TCM syndromes of CHD were generally limited to regional observation in small areas, which could not represent the distribution characteristics of TCM syndromes of CHD patients in China. Due to the differences in environment, climate,

Table 3: Comparison of the presence/absence of each of the 7 syndrome types between North and South China.

	Qi deficiency syndrome		Yang deficiency syndrome		Yin deficiency syndrome		Cold congelation syndrome		Qi stagnation syndrome		Blood stasis syndrome		Turbid phlegm syndrome	
	NO	Yes	NO	Yes	NO	Yes	NO	Yes	NO	Yes	NO	Yes	NO	Yes
North China	236 (69.2%)	105 (30.8%)	318 (93.3%)	23 (6.7%)	291 (85.3%)	50 (14.7%)	314 (92.1%)	27 (7.9%)	316 (92.7%)	25 (7.3%)	115 (33.7%)	226 (66.3%)	189 (55.4%)	152 (44.6%)
South China	46 (16.3%)	237 (83.7%)	257 (90.8%)	26 (9.2%)	243 (85.9%)	40 (14.1%)	276 (97.5%)	7 (2.5%)	268 (94.7%)	15 (5.3%)	24 (8.5%)	259 (91.5%)	88 (31.1%)	195 (68.9%)
χ^2	175.10		1.28		0.04		8.90		1.06		56.92		37.09	
P	0.00		0.26		0.85		0.003		0.30		0.00		0.00	

Table 4: Comparison of the frequencies of syndrome combinations and deficiency or excess syndrome between North and South China.

	Syndrome combinations				Deficiency or excess syndrome		
	Single syndrome	Two-syndrome combination	Three-syndrome combination	Four-syndrome combination	Deficiency syndrome	Excess syndrome	Primary deficiency and secondary excess syndrome
North China	130 (38.1%)	164 (48.1%)	38 (11.1%)	9 (2.7%)	45 (13.2%)	193 (56.6%)	103 (30.2%)
South China	15 (5.3%)	68 (24.0%)	172 (60.8%)	28 (9.9%)	10 (3.5%)	29 (10.3%)	244 (86.2%)
χ^2	222.73					197.03	
P	0.00					0.00	

diet, and body constitution, potential regional difference may exist in the TCM syndromes of CHD patients. Previous studies showed that, the most common TCM syndrome of CHD patients was blood stasis (81.4%), followed by Qi deficiency (56.8%), turbid phlegm (48.5%), and Yin deficiency (25.1%) in Beijing/Tianjin (North China) [10], while in Changsha (South China), the commonly seen syndromes included heart-blood stagnation, phlegm blocking heart vessel, cold congelating heart vessel, Qi stagnating heart vessel, Qi deficiency of heart, Yang deficiency of heart, Yin deficiency of heart [11], which was generally consistent with the findings of our study. Previous studies have not investigated the distribution of TCM syndromes of CHD. In this study, we chose Beijing Fuwai Cardiovascular Hospital and Guangdong Provincial Hospital of TCM as the study sites because the former is located in North China while the latter in South China; both sites are leading cardiovascular centers with a great number of CHD patients from North and South China, respectively. Thus, based on these two sites, it is possible to characterize the distribution of TCM syndromes of CHD patients in China.

Our study found that the proportions of patients with a Qi deficiency syndrome, turbid phlegm syndrome, or blood stasis syndrome were generally higher in South China, while the proportion of patients with a cold congelation syndrome was identified to be higher in North China. In terms of syndrome combinations, the observed frequencies of the 4 patterns in North China were two-syndrome combination > single syndrome > three-syndrome combination > four-syndrome combination, while those in South China were three-syndrome combination > two-syndrome combination > four-syndrome combination > single syndrome. Overall, the number of combined syndromes in North China tended to be fewer than that in South China. As for the deficiency or excess properties of TCM syndromes, excess syndrome was the most common type in North China, while primary deficiency and secondary excess syndrome had the highest rate in South China. These results could be explained by the following two reasons: on one hand, the environmental factors have contributed to the North-South differences. The Lingnan region is located within the subtropical zone characterized by hot and humid climate throughout the year, which is likely to consume Qi and injury Yin; and people there like cold food and cold environments, which may result in spleen and stomach injuries, as well as phlegm-damp retention. Therefore, turbid phlegm and Qi deficiency are common in patients from the Lingnan region. Whereas the weather in the northern region is much colder, which may cause congelation and stagnation; the attack of cold pathogens may result in impeded circulation of the blood and Qi, manifested by the symptom of angina, for this reason cold congelation syndrome is common in patients from North China. On the other hand, constitution factors also have an impact on the incidence trend of CHD. People from North China are relatively strong, and the attack of various pathogens always results in stagnation of Qi activity and blood circulation, thus the single excess syndrome of Qi stagnation or blood stasis is common in North China; while primary deficiency and secondary excess syndrome has a higher incidence in South China.

5. Conclusion

It is concluded that a regional difference exists in the TCM syndromes of patients with CHD between North and South China, indicating that the prevention and treatment of CHD

in South China should not only focus on promoting blood circulation and removing blood stasis, but also include supplementing Qi and eliminating phlegm.

Limitations of the Study

Although our study revealed the potential regional differences in TCM syndromes of CHD patients in China, there are several limitations of the study. Firstly, due to the limited budgets, the duration of study period was short which resulted in a relatively small sample. Secondly, we only chose a single medical center from North and South China, respectively, which may not fully reflect the distribution characteristics of TCM syndromes of CHD patients across China. Therefore, multicenter large-scale studies are needed to further define the potential regional differences in TCM syndromes of CHD patients.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgments

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Review Article

A Study of Prognosis, Outcome, and Changing Tendency of Hospitalized AMI Patients in Beijing Third-Grade A-Level Traditional Chinese Medicine Hospitals from 1999 to 2008

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Objectives. To survey and analyse the prognosis, outcome, and changing tendency of the Acute Myocardial Infarction (AMI) patients in Beijing third-grade A-level Traditional Chinese Medicine (TCM) hospitals. *Methods*. We collected the clinical datum of hospitalized AMI patients in Beijing 6 TCM hospitals from January 1999 to December 2008 and then analysed the clinical datum. *Results*. (1) The mean age of patients had showed a slowly rising tendency during this ten years. The patients who had previous history of cerebrovascular diseasea and multiple comorbidities had increased year by year. (2) The rate of reperfusion therapy, revascularization and standardized using of drug, and usage of TCM of AMI patients presented a significant increasing tendency in these hospitals. (3) The proportion of AMI patients combined with cardiac arrhythmia and heart failure had decreased significantly. (4) The AMI mortality presented a decreasing tendency in 10 years. *Conclusions*. The AMI patients in Beijing TCM hospitals had their own unique clinical features, and it can improve their prognosis by combined therapy of Western Medicine and Traditional Chinese Medicine.

1. Objectives

Cross-sectional registry was used to collect the hospitalized AMI patients' clinical datum in third-grade A-level TCM hospitals from 1999 to 2008 in Beijing, so as to dynamically analyse their clinical characteristics in recent 10 years and provide preliminary epidemiological datum of standardized treatment in TCM hospitals.

2. Participants

2.1. Source of Cases. The subjects we enrolled were hospitalized patients suffering from AMI in Beijing Third-grade A-Level TCM hospitals from January 1, 1999 to December 31, 2008. We totaly recovered 2056 case report form (CRF) handbooks, and by screening there were 2053 within registered. See Table 1.

2.2. Inclusion Criteria. (1) All participants in the study hospital's records and statistics room in the computer management system of the international classification of diseases (ICD) were coded for AMI; (2). All these patients were diagnosed as AMI by reference of the Chinese society of Cardiology, Chinese Journal of cardiovascular medicine, Editorial Committee of Chinese Circulation Journal jointly developed the *Guidelines for the Diagnosis and Treatment of Acute Myocardial Infarction* (2001) [1] (the following referred to as the *Guideline*). (3) The information of research-related syndromes of Chinese medicine were filled or by adding completely then could be used. Conforming to the above three criteria can be inclused into the survey.

2.3. Exclusion Criteria. (1) Old AMI patients. (2) Patients'. TCM syndrome information was seriously uncomplete and unable to complement. In accordance with neither of the two criteria, it would be been exclused.

Table 1: List of hospitals participating in the survey.

Hospital name	Cases	Proportion (%)
Beijing TCM Hospital	479	23.33
Xi Yuan TCM Hospital	584	28.45
Dong Fang TCM Hospital	456	22.21
Guang, anmen TCM Hospital	248	12.08
Dongzhimen TCM Hospital	204	9.94
Wangjing TCM Hospital	82	3.99

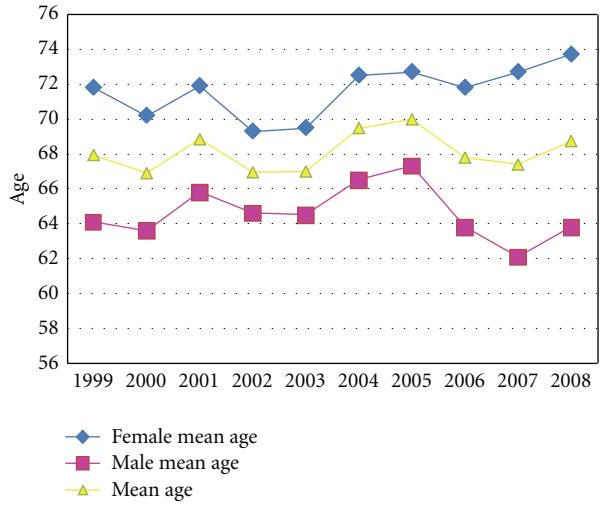

Figure 1: The age change trend of AMI patients in third-grade A-level TCM hospitals in Beijing from 1999 to 2008.

3. Methods

First of all the Capital Research Foundation for Medical Development group designed the CRF handbook which included the general characteristics, hospitalized therapeutic conditions and the prognosis, and outcome of AMI patients. Then we gave the CRF to the doctors of cardiovascular department of each hospital. In every hospital, elected cases were filled according to the actual situation by the first doctor who had been trained. About every six months, all the cases of cooperate units were summarized to the cardiovascular department of Beijing TCM Hospital, then the task group inspected the datum and eliminated invalid datum.

Next the datum was entered to the survey database which was established based on ACESS 2000 by China Department of Epidemiology, Capital Medical University Beijing Anzhen Hospital. The datum was entered by the trained persons. We used double datum entry, consolidated datum, and then checked error by the logic difference method. Statistical software SPSS15.0 (SPSS Base15.0S, PN: 32119001, SN: 5045602, L20080005) was used in descriptive analysis for general datum, measurement datum was analyzed by F test and Q test, and two sets of measurement datum were analyzed by T test; categorical data was analyzed by X^2 test.

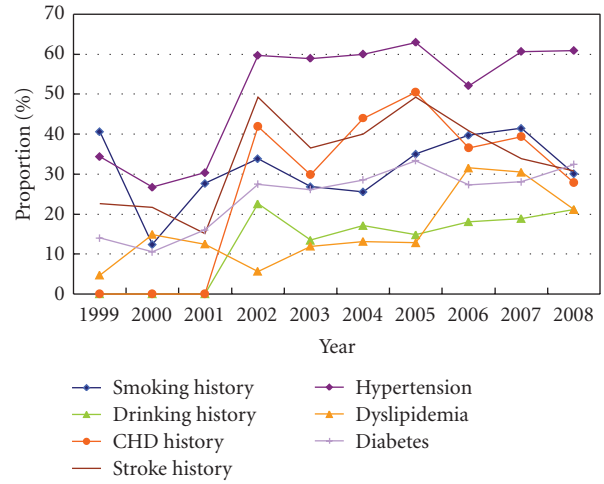

Figure 2: The risk factors change trend of AMI patients in third-grade A-level TCM hospitals in Beijing from 1999 to 2008.

4. Results

4.1. General Characteristics

4.1.1. Age. A total of 2053 AMI patients were entered, of these, 2015 cases had clearly recorded the age of onset, the minimum age was 18 years old, the maximum age was 105 years old, and the mean age was 67.37 ± 12.10 years old. The average onset age showed a slowly rising tendency from 1999 to 2008. 1316 cases were male patients, 737 cases were female patients, and the gender ratio was 1.8 : 1. For the changing tendency of AMI patients' see Figure 1.

4.1.2. The History and Risk Factors. There were total 680 patients with a clear history of smoking and 299 cases with a history of clear drinking. Patients with a history of smoking drinking in the proportion of overall show a slow ascending tendency. In 638 cases with clear history of coronary heart disease, the proportion had decreased. 695 patients had a clear stroke history, the proportion had gone up. 1130 patients combined with hypertension, and 438 patients had dyslipidemia, 542 patients combined with diabetes mellitus; the proportion of those patients to the overall was in a upward trend from 1999 to 2008 (see Figure 2).

4.1.3. AMI Status. The total anterior wall myocardial infarction (MI) cases was 1324, and the proportion had no obvious change; the rear wall MI cases were 1147, right ventricular MI cases were 170, and the proportion of inferior wall and right ventricular MI decreased slowly. Clear ST segment elevation cases were 1287; clear Q wave MI cases were 1231; 1616 cases were the first MI, 243 cases were second MI, 16 cases were third MI, and 178 cases were unspecified (see Figure 3).

A total of 656 patients underwent coronary angiography, accounted for 31.95 percentage of the observed cases; patients undergoing coronary angiography increased year by year from 2001 to 2008.

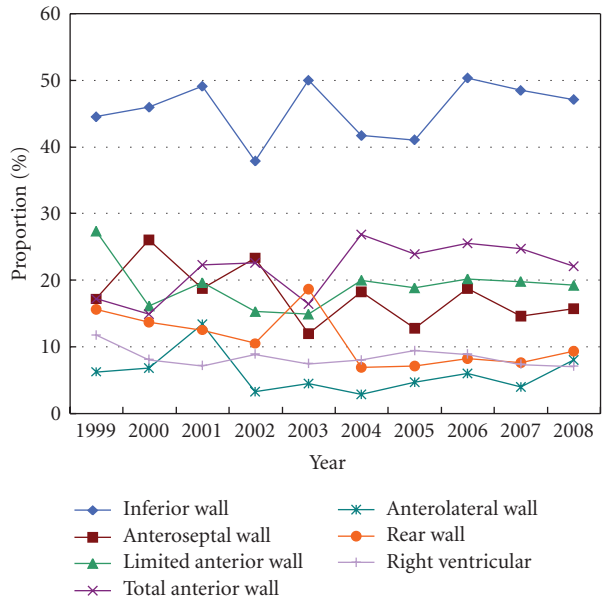

Figure 3: The proportion of MI location of hospitalized AMI patients from 1999 to 2008.

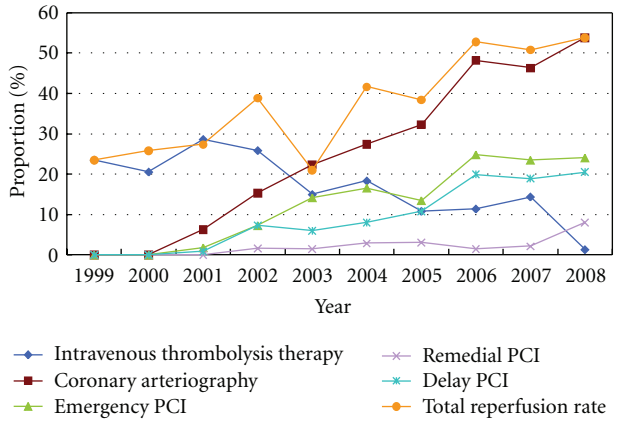

Figure 4: the reperfusion therapy conditions of AMI patients in third-grade A-level TCM hospitals in Beijing from 1999 to 2008.

4.2. Hospitalized Therapeutic Conditions

4.2.1. Reperfusion Therapy. In 2003, the usage rate of reperfusion therapy was the lowest (20.97%), while in 2008, it had gone up to 53.80%, which presented an upward tendency from 1999 to 2008. The usage rate of intravenous thrombolysis therapy was the lowest in 2008 (1.28%), while in 2008, it was the highest up to 28.57%, which presented a downward tendency from 1999 to 2008.

There was no emergency PCI patient in 1999 and 2000. The rate of underwent emergency PCI was the lowest in 2008 (1.79%), while in 2006, it was the highest up to 24.82%, which presented an upward tendency from 1999 to 2008. The rate of underwent early reperfusion was the lowest in 1999 (23.44%), while in 2007, it was the highest up to 37.80% which presented an upward tendency from 1999 to 2008.

There was no patients undergoing CABG in 2000, 1999, 2007, and 2008, CABG patients have the highest proportion (1.61%) in 2002, and from 1999 to 2008 there had been no obvious regularity. There was no patients undergoing remedial PCI in 1999 and 2001. The remedial PCI patients covered the lowest proportion in 2003 as 1.49% and the highest proportion in 2008 as 8.01%, which presented an ascendant tendency from 1999 to 2008. There was no patient undergoing selective PCI in 1999 and 2000; the selective PCI patients covered the lowest proportion as 0.89% in 2001 and the highest as 20.51% in 2008, which also presented an ascendant tendency from 1999 to 2008. Specific reperfusion form is shown in Figure 2.

4.2.2. Oral Drug Therapy. Western medicine intervention: the usage rate of drugs followed the *Guideline* recommendations from 1999 to 2008 which were the following: aspirin was 86.64%, clopidogrel was 37.91%, nitrate was 88.91%, beta blockers were 67.84%, angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blocker (ARB) together were 77.75%, low molecular weight heparin was 82.38%, and adjustable lipid drug was 54.37% see Figure 3.

The usage rate of other drugs: from 1999 to 2008 the usage rate of these drugs which were not recommended by the *Guideline* was the following: unfractionated heparin was 14.4%, antiarrhythmic drug was 16.1%, CCB was 23.4%, diuretic was 39.9%, digitalis cardiac drug was 16%, GIK was 20.4%. See Figures 4 and 6.

4.2.3. The Intervention's Conditions of Chinese Medicine. From 1999 to 2008, within 2053 patients, 1851 patients have used intravenous preparation of Chinese medicine in TCM hosptitals, and the total usage rate was 90.2%, which presented a tendency of escalation. 400 patients used oral proprietary Chinese medicines, and the general usage rate was 19.5%, which presented a decline in general from 1999 to 2008. 1056 patients using oral decoction, the total usage rate was 51.4%, and the usage rate of decoction of Chinese medicine was increasing in general from 1999 to 2008. The changing tendency of Chinese medicine intervention was shown in Figures 5 and 7.

4.3. Complications. Arrhythmia, heart failure, and cardiogenic shock are the common clinical complications of AMI patients, and are also the major cause of death. Based on the datum of 10 years in Beijing third-grade A-level TCM hospitals, the proportion of patients complicated by arrhythmia had dropped from 42.91% in 1999 to 24.04% in 2008, presented a fluctuating downward tendency; the proportion of patients complicated by heart failure had dropped from 60.16% in 1999 to 41.03% in 2008, and presented a significant downward tendency; patients complicated by cardiogenic shock had no obvious changes in the proportion.

4.4. Mortality. This survey showed that the mortality of AMI patients presented a fluctuating tendency of decline from 1999 to 2008 in Beijing Third-grade A-Level TCM hospitals, the main death due to cardiogenic death. With the

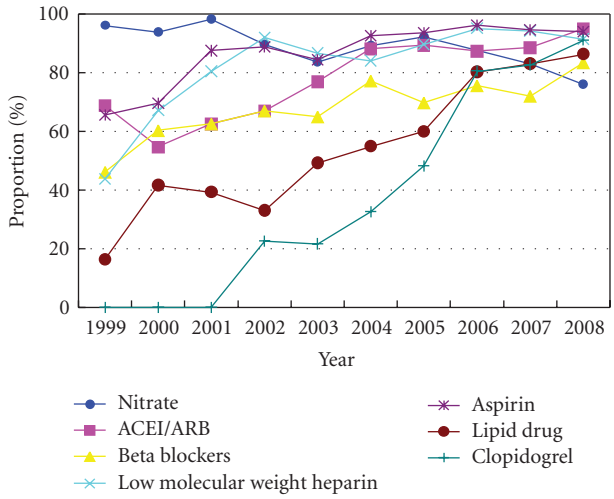

Figure 5: The status of oral drug usage followed the *Guideline* recommended to AMI patients in Third-grade A-Level TCM hospitals in Beijing from 1999 to 2008.

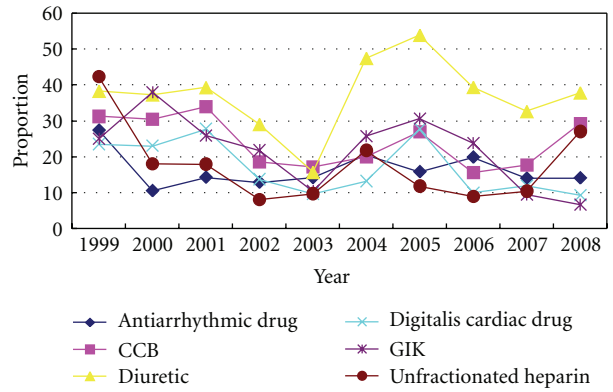

Figure 6: The rate of oral drugs which were not recommended by the *Guideline* to AMI patients in Third-grade A-Level TCM hospitals in Beijing from 1999 to 2008.

increase of age, AMI's mortality gradually increased, and the hospitalized mortality of patients older than 75 years old was up to 22.91%.

Female AMI patients' hospitalized mortality was higher than that of male, which was 19.15%.

The survey also showed that the mortality of patients accompanied by arrhythmia, heart failure was significantly higher than in those without the complications (22.5% versus 8.8%, 37.8% versus 4.4%), and especially patients complicated with cardiogenic shock had a high mortality which was upto 56.9%.

5. Discussion

AMI is a serious cardiovascular disease which is hazard to human health and is the leading cause of death worldwide. In recent years, with the standardization of early reperfusion and drug treatment AMI mortality declined but it is still a high-mortality disease.

5.1. Clinical Characteristics. This survey presented that the average age of AMI patients was 67.37 years old, the patients older than 65 years old account for 64.40% of all observed cases, and the male to female ratio was 1.8 : 1.

A 26-year follow-up survey showed that the incidence of AMI of population aged 35 to 84 years old, for males was 71‰, for women, was 22‰. For the group from 55 to 64 years old, the incidence for male and female was 91‰ and 25‰, it were 119‰ and 51‰ in the 65~74 years old group and; was 168‰ and 90‰ in the 75~84 age group [2].

Compared with AMI patients on the study which was also sponsored by our study group to survey the therapeutic situations of TCM hospitals and Western medicine hospitals in Beijing in 2005 [3], we found that AMI patients in thrid-grade A-level TCM hospitals in Beijing area tended to be older, and the female proportion was much higher in TCM hospitals than that in western medicine hospitals.

5.2. Therapeutic Conditions. Reperfusion therapy has become the most important means of treatment on AMI. Based on the datum of 10 years, the proportion of reperfusion therapy showed rising, the proportion of intravenous thrombolytic treatment had descended, all these presented the whole reperfusion technology, and levels rose apparently in TCM hospitals in Beijing.

The usage rate of drugs recommended by Guideline presented obviously increased, while the usage rate these drugs not recommended by Guideline presented a fluctuated decline, which prompted the gap between Beijing Third-grade A-level TCM hospitals and the Guideline demand narrowed gradually.

In the development of China Traditional Chinese medicine had been playing an important role in preventing and treating all kinds of diseases. In the treatment of AMI, Chinese medicine also plays its role. This survey showed that Chinese medicine intravenous preparations currently have been widely used in clinically; this may be related to their convenient usage and the effective component being relatively single. Other several studies of our study group all show that Chinese medicine intravenous preparations can lower the mortality [3–6].

5.3. Complications. Arrhythmia, heart failure, and cardiogenic shock are the common clinical complications of AMI patients and are also the major cause of death. The datum showed the proportion of patients complicated by arrhythmia and heart failure presented a significant downward tendency during this 10 years in Beijing third-grade A-level TCM hospitals. All these showed that TCM hospitals in Beijing area have made unceasing progress in the treatment concept, treatment means, and so on, with the early reperfusion level rising ceaselessly and Chinese medicine vein preparation widely used clinically in AMI patients, patients can receive timely and effective treatment, and hospitalized complication will decrease gradually.

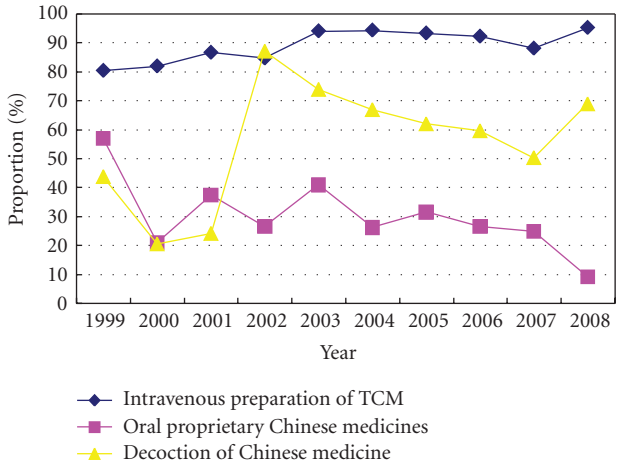

Figure 7: Chinese medicine intervention in AMI patients in Beijing Third-grade A-Level hospitals from 1999 to 2008.

5.4. Mortality. The mortality of AMI correlates to age, gender, and complications. Our survey showed that AMI patients who had a history of stroke and coronary heart disease, combined with hypertension, diabetes mellitus patients and many hospitalized history had a relatively higher mortality, considering related to the factors as combined with more disease, relatively complex and worse vascular condition.

Arrhythmia, heart failure, and cardiogenic shock are common complications in patients with AMI; AMI complications and mortality are also highly correlated. The survey also showed that the mortality of patients accompanied by arrhythmia, heart failure was significantly higher than in those without the complications, and especially patients complicated with cardiogenic shock had a high mortality which was up to 56.9%.

6. Conclusions

Through this survey, we found patients who suffered from AMI in TCM hospitals in Beijing area were mainly old patients and the female patients were more than those in Western Medicine hospitals, and they had more complications. Our other studies have shown that [7–9] elder AMI patients either in clinical features, combined with risk factors, treatment, or on long- or short-term prognosis were significantly different from young patients, and especially for older female patients, it was different from male patients.

Traditional Chinese medicine had been playing an important role in preventing and treating all kinds of diseases for Chinese people. In the treatment of AMI, Chinese medicine also plays its role. Integrative medicine treatment, combining TCM and conventional medicine, has been the most representative characteristic for AMI. However, the potential benefit of integrative medicine therapy in improving AMI prognosis is still under studying. Our study showed that, by combined therapy of Western Medicine and Traditional Chinese Medicine, the mortality of AMI patients in Beijing TCM Hospitals presented a decreasing tendency, which presented that the integrative medicine might have potential benefit for AMI patients.

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Research Article

Design and Methods for a Pilot Study of a Phone-Delivered, Mindfulness-Based Intervention in Patients with Implantable Cardioverter Defibrillators

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Background. Meditation practices are associated with a reduction in adrenergic activity that may benefit patients with severe cardiac arrhythmias. This paper describes the design and methods of a pilot study testing the feasibility of a phone-delivered mindfulness-based intervention (MBI) for treatment of anxiety in patients with implantable cardioverter defibrillators (ICDs). *Design and Methods*. Consecutive, clinically stable outpatients ($n = 52$) will be screened for study eligibility within a month of an ICD-related procedure or ICD shock and will be randomly assigned to MBI or to usual care. MBI patients will receive eight weekly individual phone sessions based on two mindfulness practices (awareness of breath and body scan) plus home practice with a CD for 20 minutes daily. Patients assigned to usual care will be offered the standard care planned by the hospital. Assessments will occur at baseline and at the completion of the intervention (between 9 and 12 weeks after randomization). The primary study outcome is feasibility; secondary outcomes include anxiety, mindfulness, and number of administered shocks during the intervention period. *Conclusions*. If proven feasible and effective, phone-delivered mindfulness-based interventions could improve psychological distress in ICD outpatients with serious cardiovascular conditions.

1. Introduction

Sound evidence supports the value of meditation-based interventions in patients with cardiovascular disease. As far back as 1971, Wallace et al. [1] described the physiological characteristics of the "relaxation response" associated with meditative practices. These included a general decrease in sympathetic nervous system (SNS) activity, oxygen consumption, and blood lactate levels. Later studies comparing subjects practicing meditation with nonpractitioners found reduced endorgan sensitivity to catecholamines, possibly mediated by a lower percentage of functional lymphocyte beta-adrenergic receptors [2]. This overall reduction in the SNS activity may be beneficial in patients affected by cardiovascular disease as suggested by a number of studies of transcendental meditation [3–8]. Fewer studies, however, have explored the possible effect of mindfulness meditation interventions in this population [9–11].

The most widely available mindfulness training course is the mindfulness-based stress reduction (MBSR) program [12] created in the early eighties by Jon Kabat-Zinn. MBSR offers training in traditional mindfulness meditation adapted to a non-Buddhist, clinical context. While MBSR has shown positive results in a wide range of medical and psychological conditions, including anxiety and depression [13, 14] some features of the program, such as the need to attend eight weekly classes and the recommended duration of daily home mindfulness practice (usually 45 minutes), may constitute a barrier to participation for patients with severe chronic cardiovascular disease.

With this study, we sought to evaluate whether a new delivery method (phone delivery) would overcome some

of the above-mentioned barriers to mindfulness training in patients with implantable cardioverter defibrillators (ICDs). This is a population with severe underlying cardiac conditions and considerable prevalence of psychological distress, with figures reaching up to 38% for clinically significant anxiety and depression [15, 16] for which mindfulness training may be beneficial. We describe here the design and methods of a pilot randomized clinical trial examining the feasibility of a mindfulness-based, phone-delivered intervention in a group of ICD patients Secondary outcomes will include the preliminary estimation of efficacy of the intervention on anxiety and mindfulness scores.

2. Design and Methods

The "Coping with ICD study" (Clinicaltrials.gov NCT01035 294) is a pilot randomized clinical trial designed to evaluate the feasibility of a phone-administered, mindfulness-based training program for the treatment of psychological distress in patients undergoing an ICD procedure, or reporting an ICD-related event (shocks).

2.1. Setting. The study will be conducted at the university campus of the UMass Memorial Medical Center (UMMMC), a tertiary care medical center located in Worcester, MA admitting more than 300 patients every year for ICD procedures.

2.2. Recruitment and Screening Procedures. Patients scheduled for an ICD-related procedure or who recently had an ICD-related event (shocks) will be screened for study eligibility within a month of the procedure/event. All potentially eligible patients will receive a letter inviting them to participate and asking them to call a dedicated phone number to communicate their possible interest. To ensure an unbiased presentation of the study, we developed a script of the first phone contact call. If the patient expresses interest, a screening visit will be scheduled.

Patients will be eligible if they meet the following criteria: age ≥21, ICD-related procedure or recent ICD shocks, ability to understand and speak English, and access to a telephone. Patients will be excluded from the study under the following conditions: inability/unwillingness to give informed consent, cognitive impairment, New York Heart Association (NYHA) functional class >III or Canadian Cardiovascular Society (CCS) angina class III or IV or otherwise clinically unstable, pending coronary bypass or heart transplantation, comorbid life threatening conditions, and ongoing severe depression or psychosis. The Blessed Orientation Memory and Concentration test [17] will be used to screen patients for cognitive impairment. Patients scoring ≥10 will be excluded from the study since mindfulness training requires a normal cognitive function and ability to focus the attention; cognitive impairment would limit the ability of the subjects to adequately participate in the intervention. Screening for ongoing depression and psychosis will be based on DSM criteria of major depressive disorder or psychosis as documented by the physician in the most recent medical evaluation. Once eligibility is confirmed, we will obtain informed consent in person. Since the study requires access to protected health information (PHI), a HIPAA authorization will be signed by each study subject in order to access his or her medical records. After baseline data collection is completed, participants will be randomly assigned to the intervention or to the control group.

2.3. Randomization. The randomization sequence will be generated using STATA [18] "ralloc" command, which produces a sequence of group assignments randomly permuted in blocks of several sizes. Block sizes of 4 and 6 will be used in this study. A programmer will generate the random allocation sequence and upload the table containing the random sequence of group assignments to an Access database. Based on this table, the participant will be automatically assigned to a group by clicking the "Randomize" button.

2.4. Follow-Up. To maximize retention, patients in both study arms will receive a weekly phone call inquiring as to whether they had questions regarding their participation. When a participant misses an intervention session, he/she will be immediately contacted. After three missed contacts, nonresponders will be sent a letter encouraging them to discuss their status. Participants will not be expected to stop any of their usual support services, for example, professional counseling, support groups, or any antianxiety or antidepressant treatment.

The study protocol and the study materials were approved by the Committee for the Protection of Human Subjects at the University of Massachusetts Medical School (Docket H-13078).

2.5. Mindfulness-Based Intervention (MBI)

2.5.1. Rationale. The study intervention will adapt elements of the MBSR [12] program (whose standard curriculum includes participation in eight weekly classes, lasting two and a half hours; an all-day retreat; practicing mindfulness and yoga exercises at home for 45 minutes/day) to the needs of patients with ICDs. Changes will include in person phone delivery versus in class delivery of the intervention; shortening of the duration of the training sessions and of the individual home practice; the exclusion of the all-day retreat and of yoga exercises.

Several conditions suggested the need for a modification of the standard MBSR program for ICD patients. (1) Driving is usually discouraged in ICD patients during the months immediately following ICD surgery [19] since up to 8% of patients experience a shock while driving [20]. This circumstance would limit attendance at regular MBSR classes. (2) Psychological distress is higher soon after ICD implantation [15], and it is important to start the intervention as close as possible to the ICD procedure to help alleviate symptoms when they are more intense. Since most MBSR programs are usually offered at discrete times, it may not be possible for many ICD patients to receive the intervention when it is most

Table 1: Characteristics of the study intervention.

Component	Duration	Objectives/content	Strategies/materials
Screening visit	10 minutes	(i) Patient receives general instructions about the intervention by the study manager (ii) Patient receives study CD player if needed, intervention CD, and mindfulness diary	Study CD player Intervention CD Mindfulness diary
Phone sessions (8)	30 minutes	(i) Instructor checks on patient ability to practice specific mindfulness technique(s) taught during previous session(s) (ii) Instructor inquires about symptoms/side effects (iii) Instructor guides patient in mindfulness exercise and receives feedback from patient (iv) Instructor and patient develop goals for next session (v) Instructor encourages participant to practice mindfulness technique with help of study CD (specifies track, 20′ every day) (vi) Instructor arranges next phone session (vii) Instructor completes intervention checklist (viii) Instructor reports problems to study manager	Intervention checklist Digital recorder for session recording by the instructor

needed. (3) Physical activity may trigger arrhythmias and shocks [21], and it is often avoided by patients at this early stage for fear of ICD discharges. (4) The clinical condition of this population with severe underlying cardiovascular disease may limit their ability to attend classes and to practice mindfulness exercises for longer periods.

2.5.2. Intervention Content. The conceptual background informing the intervention was guided by the "parsimonious" model recently proposed by Carmody [22]. Briefly, in this model mindfulness interventions are described as training in self-regulation of attention and recognition of the sensations, cognitions, and feelings that comprise daily experience. In the untrained individual, negative cycles of associated thoughts, sensations, and feelings are maintained by attention being absorbed in their content and/or meaning. For example, the presentation of a frightening thought generates an associational cycle in which the thought leads to unpleasant sensations of constriction. This negative cycle can begin with a thought, a feeling, or a sensation and is then self-maintained as long as the subject's attention remains engaged with the content of any of its components. In the process of mindfulness training, the subject learns to *notice* which component the attention is directed toward in any given moment and to *choose* to keep the attention where it is or to redirect it to another object, usually an arousal "neutral" object such as the sensations of breathing.

Consistently with our endeavor of adapting elements of the MBSR curriculum to meet the needs of ICD patients, the intervention's content will be simplified to include two basic components: (1) the body scan, a technique based on the cultivation of attention to bodily sensations and cognitions that would normally go unnoticed and (2) training in the awareness of the sensations of breathing. In addition, participants will be gradually taught to direct their attention to simple activities of daily life (such as eating and drinking), to sounds, visual objects, thoughts, and emotions and to recognize when their attention is no longer focused on that specific object of attention. At the final session, participants will practice "open awareness" in which they will be instructed to just notice which events (physical sensation, sound, visual object, and/or thought) their attention will be spontaneously drawn to from moment to moment. Patients will not receive additional materials usually provided to MBSR trainees in the form of poetry or other readings. In addition, lovingkindness ("metta" practice—a technique based on deliberately generating feelings of compassion, benevolence, and acceptance towards self and others) will not be a component of the study intervention. This technique was excluded because there is insufficient evidence for a benefit of such a practice on psychological well-being in patients with cardiovascular disease and because it would imply a different study hypothesis that deserves to be tested in a separate investigation.

2.5.3. Intervention Format. The study intervention will consist of eight phone-delivered, individual training sessions each lasting 30 minutes (Table 1). Twenty minutes will be spent on intervention delivery, and 10 minutes on questions, answers, and scheduling the next intervention. At the beginning of the study patients will receive an audio CD consisting of two different mindfulness practices, each lasting about 20 minutes, consistent with the techniques learned during each session with the instructor: track 1: sitting practice; track 2: body scan practice. After the delivery of the first intervention, participants will be encouraged to listen to the audio CD every day, at least once a day, and then throughout the study. The CD can be played using a regular CD player or a computer, and a portable CD player will be given to participants when needed.

To ensure that the delivery of the intervention will be similar across instructors we developed a script of each session. Although instructors will not have to follow the script verbatim, they will be expected to follow the sequence indicated in the script. Figure 1 shows the components of the intervention, each in a different color, and the session at which it was introduced. By looking at each row in this table it is possible to identify the content of each individual session.

2.6. Instructors. The instructors will be healthcare professionals and graduates of the Center for Mindfulness Professional Training Program with at least five-year experience in mindfulness training and a personal mindfulness practice. Prior to the study beginning, they will receive three hours of training, including a detailed review of the intervention

Sessions	Intervention components							
	Awareness of breath	Mindful eating exercise	Body scan	Mindful drinking exercise	Awareness of sounds	Awareness of emotions	Awareness of thoughts	Open awareness
1								
2								
3								
4								
5								
6								
7								
8								

Figure 1: Overview of the study intervention component by session number. Each color indicates a different component of the intervention.

script. We will hold bimonthly meetings to discuss any questions or difficulties that might be arising during the intervention sessions. Each patient will be trained by the same instructor throughout the intervention, and although not blinded to group assignment, instructors will be blinded to the study outcomes. At the end of each session, the instructors will complete an attendance form and a checklist in which duration and delivery of the intervention as specified in the intervention script as well as their perception of the patient's level of engagement during the session will be recorded. Patient's engagement will be evaluated immediately after each session and scored on a scale of 1 (completely unengaged) to 10 (extremely engaged). In order to monitor the provider's adherence to the protocol and the consistency of the delivery of the intervention across providers, each session will be digitally recorded by the instructor. Electronic copies of the attendance form, the checklist, and the MP3 file of the recorded session will be emailed weekly to the study manager.

2.7. Control Group: Usual Care (UC). Patients in the control group will receive the usual care provided by UMMMC to all ICD patients. Due to budgetary constraints, it was not possible to use an active control condition. To offset this limitation at least partially, patients in the usual care arm will receive a weekly phone call (duration: 5–10 minutes) that, although not designed to offer a specific intervention, will be aimed at addressing patients' possible concerns regarding their health or the ICD. If such concerns presented, the patient will be advised to contact his/her physician or nurse at the electrophysiology clinic. This phone call will also help to equalize the amount of study contact between study arms.

2.8. Study Assessments. Data collection will be performed at the baseline interview immediately after consent is provided, and nine weeks after enrollment once the intervention is completed.

2.8.1. Primary Outcome: Feasibility. Feasibility metrics include eligibility and recruitment rates, retention rates, intervention adherence rates, treatment fidelity, and patient's experience with the intervention. Recruitment metrics include number of screened and eligible patients, number of eligible patients who refused to participate, and reasons for refusal. Retention measures will be the number of subjects who dropped out or were lost to followup and reason(s) for dropping out. Adherence metrics include number of sessions attended and total time spent in mindfulness practice in hours over the intervention period. In addition, the time spent engaging in each separate technique will be collected. Mindfulness practice will be self-reported by means of a daily diary that patients will receive at the consenting visit, and will be instructed to mail them back using prestamped envelopes. A similar diary was successfully used in a study [23] of the effect of mindfulness training on hot flashes in menopausal women. Treatment fidelity (developed following Treatment Fidelity Workgroup guidelines) [24] will be both self-reported by the instructors (by means of a checklist to be completed at the end of each session) and objectively evaluated by reviewing a random sample (10%) of all recorded sessions, and will be defined as the average of the ratio between the number of objectives achieved versus the number of objectives planned for each session, multiplied by 100, calculated from the checklist form.

2.8.2. Secondary Outcomes

Mindfulness. Baseline and postintervention mindfulness scores will be measured using the Five Facets of Mindfulness (FFM) questionnaire [25], an instrument derived from a

Table 2: Sample size calculations[§].

Measure	Instrument	Definition	Hypothesized mean (SD) control group	Hypothesized mean (SD) intervention group	Sample size (total)
Anxiety	HADS	Mean differences between baseline and postintervention	3.0 (2.2)	5.1 (2.6)	42

[§]Null hypothesis Ho = mean difference in pre- /post-HADS score in intervention group = mean difference in pre- /post-HADS score in control group; α (two-tailed) = 0.05 and $1-\beta = 0.8$.

factor analysis of questionnaires measuring mindfulness in daily life. It consists of 39 items, exploring different aspects of mindfulness: observing, describing, acting with awareness, nonjudging of inner experience, and nonreactivity to inner experience. Each item is rated on a Likert scale ranging from 1 (never or very rarely true) to 5 (very often or always true). The FFM has shown good internal consistency [25].

Anxiety. Anxiety will be measured using the Hospital Anxiety and Depression Scale, [26] a 14-item self-administered questionnaire with two subscales measuring anxiety and depression, with higher scores indicating greater psychological morbidity. A cutoff point of 8 is usually recommended to screen patients for clinically significant depression and anxiety [26–28]. A correlation between 0.6 and 0.8 has been reported between the HADS and other commonly used questionnaires for anxiety and depression such as the Beck Depression Inventory and the State-Trait Anxiety Inventory [27], and its validity has been confirmed both in hospital settings and primary care medical practice [29]. Furthermore, the HADS offers the advantage of focusing on cognitive symptoms of anxiety instead of physical symptoms; this is particularly useful in cardiac patients where symptoms of the underlying cardiac disease may be similar or identical to those resulting from somatic manifestations of anxiety.

Number of Shocks. The number of delivered shocks (if any) will be abstracted from the electronic version of the follow-up visit 9 weeks after enrollment. While patients can receive care in other centers during the follow-up period, ICD-specific follow-up visits are mostly performed at UMMMC, thus limiting the chance of missing important information about ICD functioning and arrhythmic events.

Covariates. Information will be collected on demographics (age, gender, ethnicity, education, marital status, and financial status), type of ICD, indication for and time from the ICD procedure or shocks, prior history of anxiety and depression, ejection fraction and cardiac functional status, hospital readmissions during the study period, ongoing medications as well as other relevant data such as physical activity, use of other complementary medicine treatments, and life events (i.e., death or illness of spouse or relative) during the study period.

2.9. Data Sources. Demographic data, physical activity, use of other complementary/alternative therapies, anxiety, and mindfulness scores will be obtained from self-administered standardized questionnaires. Medical history, including past history of anxiety or depression, prescription of psychotropic medications and antiarrhythmic drugs, indication for ICD implantation, functional class, and number of shocks/arrhythmic episodes and hospital readmissions will be abstracted directly from the medical record. Study questionnaires will be administered by in-person interview at baseline and by phone interview at week 9. Questionnaires delivery via phone interview (following intervention) was preferred to mailing of questionnaires because response rates tend to be higher using the telephone as compared with mailed surveys [30]. Furthermore, the study population will likely include older individuals who may have difficulties reading the surveys. There is evidence that for some people, particularly those of low literacy and education, telephone interview is less intimidating.

2.10. Data Management. Daily management of study activities will be facilitated by the use of Microsoft Access 2007 tracking system software. Scores from study questionnaires will be immediately calculated, copied into abstraction forms together with other relevant study variables, and then entered into STATA software [18]. The study database will be kept on a server at the University of Massachusetts Medical School, with multiple levels of password protection ensuring data security. All statistical analyses will be performed using STATA version 10 statistical software [18].

2.11. Statistical Analysis. Descriptive statistics will be used to describe retention and adherence indices; a graphical examination of the distribution of the continuous variables will be used to assess the need for transformation and to show patterns (e.g., whether the amount of self-reported daily mindfulness practice shows preferential "patterns" of practice). Correlations between duration of individual mindfulness practice and baseline characteristics such as age, gender, education, and severity of cardiac illness will be evaluated using Spearman's correlation. We will assess the preliminary estimates of effect sizes of the MBI intervention on pre-/postintervention differences in mindfulness and anxiety scores using multivariate linear regression models (shown here for FFM scores): y (pre-/postintervention difference in FFM scores = $\beta_0 + \beta_1$ TX (0 control, 1 treatment) $+ \beta_2$ age $+ \beta_3$ gender (0,1) $+ \beta_4$ baseline FFM $\cdots + \varepsilon$.

2.12. Sample Size. Our planned sample size ($n = 52$, 26 for each arm) has been calculated (Table 2) using hypothetical estimates of effect size on anxiety (secondary outcome), based on data from two studies using HADS anxiety scores as the outcome variable in similar patients [31, 32]. Based on

our previous experience with mindfulness-based interventions in menopausal women and bone-marrow transplant candidates, a potential loss of 20% of patients may be expected during the study, and consequently we will need to enroll 52 patients in order to leave 42 for the final analysis.

3. Discussion

The purpose of this study is to evaluate whether a mindfulness-based behavioral intervention delivered over the phone and adapted to the needs of ICD patients would be feasible and acceptable to these individuals. Since the proposed intervention will involve several changes from the traditional MBSR program, the question arises of whether such changes are legitimate. Since its inception in the early eighties the MBSR program has been modified several times to meet the needs of hospitalized patients [33] or to minimize the time commitment required [34]. Carmody et al. [35] found no association between the number of class hours employed in published trials of MBSR and the effect sizes for measures of psychological distress. Furthermore, different psychological interventions, including cognitive behavioral therapy, [36–45] have been successfully implemented over the phone. Given this, it seems reasonable to hypothesize that mindfulness training could be delivered over the phone, and, in fact, phone delivery may have an important impact on retention: the attrition rate reported in a meta-analysis of studies evaluating the effect of telephone-administered psychotherapy on symptoms of depression was only 7.6%.

This study presents some limitations, which for the most part reflect its pilot nature and budgetary constraints. First, we did not have the financial and personnel resources to plan for the recruitment from additional clinical centers to achieve an ethnically diverse population. For similar reasons we will not be able to have an active control comparison group. Under ideal conditions, a three-arm randomization to a nurse education intervention, a mindfulness intervention, and usual care would probably be the optimal design. A usual care comparison group does not control for the possible effect deriving from the interaction with the instructor, independently of the intervention administered. Second, study assessments will occur only before and after the intervention. Further data collection points (i.e., at six months and one year) would provide useful information about a possible long-term effect of the intervention on anxiety and possibly on the number of shocks. However, this pilot study is a very preliminary exploration of the possible effects of a mindfulness-based intervention on anxiety in cardiac patients and specifically, in ICD-implanted patients. It seems that evidence of a short-term effect is warranted before the analysis can be carried on further. Third, study participants will not be blinded. This is a common limitation of behavioral interventions; however, assessors will be blinded to patients' treatment allocation status and the instructors will be blinded to study outcomes. Finally, the individual mindfulness practice will be self-reported. With adequate funding, it would be possible to develop techniques to track the amount of time that each participant listens to the study CD, such as the device used by Bauer-Wu et al. [33] or by posting each recorded session on a dedicated intervention website and then tracking the time that each participant is logged onto the website.

In conclusion, this project has potentially great significance considering the prevalence of anxiety in this population (up to 40%) [15] and the increased number of candidates for ICD implantation due to a broadening of ICD indications to include primary prevention of sudden death [46]. To date, there have been no published studies of mindfulness-based interventions in ICD patients. A mindfulness-based intervention, adapted to this group of patients, is relatively inexpensive and, once learned, could be easily self-administered by the patient. If proven feasible and effective, it could positively impact the psychological well-being and the quality of life of these patients.

Conflict of Interests

The authors declare that there is no conflict of interests.

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Research Article

Refined Qingkailing Protects MCAO Mice from Endoplasmic Reticulum Stress-Induced Apoptosis with a Broad Time Window

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In the current study, we are investigating effect of refined QKL on ischemia-reperfusion-induced brain injury in mice. *Methods.* Mice were employed to induce ischemia-reperfusion injury of brain by middle cerebral artery occlusion (MCAO). RQKL solution was administered with different doses (0, 1.5, 3, and 6 mL/kg body weight) at the same time of onset of ischemia, and with the dose of 1.5 mL/kg at different time points (0, 1.5, 3, 6, and 9 h after MCAO). Neurological function and brain infarction were examined and cell apoptosis and ROS at prefrontal cortex were evaluated 24 h after MCAO, and western blot and intracellular calcium were also researched, respectively. *Results.* RQKL of all doses can improve neurological function and decrease brain infarction, and it performed significant effect in 0, 1.5, 3, and 6 h groups. Moreover, RQKL was able to reduce apoptotic process by reduction of caspase-3 expression, or restraint of eIF2a phosphorylation and caspase-12 activation. It was also able to reduce ROS and modulate intracellular calcium in the brain. *Conclusion.* RQKL can prevent ischemic-induced brain injury with a time window of 6 h, and its mechanism might be related to suppress ER stress-mediated apoptotic signaling.

1. Introduction

Ischemic stroke is a life-threatening disease featured by high morbidity and mortality. Investigating new drugs is a hotspot and the difficulty of ischemic stroke research. The committee of the Stroke Therapy Academic Industry Roundtable proposed that studies of drugs for brain ischemia should pay attention to their pharmaceutical window [1, 2]. In clinical practice, treatment for stroke patients is always delayed after onset, and the time at which treatment is administered is highly correlated with therapeutic effect.

Qingkailing (QKL) injection was originally prepared by the Beijing University of Chinese Medicine in the 1970s, by modifying a traditional Chinese medicine, Angongniuhuang pills, composed of *Radix isatidis*, *Flos Lonicerae*, *Concha Margaritifera Usta*, baicalin, *Fructus gardeniae*, cholic acid, hyodeoxycholic acid, and *Cornu Bubali* [3]. It has been extensively used to treat the acute stages of cerebrovascular disease and has performed excellently in improving neurological function [4]. Animal experiments have shown that QKL injection can promote endothelial nitric oxide synthase expression, reduce calcium overload, regulate matrix metallopeptidase 9 expression, and inhibit inflammation in the murine model of cerebral ischemia/reperfusion [5–8]. However, QKL is confronted with handicaps from drug safety regulations in recent years, which has severely embarrassed its clinical application [9]. QKL being composed of complex components is the central problem of quality stabilization and clinic safety. Based on this, we developed refined Qingkailing (RQKL), aimed at acute ischemic stroke.

Refined Qingkailing (RQKL) injection consists of cholic acid, hyodeoxycholic acid, baicalin, and jasminoidin (Figure 1). Baicalin is derived from the dried root of *Scutellaria baicalensis* Georgi which is named as Huang Qin in traditional Chinese medicine (TCM), and jasminoidin is derived from the dried fruit of *Gardenia jasminoides* Ellis which is named as Zhi Zi in TCM. Both herbs provide excellent antioxidative and anti-inflammation effects [10–13]. Cholic acid and hyodeoxycholic acid are both bile acids and have neuron-protective effects [14, 15]. Furthermore,

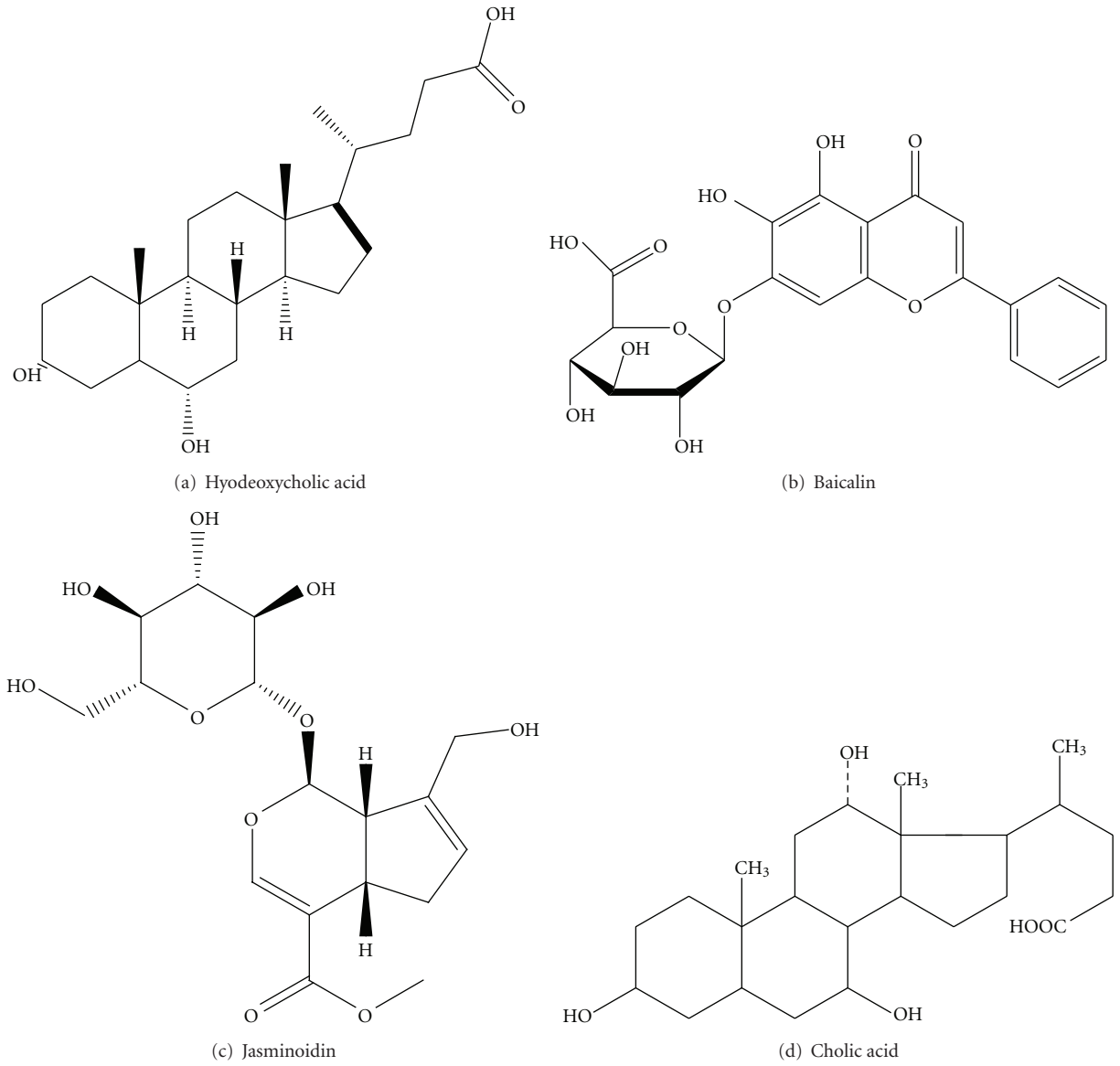


Figure 1: Molecular structure of the four components of RQKL.

the combination of the four components can significantly improve their effectiveness [16].

The endoplasmic reticulum (ER) regulates protein synthesis, protein folding and trafficking, cellular responses to stress, and intracellular calcium levels [17]. The conditions that impair the function of the ER, designated "ER stress", can lead to an accumulation of unfolded proteins in the ER lumen [18]. However, if ER stress is too severe, the unfolded protein response ultimately initiates an apoptotic pathway [19]. ER stress-induced cell death has been shown to involve the activation of caspase-12 [20, 21], while another component of the ER stress-mediated apoptotic pathway is elf-2a [22]. Several studies have shown that cerebral ischemia is a pathological ER stressor, which can trigger the shutdown of protein translation and apoptosis, suggesting that ER plays an important role in cerebral ischemia [23, 24]. Thus, reducing ER stress may provide a therapeutic way to block the pathological process induced by cerebral ischemia [18, 24].

This paper is designed to explain the dose effect and therapeutic time window of RQKL on MCAO rodents, as well as to elaborate intervention effect of RQKL on neuron apoptosis due to ER stress after cerebral ischemia.

2. Materials and Methods

2.1. Animals. We used 154 healthy, male, Kunming mice weighing 25–28 g, and 90 healthy, male, C57BL/6 mice weighing 25–30 g, purchased from Vital River Laboratories, Beijing, China (no. SCXK (Beijing) 2006-0009) and housed in the Central Laboratory, Beijing University of Chinese Medicine on a 12 h light: dark cycle at 25 ± 1°C, with relative humidity of 40–60% and automatic day-night rhythm, the mice had free access to standard lab chow and tap water.

Procedures involving animals and their care were conducted in compliance with institutional guidelines in accordance with NIH Guide for the Care and Use of Laboratory Animals, NIH publication no. 85-23, 1985.

2.2. Drugs. QKL injection, composed of cholic acid, *Concha Margaritifera Usta* (powder), hyodeoxycholic acid, *Fructus Gardeniae*, *Cornu Bubali* (powder), *Radix Isatidis*, baicalin, and *Flos Lonicerae* [25], was purchased from the Pharmaceutical Factory of Beijing University of Chinese Medicine (no. 813204A). RQKL injection composed of cholic acid, hyodeoxycholic acid, baicalin, and jasminoidin (Figure 1). Proportion and concentration of 4 compositions is consistent with QKL injection. RQKL injection is provided by Prof. Shouying Du, affiliated to School of Chinese Materia Medica, Beijing University of Chinese Medicine.

2.3. MCAO Model Establishment. Mice were anesthetized with 4% chloral hydrate (350 mg/kg) and kept under heating lamp to maintain the core body temperature at $36.5 \pm 0.5^{\circ}$C. Under a dissecting microscope (SXE-1, Shanghai Precision Instrument, Shanghai, China), the right common carotid artery, internal carotid artery, and external carotid artery were carefully exposed, and the external carotid artery was coagulated distal to the bifurcation. A 0.16 mm diameter nylon filament (tip diameter 0.20 ± 0.01 mm; Beijing Sunbio Biotech, Beijing, China) was inserted through the external carotid artery stump and gently advanced 10 mm to occlude the origin of the middle cerebral artery [26, 27]. The body temperature of the animals was maintained at 37°C. The filament was removed after 1.5 h. Postoperatively, the mice were housed separately. Neurological function was evaluated when the mice were awake [28]; those with scores less than 2 were excluded.

2.4. Grouping of Experimental Animals and RQKL Injection. In the dose-effect experiments, high, moderate, low dose RQKL injection and QKL injection and model groups were injected with 6, 3, 1.5 mL/kg RQKL and 3 mL/kg QKL or equal volume of normal saline, respectively, via the tail veins [7]. Both injections were diluted in saline with different concentrations and the final dose injected to each animal was 9 mL/kg. The first injection was performed immediately after model establishment, followed by administration after 4 h, and once every 12 h thereafter.

For time window experiments, the model group was injected with normal saline, and each RQKL group with RQKL (3 mL/kg) diluted using normal saline, via the tail vein. In model and 0 h groups mice, were first injected simultaneously with the middle cerebral artery, was occluding. The other groups received RQKL injections at 1.5, 3, 6, 9 h after MCAO, followed by a second injection after 4 h, and every 12 h thereafter [29], Figure 3(A).

2.5. Assessment of Neurological Function after Focal Cerebral Ischemia/Reperfusion Using Clark Scores. Mice neurological function was evaluated using a blind method 24 h after model establishment. Clark scores [28] include focal and general neurological function, which reflect ischemia foci-induced neurological function injury and general function, respectively. The focal neurological function was scored from 0–28, and the general function ranged from 0–32. Normal mice had a score of 0. High scores reflect severe neurological functional injury.

2.6. Infarct Volume Assessment. Following neurological function evaluation, mice were sacrificed, and the brain was harvested for TTC staining (Nanjing Greensynthesis Biochemical Co., Ltd., Nanjing, Jiangsu, China). The percent of infarct volume out of the entire brain represented the degree of cerebral infarction. Serial coronal sections (1 mm thickness) were prepared and soaked in 2% TTC phosphate buffer at 37°C for 10 minutes in the dark. Normal brain tissues were stained red, while infarct tissues were not stained (white). The sections were soaked in 4% paraformaldehyde phosphate buffer for 30 minutes, arranged in order and scanned (Tsinghua Unisplendour A688, Xi'an, China). Areas of red and white staining were measured using a computer color multimedia image analysis system (Image-Pro Plus6.0, Media Cybernetics, Wyoming, USA). The percent of infarction is given by the equation: %Infarct volume = Infarct volume/Total volume of slice $\times$ 100 [30, 31].

2.7. TUNEL Staining. After 24 h of recovery, the animals were euthanatized and the brain was rapidly removed, frozen, and cut into 20 μm slices. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was performed using a kit for programmed cell death (In Situ Cell Death Detection Kit, TMR Red, Roche, USA) according to the manufacturer's directions [32]. Sections of prefrontal cortex were collected. Five areas of each section were examined by fluorescence microscope (ZEISS, LSM510 meta, Germany) in the prefrontal cortex of the ischemic hemisphere and TUNEL-positive cells were quantified [18].

2.8. Measurement of ROS Generation. Brain reactive oxygen species (ROS) production was determined using dihydroethidium (DHE) microfluorography [33]. DHE is a cell permeable dye, which can be oxidized into ethidium and other products by superoxide [33, 34]. Animals were sacrificed at 24 h after MCAO, and the brains were removed, frozen, and sectioned (20 μm thickness) on a cryostat. Sections of prefrontal cortex were collected. A ROS fluorescence detection kit (Genmed, Wyoming, USA) was used. DHE solution was superfused on the brain sections for 60 minutes and fluorescence intensity was detected by fluorescence microscopy (ZEISS, LSM510 meta, Germany). The fluorescence intensities of five different fields of the brain section were averaged and expressed as relative fluorescence units (RFU) [35].

2.9. Western Blot. Mice were sacrificed after 24 h of cerebral ischemia, the forehead cortex was collected and homogenated in an ice bath. Proteins were separated on sodium dodecyl sulfate polyacrylamide gel electropheresis and transferred to a nitrocellulose membrane. Blots were

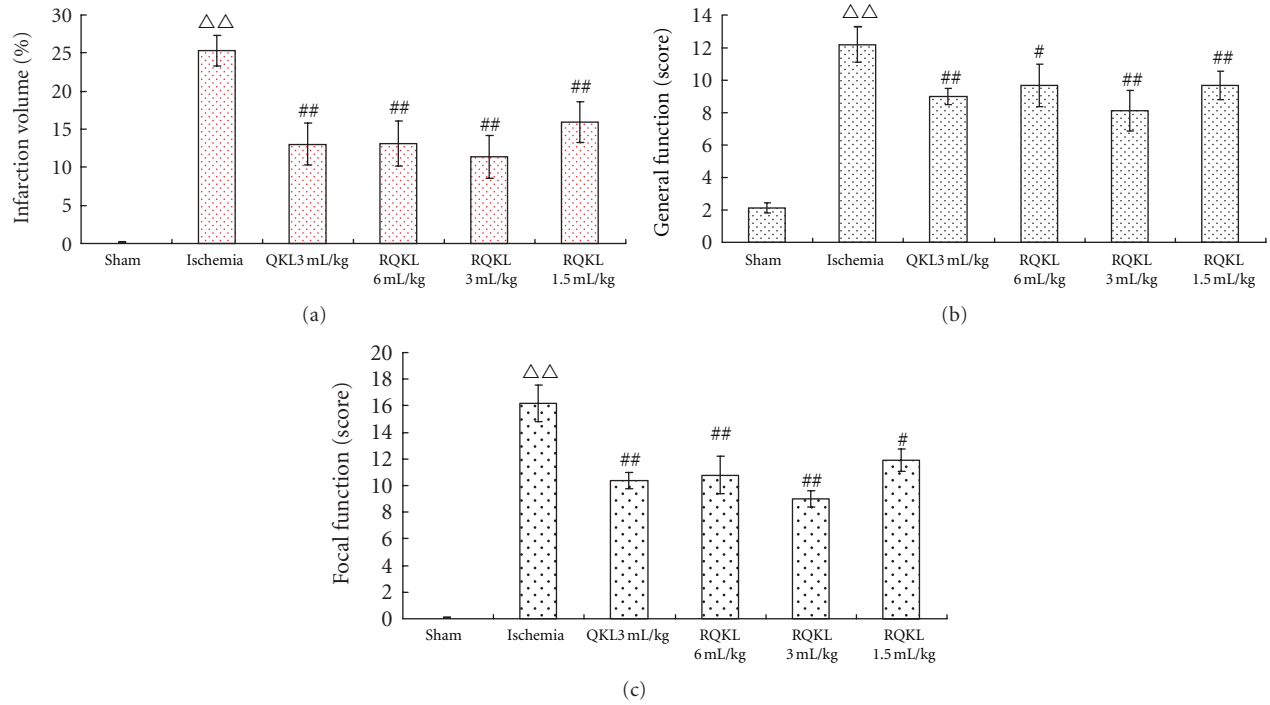


Figure 2: Effect of different doses of RQKL on infarction volume and neurological function of MCAO mouse. Five brain coronal sections, 2 mm thick, were selected for tetrazolium chloride staining. The infarct volume was quantified as a percentage of total volume, with large infarcts representing more severe injury. The percent of infarct volume was significantly less in RQKL injection groups compared with model group (a). RQKL injection at different doses significantly improve focal and general neurological function (b, c). The scores of focal neurological function and general neurological function were 0 in normal mice. High scores represent severe injury. $^{\Delta\Delta}P < 0.01$, versus model group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, versus model group. Data are expressed as mean ± SEM, $n = 10$.

blocked with 5% nonfat dry milk in phosphate buffered saline, pH 7.6, with 0.1% Tween-20 buffer and then incubated with antiphospho-eIF2α (Ser51) polyclonal antibody (Cell Signaling Technology Inc., Tokyo, Japan), anti-caspase12 polyclonal antibody (Cell Signaling Technology Inc., Tokyo, Japan), or anti-caspase3 (8G10) polyclonal antibody (Cell Signaling Technology Inc., Tokyo, Japan), subsequently incubated with secondary anti-rabbit antibody conjugated with horseradish peroxidase. Finally, membranes were processed for detection using the ECL system.

2.10. Intracellular Calcium Measurement. Intracellular calcium concentration was measured by flow cytometry using Fluo-3AM fluorescence as described previously [36, 37]. Mice were sacrificed 2 h after middle cerebral artery occlusion or 10 h reperfusion after 2 h of occlusion. Forehead cortex and hippocampus were separated in ice bath and prepared into cell suspension. Cell density was adjusted with D-Hanks solution to about 1×10^6. Suspension was incubated at 37 for 10 minutes, then 2 μL/mL Fluo-3AM dye working solution was added and mixed to uniform. Mixed solution was incubated at 37°C for 40 minutes in dark place. Flow cytometry (FACS Calibur, Becton Dickinson, USA) analysis was performed with excitation wavelength 488 nm and emission wavelength 528 nm. The results are expressed as mean fluorescence intensity (MFI) [37, 38].

2.11. Statistical Analysis. Data were analyzed using SPSS 16.0 (SPSS, Chicago, IL, USA). One-way analysis of variance was used followed by post hoc analysis for significance with the Student-Newman-Keuls multiple comparison test. All values are expressed as mean ± SEM. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Dose-Response of RQKL for Focal Cerebral Ischemia/Reperfusion

3.1.1. Dose-Response Effects on Infarct Volume. Mice with focal cerebral ischemia/reperfusion were injected with different doses of RQKL. Infarct foci were evident in the brain of mice at 24 h after ischemia/reperfusion. Compared to model group, the infarction size was reduced by 49%, 48%, 55% and 37% ($P < 0.01$) in QKL and high, moderate, and low dose RQKL injection groups, respectively (Figure 2(a)).

3.1.2. Dose-Response Effects on Neurological Function. Prior to MCAO, scores of focal neurological function and general neurological function were 0. Mice developed neurological functional injury 24 h after MCAO; however, QKL and all doses of RQKL ameliorated this injury compared with the

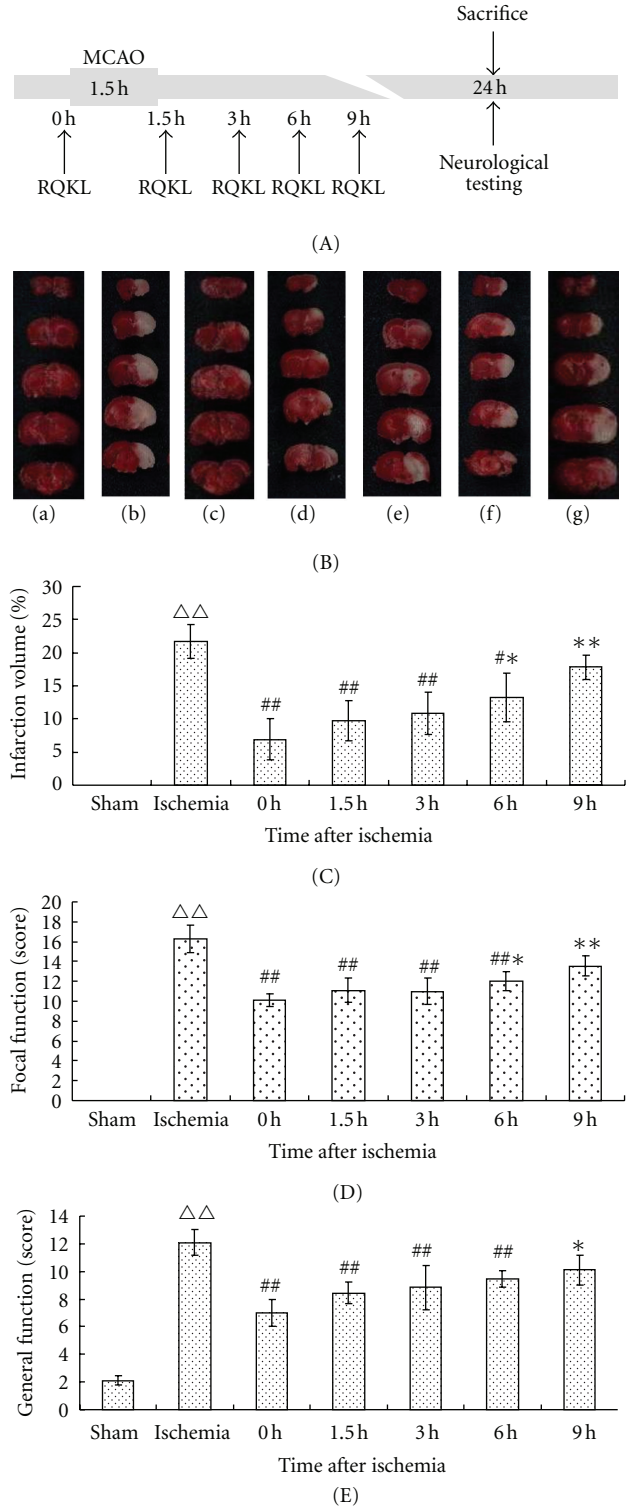


Figure 3: Effects of *RQKL* injection at different time points in mice undergoing middle cerebral artery occlusion. Flow chart of time window experiment of RQKL (A). Cerebral infarct volume (B, C) and neurological function scores (D, E) were evaluated 24 h after brain ischemia. Five brain coronal sections, 2 mm thick, were selected for tetrazolium chloride staining. Red stain represents normal tissues; white represents the infarct region (B). In panels (D) and (E), high scores represent serious injury. RQKL injection at 0, 1.5, 3, or 6 h after infarction significantly reduced infarct volume and improved focal and general neurological function, but 9 h group had no effects. $^{\Delta\Delta}P < 0.01$, versus model group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, versus model group. Data are expressed as mean ± SEM, the numbers of each group were 10, 11, 12, 9, 9, 12, 9, respectively.

model group ($P < 0.05$ or $P < 0.01$). The results are consistent with our preliminary experiments [29]. The groups receiving the moderate QKL and RQKL dose (3 mL/kg) exhibited the greatest improvement ($P < 0.01$). Moderate and high dose RQKL injection was able to restore focal neurological function ($P < 0.01$); however, the effect of low dose group was relatively poor ($P < 0.05$), Figures 2(b) and 2(c).

3.2. The Therapeutic Time Window of RQKL

3.2.1. Cerebral Infarct Volume after Treatment with RQKL at 0, 1.5, 3, 6, 9 h after Ischemia. In preliminary experiment, we found that QKL had a wide therapeutic time window [29]. In this study, RQKL also showed a broad time window. Injection of 3 mL/kg RQKL was most effective at reducing infarct volume and improving neurological function, so this dose was used in time window experiments. RQKL injection at 0, 1.5, 3, and 6 h after ischemia significantly reduced cerebral infarct volume ($P < 0.01$ or $P < 0.05$). The largest reduction of infarct volume (68%) was in the 0 h group compared with model group ($P < 0.01$). Percent reduction of infarct volume gradually decreased with increasing delay before QKL injection (infarct size: 55%, 50%, 39% in the 1.5, 3, and 6 h groups, resp.). However, 9 h group reduced cerebral infarct volume 18% than model group, but with no significance ($P > 0.05$) (Figures 3(A) and 3(B)).

3.2.2. Neurological Function Scores after Treatment with RQKL at 0, 1.5, 3, 6, 9 h after Ischemia. Consistent with dose-effect experiments, the general and focal neurological functions were significantly improved in the 0 h group ($P < 0.01$). In other treatment groups, RQKL injected at 1.5, 3, and 6 h after MCAO significantly enhanced both general and focal neurological functions ($P < 0.01$), but administration at 9 h was not effective. However, with the first treating time delay, the effects gradually decreased. The focal neurological function of 6 and 9 h groups were significantly lower than 0 h group ($P < 0.05$, $P < 0.01$, resp.), while general neurological function of 9 h group was significantly lower than 0 h group ($P < 0.05$) (Figures 3(C) and 3(D)).

3.2.3. Effects of RQKL on Neurons Apoptosis. Cellular apoptosis is an important mechanism of nerve injury after brain ischemia/reperfusion [18]. The TUNEL method was used to investigate whether an antiapoptotic effect was involved in the neuroprotection by RQKL. Few apoptotic cells were observed in brain tissues of sham mice. A large number of TUNEL-positive neurons were observed in the prefrontal cortex 24 h after ischemia. Following QKL (3 mL/kg) or RQKL (6, 3 and 1.5 mL/kg) injections, the number of TUNEL-positive neurons was reduced significantly (Figures 4(a), 4(b), and 4(c)). This showed that RQKL reduced the number of apoptotic cells in brains of MCAO mice. Meanwhile, the antiapoptosis effect of RQKL showed dosedependence.

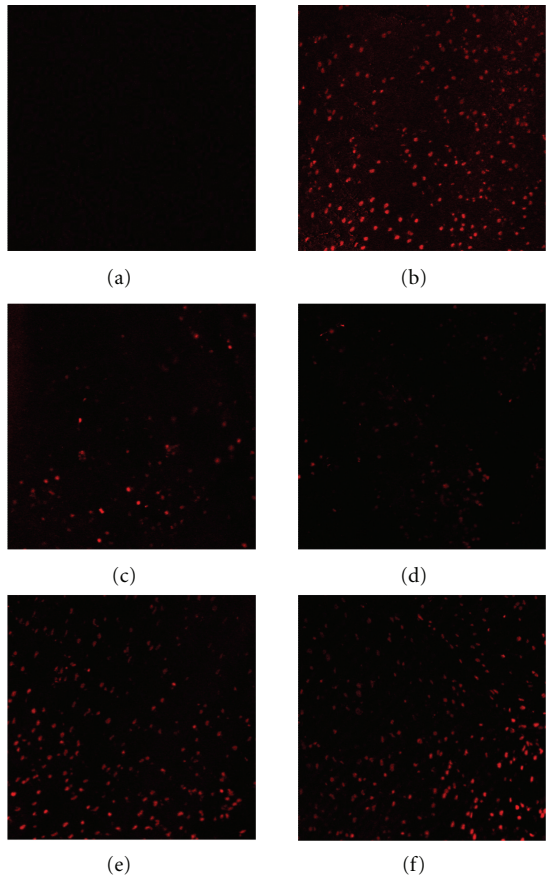
3.2.4. Effects of RQKL on Protein Level of Caspase-3, Pro-Caspase12, and P-eIF2α. Caspase-3 is an important key protein in cell apoptosis. Moreover, it is well known that caspase-3 is induced after ischemic insults [39]. In this study, caspase-3 in the cortex increased markedly, whereas QKL and RQKL injections noticeably reduced the protein level (Figures 5(a) and 5(b)). Caspase-12 plays a role in apoptotic cell death by ER stress [20]. Therefore, we examined the effects of RQKL treatment on the induction of caspase-12 after ischemia. Western blotting analysis showed that caspase-12 was activated 24 h after hypoxia-ischemia, as evidenced by a decrease in the level of the procaspase-12, which was largely restored by QKL and RQKL (about 50% restoration compared with the model group) (Figures 5(a) and 5(c)). A key feature of ER stress induced by cerebral ischemia is the blocking of translation at the initiation step, as indicated by increased phosphorylation of eIF2α [40, 41]. Therefore, we examined whether RQKL affects the phosphorylation of eIF2α. The level of phospho-eIF2α in injured cortex was markedly increased 24 h after ischemia, whereas the injection of QKL or RQKL noticeably reduced the levels of phospho-eIF2α (approximately 30 to 60% reduction, compared with the model group) (Figures 5(a) and 5(d)).

3.2.5. Antioxidative Effects of RQKL Injection. Reactive oxygen species production was detected using DHE staining. As shown in (Figures 6(A) and 6(B), no fluorescence was evident in sham mice brain. A large number of fluorescent nerve cells contributed to significantly enhanced fluorescence in the prefrontal cortex at 24 h after MCAO. Following treatment with QKL and RQKL, the number of fluorescent nerve cells was reduced, and the fluorescence intensity was weakened, indicating that RQKL reduced ROS production. Quantitative analysis showed that fluorescence in the cortex was significantly decreased in all the RQKL doses groups compared with the model group ($P < 0.01$, Figure 6(c)). This was consistent with our previous work that QKL injection has antioxidative effects [29].

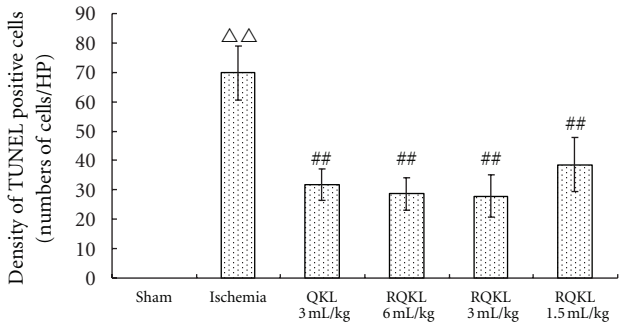
3.2.6. Intracellular Calcium. As shown in Figure 7, intracellular Ca^{2+} markedly increased 2 h after ischemia, significantly compared with the sham group ($P < 0.01$), whereas the injection of RQKL noticeably reduced the Ca^{2+} content. However, for mice suffering 2 h ischemia following 10 h reperfusion, only intracellular Ca^{2+} of hippocampal cells increased, and RQKL did not significantly reduce it. So RQKL only performed modulation effect of intracellular calcium in the early period after ischemia.

4. Discussion

QKL injection is a famous Chinese medicine widely used in China for more than thirty years. However, since the National Accident Data Recorder Monitoring Center noticed the first case of anaphylaxis following administration of QKL in November 2001, medical journals have published some case reports about the adverse drug reactions and adverse



(A)



(B)

Figure 4: Effect of *RQKL* injection on cell apoptosis in prefrontal cortex of mice (TUNEL staining). After middle cerebral artery occlusion for 24 h, apoptotic cells were detected in the prefrontal cortex ((A), ×400). Apoptotic cells were labeled with red fluorescence. (a) sham, (b) ischemia, c-ischemia+QKL3ml/kg, d-ischemia+RQKL 6 mL/kg, (e) ischemia+RQKL3ml/kg, (f) ischemia+RQKL1.5 mL/kg. Five animals were selected from each group; three sections were selected from each site; and five 400-fold fields of view were randomly selected from each section to quantify the mean of positive cells. Results are expressed as mean ± SEM (B). $^{\Delta\Delta}P < 0.01$, versus sham-surgery group; $^{\#\#}P < 0.01$, versus model group.

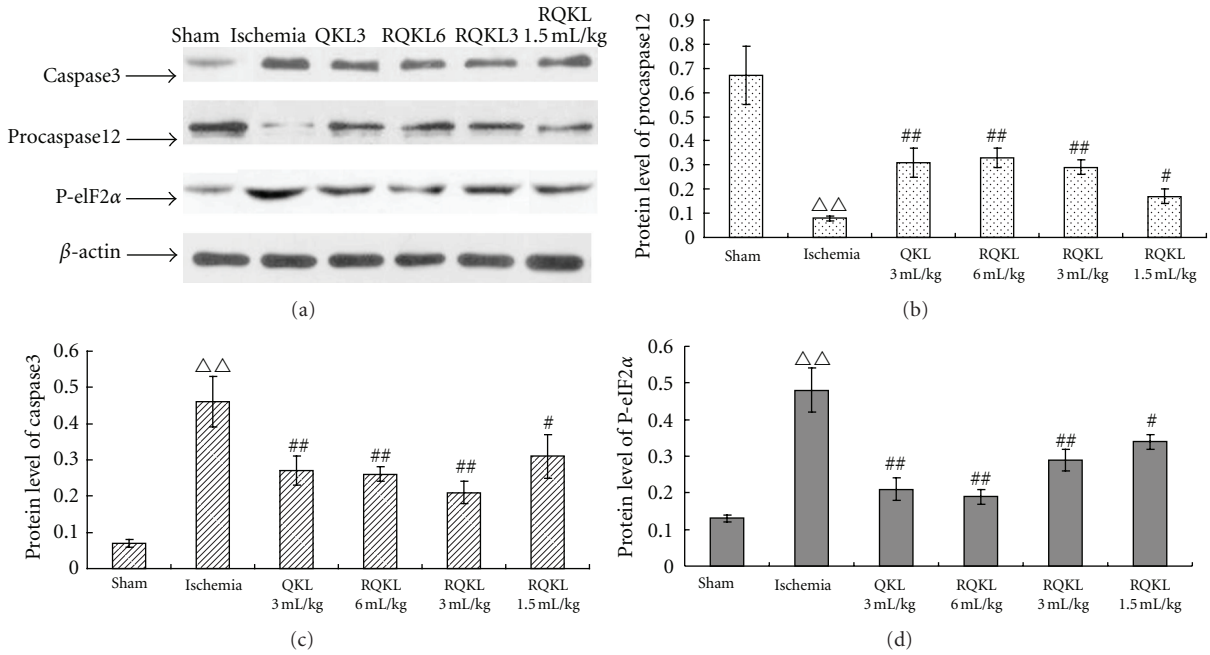


Figure 5: Effect of RQKL on protein levels of caspase3, procaspase12, and P-elF2a in cortex of MCAO mouse. Mice were subjected to 1.5 h ischemia followed 22.5 h reperfusion. QKL or RQKL was injected immediately after ischemia. The tissue samples were from the cerebral cortex. The panels are representative Western blotting analyses of caspase3, procaspase12 and P-elF2a. The gray values were calculated, and protein levels were expressed by the ratio of aim protein/β-actin as mean ± SEM. Notes $^{\Delta\Delta}P < 0.01$, versus normal, $^{\#\#}P < 0.01$, versus ischemia, $^{\#}P < 0.05$, versus ischemia, $n = 5$. Primary antibodies were diluted, caspase12 1 : 400, caspase3 1 : 2000, and P-elF2a 1 : 500.

events due to its use, so the safety of QKL injections became a focus of public opinion [9]. Much more attention has been attached to drug safety of traditional Chinese medicine (TCM), as well as efficiency. Redeveloping famous Chinese medicine formulas is a new way to improve traditional Chinese medicine, which is important for the modernization and globalization of TCM. Aimed at acute ischemic stroke, one of the indications of QKL, we refined QKL into a novel medicine, namely RQKL. It is composed of only four components of QKL, which has an advantage in quality stabilization and drug safety. In this study, we researched the efficiency and therapeutic time window of RQKL using MCAO mice, compared with QKL. The results showed that the injection of RQKL at doses of 1.5, 3, 6 mL/kg protected the brain from ischemic injury, as evidenced by reductions in infarct volume and neurological function. Most importantly, administration of RQKL provided a wide therapeutic window of 6 h.

The time window in which a drug is effective varies between drugs, commonly ranging between 2–4 h for ischemic stroke drugs [42–44], but occasionally extended to 12 h [45]. Administration beyond the therapeutic window can reduce or even abolish the pharmacodynamic action. This present study has defined the therapeutic window for RQKL injection in the treatment of brain ischemia. Incompatible with QKL, RQKL can also significantly reduce infarct volume and improved focal and general neurological function when administered in a broad time window after ischemia. RQKL injection at 6 h after ischemia significantly reduced infarct volume and improved neurological function, so the therapeutic window for RQKL injection for MCAO mice can be said to extend to over 6 h. This may be profited from multimechanisms of the compound Chinese medicine, for it showed remarkable effects of promoting endothelial nitric oxide synthase expression, reducing calcium overload, regulating matrix metallopeptidase 9 expression and inhibiting inflammation in a murine model of cerebral ischemia/reperfusion [5–8].

In this study, we found that RQKL can effectively suppress apoptosis *in vivo*. RQKL treatment significantly inhibited apoptosis under *in vivo* ischemic conditions, as indicated by the results of TUNEL assays. Moreover, the present findings indicated that the protective effect of RQKL on ischemic injury may be medicated in part by restoration of ER dysfunction. Previous studies showed that mitochondria play a central role in neurons apoptosis after ischemia. However, recent studies suggested that ER damage is involved in neuronal cell death induced by cerebral ischemia [23, 24, 46]. In the present study, we investigated the effect of RQKL on ER dysfunction under pathological conditions. Consistent with the results reported in recent years [18, 47], in mice subjected to 1.5 h ischemia and 22.5 h reperfusion, we observed an remarkable increase in the level of the eIF2α-phosphorylation in the ischemic cortex, which indicated that ischemia/reperfusion caused severe ER damage. On the other hand, treatment with RQKL significantly inhibited p-eIF2α induction. Therefore, the protective effects of RQKL in ischemia/reperfusion injury may be partly due to inhibition of ER stress and subsequent apoptotic signaling pathway.

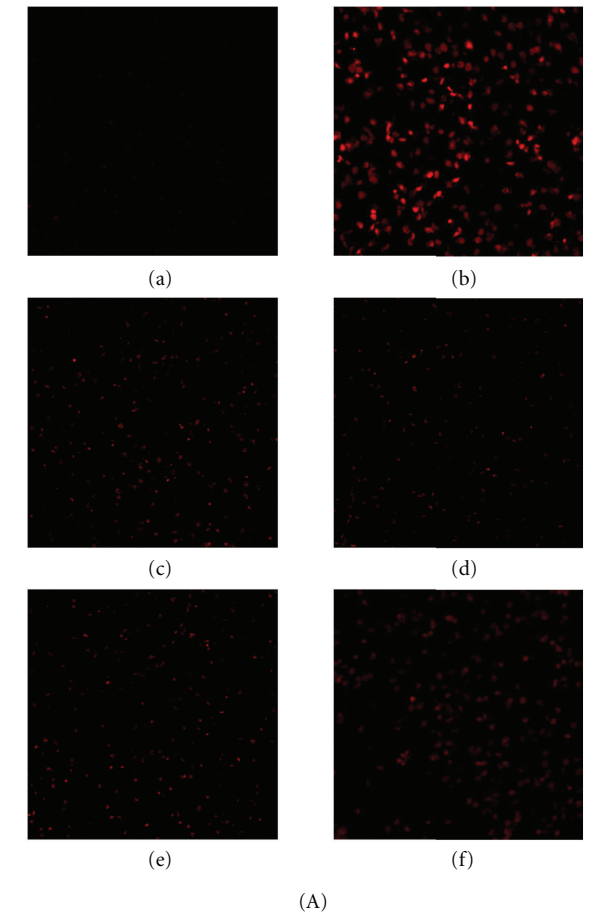


Figure 6: Effects of RQKL injection on reactive oxidative species in mice undergoing middle cerebral artery occlusion. Relative intensity of red fluorescence represents reactive oxygen species (ROS) content in the prefrontal cortex ((A) ×200) regions of the injured hemisphere. (a) sham, (b) ischemia, (c) ischemia+QKL3ml/kg, (d) ischemia+RQKL 6 mL/kg, e-ischemia+RQKL3ml/kg, (f) ischemia+RQKL1.5 mL/kg. Relative fluorescence intensity in five sites from one section was determined by fluorescence microscopy. The mean value of ROS content was calculated and expressed as mean ± SEM (B). $^{\Delta\Delta}P < 0.01$, versus sham, $^{\#\#}P < 0.01$, versus ischemia, $n = 5$.

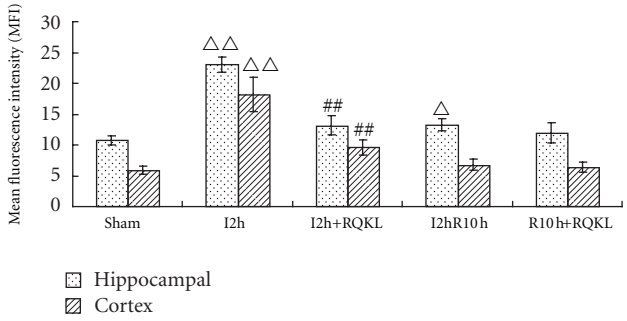


Figure 7: Effect of RQKL on intracellular Ca^{2+} in brain cells. Mice were divided into five groups: sham group, I2h group for cerebral ischemia 2 h, I2hR10h group suffering ischemia 2 h followed 10 h reperfusion. RQKL therapeutic group received RQKL (3 mL/kg) immediately after ischemia. Neurons of hippocampal and forehead cortex regions were detected by flow cytometry analysis. The results are expressed as mean fluorescence intensity (MFI). Note. $^{\Delta\Delta}P < 0.01$, versus sham, $^{\Delta}P < 0.05$, versus sham, $^{\#\#}P < 0.01$, versus ischemia, $n = 6$.

ER stress-induced cell death has been shown to involve the activation of caspase-12, which subsequently activates executer caspases such as caspase-3 [20, 21, 48, 49]. Caspase-12 is specific to insults that elicit ER stress and is not proteolytically activated by other death stimuli [20]. Previous studies have shown that caspase-12 is activated after permanent and transient middle cerebral artery occlusion, and many caspase-12 positive cells exhibited DNA fragmentation; on the other hand, mice that are deficient in caspase-12 are more resistant to ER stress-induced apoptosis [20]. This indicated the activation of caspase-12 is involved in ischemia-induced apoptosis. We found caspase-12 was activated 24 h after MCAO and RQKL remarkably suppressed the activation, which indicated that RQKL inhibits caspase-12 dependent apoptotic pathway. Furthermore, RQKL also decreased caspase-3 level significantly. Caspase-3 activation is involved in caspase-12 mediated apoptotic cascade, while activated caspase-3 is directly responsible for DNA fragmentation [20, 21, 48]. It is possible that caspase-3 activation is inhibited by suppression of caspase-12. Thus, RQKL might inhibit caspase-3 activation by suppressing ER stress-mediated apoptotic signaling and therefore reduce the extent of apoptosis.

Studies suggested that multiple causes of ER stress occur in neurons following cerebral I/R: intracellular calcium homeostasis, aggregation of proteins, decreased protein degradation, and accumulation of ROS in ER and Golgi structures [50, 51]. Recent studies suggested that disruption of intracellular calcium homeostasis could induce ER stress and kill cells [52]. In this study, we found intracellular calcium raised markedly 2 h after ischemia; on the other hand, RQKL can significantly depress intracellular Ca^{2+}. Like intracellular calcium homeostasis, ROS also played an important effect in causing ER stress [51, 53]. RQKL showed excellent antioxidative effects, evidenced by notably reducing ROS. So the inhibition effect to ER stress of RQKL may be immediate or mediated by anti-oxidative as well as intracellular calcium modulation. Considering the findings of the present study, we speculate that RQKL is a promising novel drug for acute ischemia stroke, and it has multiple neuroprotective effects, although the mechanism of its actions needs to be elucidated further.

Abbreviations

MCAO: Middle cerebral artery occlusion
ROS: Reactive oxygen species
ER: Endoplasmic reticulum
RQKL: Refined Qingkailing.

Authors' Contribution

Q. Wang was in charge of funding and authorized this study. F. Cheng conducted experiments, conceived and designed the study, and wrote the draft of the paper. X. Zhong revised the paper and provided technical support. Y. Lu conducted animal experiments, conceived and designed this study, and participated in paper writing. W. Song and D. Wang contributed to evaluation of the study. S. Guo, X. Wang, D. Liu, and W. Zhao participated in animal experiments and index detections. F. Cheng and X. Zhong equally contributed for this paper.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

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Research Article

Cardioprotective Effect of the Compound *Yangshen* Granule in Rat Models with Acute Myocardial Infarction

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The protective effect of Compound *Yangshen* Granules was observed in myocardial infarction rat model. Rats were randomly divided into 6 groups: the model group, the control group (sham operated), the positive drug group, and small, medium, and large dosage of the *Yangshen* granule groups, respectively. The rats in the 3 *Yangshen* granule groups were orally administrated with 0.7 g/kg, 1.4 g/kg, and 2.8 g/kg for 7 consecutive days, whereas the rats of the positive drug group treated with 0.14 g/kg of Danshen Dropping Pills, and rats in the control and model groups orally administrated with saline. The rat model of acute myocardial infarction was established with ligation of coronary artery. Electrocardiograms at different time points, the blood rheology, myocardial enzymes, infarct size, and myocardial morphologic changes were measured. The results demonstrated that the granules could improve blood rheology, decrease st-segment of electrocardiograms and the activities of LDH and CK in serum, reduce myocardial infarction size, and alleviate myocardial histopathologic changes. In addition, the effect of the granules depended on the dose administrated orally. The results suggest that the *Yangshen* granules could produce cardioprotection effect and have potential benefits in the prevention of ischemic heart disease.

1. Introduction

Based on the principle of "nourishing the heart in summer" advocated by traditional Chinese medicine (TCM) and combined with the outlook that Chinese herbs and foods have the same source each other. Compound *Yangshen* Granule for summer health care is designed, which serves to clear the summer-heat, reinforce *qi* and nourish *yin* together protect the heart and regulate the mind as well. As a summer health formula of Chinese medicine, the granule is suitable for summer health care. In clinical, it was observed that *Shenmaiyin* which has the effect of reinforcing *qi* and nourishing *Yin* has the benefits for patients with coronary heart disease [1]. At the same time, experimental research has shown that *Shenmaiyin* have the cardioprotective effect of the rat model of acute myocardial infarction [2]. It suggested that formulas for reinforcing *qi* and nourishing *yin* may have the potential benefits in the prevention of coronary heart disease. This study is to provide the experimental evidence of the cardioprotective effect of the *Yangshen* granules in rat model of myocardial ischemia.

2. Materials and Methods

2.1. Animals. Sprague-Dawley (SD) rats (weight approximately 200–220 g) were purchased from Anhui Animal breeding laboratory of Animal Center, China (certificate no. SCXL (Anhui) 2005-001).

2.2. Herbs and Reagents. The formula of the Compound *Yangshen* Granule was provided by the Formula Laboratory of the Beijing University of Chinese Medicine (batch no: 20100202). The formula consisted of *ginseng* (Ren-Shen in Chinese), *Radix ophiopogonis* (Mai-Dong in Chinese), *Radix Puerariae* (Ge-Gen in Chinese), *Ziziphus jujuba seeds* (Suan-Zao-Ren in Chinese), *Japan-ese Honeysuckle* (Yin-Hua in Chinese), *Green tea* in proportions of 4 : 5 : 3 : 2 : 1 : 1. The processing flow of herbs is as follows: all the herbs were soaked in water for 2 hours, boiled and extracted for 3 times, 1 hour each time. Each time the proportions of water to herbs were 10 : 1, 8 : 1, and 8 : 1, and the decoction obtained was strained to put the filtrate together. After decompressed concentration (temperature $\leq 70^{\circ}$C) the relative density was

1.02∼1.04 (60°C). Then 1% chitosan solution was added to be keept overnight and the solution was filtered. After decompressed concentration (temperature ≤ 70°C) to the relative density of 1.20∼1.25 (60°C), the extracted liquid was dried (temperature ≤ 70°C) to get dry extract, which was crushed into fine dust and appropriate dextrin was added 90% ethanol was added to make granules go through a 14-meshed sift, and then dried (temperature ≤ 70°C) into granules through a 12-meshed sift. Each pouch contains 8.5 g granules (equal to 18 g crude drugs). Danshen Dropping Pills were produced by Tianjin Tasly Pharmaceutical Co, LTD (the product no. 20090722, Tianjing, China). The pills and Chinese granules were grinded and then mixed with distilled water before use. The kits of lactate dehydrogenase (LDH) and creatine kinase (CK) were provided by Nanjing Building Research Institute of Biological Engineering (the batch no. 201000118). TTC was purchased from Sigma (the batch no. T8877, USA).

2.3. Experimental Instruments. The 754-A Spectrophotometer (Shanghai Scientific Instrument Company, Shanghai, China); Automatic Chemistry Analyzer (Olympus-400, USA); YDA-IV type machine of blood rheology (Beijing Hongrunda Sci-Tech Development Company, Beijing, China); XD-7100 Single Channel Electrocardiograph (Shanghai High-tech Medical Equipment Company, Shanghai, China).

2.4. Creation of the Acute Myocardial Ischemia Rat Model. The acute myocardial ischemia rat model was established according to [3–5]. Briefly, rats were given an operation in ether anesthesia and the chest was opened in the fourth left rib space to expose the heart. The left coronary arteries were then ligated with 5–0 lines from a distance of 2-3 mm to the root of the coronary artery.

2.5. Design and Allocation. The laboratory was set at 18°C and 55–65% humidity. Rats were randomly divided into 6 groups: the control group (sham operated), the model group, the positive drug group (Danshen Dropping Pill, DDP), and small, medium and large dose of *Yangshen* granule groups, 14 rats for each group. The rats in the small (SDG), medium (MDG) and large dose groups (LDG) were administrated orally with doses of 0.7 g/kg, 1.4 g/kg, and 2.80 g/kg (equivalent to 5, 10, and 20 times of an adult dosage of 8.5 g per day), respectively for 7 consecutive days. The rats in the positive drug group were treated with 0.14 g/kg of Danshen Dropping Pill, whereas the rats of the control and model groups were administrated orally with equivalent saline. 30 min after the last administration, rats were operated on by ligation of coronary artery. The electrocardiograms of each rat were recorded at different time points 2 h before and after operation. They were injected with 10% chloral hydrate after ligation for 24 h and the blood samples were taken from the abdominal aorta. After separation of serum and plasma (heparin anticoagulation), the relative myocardial enzymes and the blood viscosity were determined. After the heart was quickly removed, the weight of myocardial infarction was measured and myocardial pathology analyzed.

2.6. Detection of Different Markers

2.6.1. Blood Rheology. 5 S^{-1}, 30 S^{-1} and 200 S^{-1} of shear rate of the whole blood and the plasma viscosity were determined by a cone-plate-type Blood rheometer.

2.6.2. Electrocardiogram (ECG). Recorded by the II Guide League Electrocardiogram at the speed of 50 mm/s at different points 2 h after the ligation.

2.6.3. Myocardial Infarction Size. The heart was quickly removed from the body. After removal of the atrium and large blood vessels, the heart and ventricular weights were measured. The ventricule was horizontally cut into slices and incubated in 1% TTC solution in PBS at 37°C for 15 min. The red area indicated no myocardial infarction (mi), the area without color was the ischemic heart muscles. The weight of the ischemic heart muscle was measured and the rate of myocardial infarction calculated. Myocardial infarction rate (%) = infarction myocardial weight/the whole ventricular weight × 100%.

2.6.4. CPK and LDH. The abdominal aortic blood was separated by centrifugation at 3,000 rpm for 10 min. The activities of the serum enzymes were measured with the colorimetric method according to specifications of the kit.

2.6.5. Myocardial Pathological Changes. The cardiac muscles below ligation to the apex area of the heart was taken and fixed with a solution containing 10% formalin. The tissue slices of the heart were fabricated with HE dyeing. Histopathological changes were analyzed under a light-microscope and the severity was classified as described in references [6, 7].

2.7. Statistical Analysis. All data were generated by the SPSS13.0 software. The data generated from multiple samples were statistically analyzed by One-way analysis of variance (ANOVA), SNK-Q tests, and a chi-square test. The data from the multiple samples for grade materials were analyzed by Kruskal-Wallis. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. The Changes of Blood Rheology among Different Groups. Compared with the control group, the whole blood and plasma viscosities of the model group rats increase to different extent at different shear rates. It is significantly different ($P < 0.05$) at the high shear rate. Compared with the model group, the whole blood and plasma viscosities of the positive herbal group and different dose groups of granules decrease in different extents at different shear rates and those in the positive drug group and large-dose group of granule are significantly different ($P < 0.05$) at the high shear rate (see Table 1).

Table 1: The changes of blood rheology in the acute myocardial ischemia model treated by oral granules ($\bar{x} \pm s$, $n = 10$, mPa/s).

Group	Dose ($g \cdot kg^{-1}$)	Whole blood viscosity			Plasma viscosity
		$5\ s^{-1}$	$30\ s^{-1}$	$200\ s^{-1}$	
Control	—	10.45 ± 0.43	6.88 ± 0.06	4.73 ± 0.03	1.59 ± 0.03
Model	—	10.99 ± 0.63	7.01 ± 0.13	$4.84 \pm 0.04^{**}$	1.62 ± 0.02
DDP	0.14	10.50 ± 0.26	6.91 ± 0.09	$4.74 \pm 0.06^{\#\#}$	$1.59 \pm 0.02^{\#}$
SDG	0.70	10.98 ± 0.37	6.99 ± 0.05	4.80 ± 0.03	1.61 ± 0.02
MDG	1.40	10.60 ± 0.72	6.92 ± 0.16	4.76 ± 0.08	1.60 ± 0.01
LDG	2.80	10.85 ± 0.52	6.95 ± 0.08	$4.77 \pm 0.07^{\#\#}$	$1.58 \pm 0.02^{\#}$

Note: $^{**}P < 0.01$, compared with the positive drug group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with the model group.

Table 2: The changes of st-segment of electrocardiograms among different groups ($\bar{x} \pm s$, $n = 10$, mV).

Group	Dose ($g \cdot kg^{-1}$)	St-segment elevation at different time after ligation			
		0 h	0.5 h	1 h	2 h
Control	—	0.15 ± 0.19	0.09 ± 0.12	0.09 ± 0.12	0.08 ± 0.09
Model	—	$0.42 \pm 0.17^{*}$	$0.36 \pm 0.17^{*}$	$0.27 \pm 0.11^{*}$	$0.30 \pm 0.16^{*}$
DDP	0.14	$0.24 \pm 0.11^{\#}$	$0.17 \pm 0.11^{\#}$	0.20 ± 0.12	0.19 ± 0.14
SDG	0.70	$0.17 \pm 0.09^{\#}$	0.20 ± 0.19	0.21 ± 0.20	0.17 ± 0.12
MDG	1.40	0.27 ± 0.25	0.23 ± 0.19	0.18 ± 0.15	$0.16 \pm 0.14^{\#}$
LDG	2.80	$0.17 \pm 0.14^{\#}$	$0.23 \pm 0.10^{\#}$	0.19 ± 0.16	$0.14 \pm 0.12^{\#}$

Note: $^{*}P < 0.05$, compared with the positive drug group; $^{\#}P < 0.05$, compared with the model group.

3.2. The Changes of St-Segment of Electrocardiograms among Different Groups. Compared with the control group, st-segment elevation of electrocardiograms at different time points are up in the model groups and the differences are all statistically significant ($P < 0.05$). Compared with the model group, st-segment elevation of electrocardiograms in the positive herbal group and different dose groups of granule reduces to different extent. The st-segment reductions of electrocardiograms are significantly different in the positive drug group at 0 and 0.5 h after surgery ($P < 0.05$). Those are also obviously significant in the small dose group of granule at 0 h and for the large dose group of granule at 0 h, 0.5 h, and 2 h after surgery ($P < 0.05$) (see Table 2).

3.3. The Changes of the Myocardial Infarction Rate among Different Groups. The average rate of myocardial infarction in the model group is 22.48%. Compared with model group, the average rates of the positive herbal group and different dose groups of granules descend to different extent. The differences were statistically significant in the positive herbal group and the medium and large dose groups of granules ($P < 0.05$) (see Table 3).

3.4. The Changes of Serum LDH and CK Activity among Different Groups. Compared with the control group, the serum LDH and CK activities in the model group increased significantly ($P < 0.01$) after coronary artery ligation for 24 h. Compared with the model group, the LDH and CK activities in the positive herbal group and different dose groups of granules decline to different extent. It is significantly different in the positive herbal group and the large dose group of granule ($P < 0.05$) (see Table 4).

3.5. The Changes of Myocardial Histopathology among Different Groups. The myocardial cells in the control group observed are arranged in order and cytoplasmic dyeing of the control group is of uniformity with the shape of round or oval. Meanwhile, nuclear chromatin in that group is uniformly distributed (see Figure 1(a)). Myocardial cells in the model group are dead with multifocal and flake coagulative characteristics. The nuclei are also dissolved and chipped. Some nuclear areas disappear and cytoplasm dyeing looks deeper. The blood vessels are of hyperemia obviously and mesenchymal edema with numerous neutrophile granulocytes infiltrating (see Figure 1(b)). The range of coagulative necrosis is smaller than the model group. The nuclei are dissolved and chipped in the infarction area. The nuclei in local area disappear and cytoplasm deeper dyeing. Some capillary congestion and mesenchymal edema with neutrophile granulocyte infiltrating are observed in the positive herbal group (see Figure 1(c)). The myocardial cells are partially in coagulative necrosis with globular or focal shape in various dose groups of granule. The myocardial degeneration appears but infarction is not obvious. In the infarction area, there are some cells with nucleus pyknosis and swelling or dissolved and chipped. Cytoplasm dyeing is deeper and the cell volume is decreased. The blood vessel congestion, edema, and neutrophil infiltration in cardiac interstitials are also observed. The pathological changes are markedly alleviated in the medium and large dose groups of granule compared with the model group (see Figures 1(d)–1(f)).

3.6. The Severity of Myocardial Damage among Different Groups. There are coagulative necrosis, interstitial hyperemia and bleeding, and inflammatory cells infiltrating in the heart tissues to different extent in various groups ($P < 0.05$).

Table 3: The changes of myocardial infarction rate among different groups ($\bar{x} \pm s$, $n = 10$, g).

Group	Dose ($g \cdot kg^{-1}$)	Weight of heart	Weight of ventricle	Weight of infarction	Rate of infarction
Control	—	0.8272 ± 0.1346	0.5678 ± 0.0995	—	—
Model	—	0.8964 ± 0.0960	0.6314 ± 0.0705	0.1440 ± 0.0519**	22.48 ± 6.77**
DDP	0.14	0.8132 ± 0.1183	0.5822 ± 0.0968	0.0411 ± 0.0188#	7.01 ± 1.49##
SDG	0.70	0.8906 ± 0.0825	0.6235 ± 0.0597	0.1016 ± 0.0307	16.05 ± 3.31
MDG	1.40	0.9097 ± 0.0841	0.6629 ± 0.0933	0.0895 ± 0.0413#	13.77 ± 7.18#
LDG	2.80	0.8194 ± 0.0525	0.5759 ± 0.0482	0.0574 ± 0.0173##	10.10 ± 3.61##

Note:** $P < 0.01$, compared with the positive drug group; # $P < 0.05$, ## $P < 0.01$, compared with the model group.

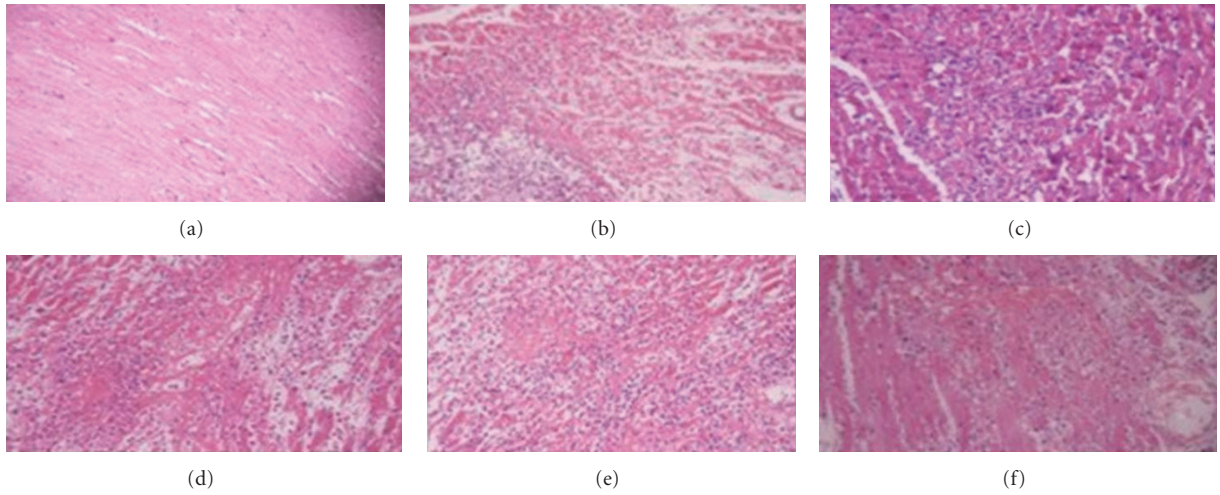

Figure 1: The myocardial protective effect of the Compound *Yangshen* Granule in the acute myocardium infarction rat model (HE, 10 × 10). (a) Control group; (b) model group; (c) positive dose group; (d) small dose group; (e) medium dose group; (f) large dose group.

Table 4: The changes of serum LDH and CK activities among different groups ($\bar{x} \pm s$, $n = 10$).

Group	Dose ($g \cdot kg^{-1}$)	LDH (U/L)	CK (U/mL)
Control	—	1755.56 ± 667.22	0.25 ± 0.13
Model	—	3481.54 ± 569.16**	0.53 ± 0.11**
DDP	0.14	2321.63 ± 602.34#	0.27 ± 0.21#
SDG	0.70	3195.65 ± 739.48*	0.47 ± 0.19**
MDG	1.40	2940.32 ± 677.04*	0.40 ± 0.21
LDG	2.80	2619.14 ± 638.03*#	0.36 ± 0.13#

Note: * $P < 0.05$, ** $P < 0.01$, compared with the positive drug group; # $P < 0.05$, compared with the control group.

Compared with the model group, myocardial histopathologic changes decrease in the positive herbal group and the three dose groups of granule to different extent. The data are statistically significant in the positive herbal group and the medium and large dose groups of the granule ($P < 0.05$) (see Table 5).

4. Discussion

In summer, people tend to perspire more than other seasons, which may cause the imbalance of water, electrolyte, and the viscous blood. Meanwhile, the heart and kidney pressure increase and the physical function declines gradually, which possibly caused the aggravation of coronary heart disease. Based on the theory of TCM, it is well known that the heart connects correspond to fire, governs blood circulation and the mind as well. Meanwhile, summer connects correspond to fire and is also closely correlated with the heart. If the body is in disorder, it will cause deficiency of *qi* or *yin* due to hyperactivity of the heart, which results in various diseases. Keeping good health in summer should follow the principle of nourishing *qi* and *yin*, protecting heart, and regulating the mind. In the light of the experience of traditional Chinese medicine and according to the reference of guidebook for using herbs of health care products in Chinese homology of medicine and food [8], the formula of Compound *Yangshen* Granule for health care in summer is designed and it contains *Ginseng, Radix ophiopogonis, radix puerariae, Ziziphus jujuba seeds, Japan-ese Honeysuckle, green tea,* and so forth. This formula has potential effects such as clearing heat, or summer-heat, supplementing *qi* and nourishing *yin*, invigorating blood circulation and nourishing the heart.

Several studies have shown that *Ginseng* has obvious reaction to relieve stress [9]. It also has the protective effect for the myocardial cells with less oxygen and sugar [10] and can improve the blood rheology in the elderly [11]. *Radix ophiopogonis* can increase the tolerant ability of the body in hypoxia [12], repair ischemia myocardial, and improve the cardiac hemodynamic [13]. *Radix puerariae* may expand

Table 5: The myocardial histopathological changes among different groups.

Group	Dose ($g \cdot kg^{-1}$)	Early coagulation necrosis				Hyperemia bleeding				Inflammatory cells infiltrating			
		–	+	++	+++	–	+	++	+++	–	+	++	+++
Control	—	8	0	0	0	8	0	0	0	8	0	0	0
Model	—	0	1	6	1	0	0	7	1	0	4	4	0*
DDP	0.14	0	6	2	0	1	4	3	0	0	4	4	0#
SDG	0.70	0	1	7	0	0	2	5	1	0	4	4	0#
MDG	1.40	0	7	1	0	1	5	2	0	0	7	1	0#
LDG	2.80	2	5	1	0	0	6	2	0	2	6	0	0#

Note: $^{*}P < 0.05$, compared with the positive drug group; $^{\#}P < 0.05$, compared with the control group.

the coronary artery, protect the heart from myocardial ischemia, and improve the blood rheology [14, 15]. *Ziziphus jujuba seeds* can repair ischemia myocardial and reduce myocardial ischemic injury [16, 17]. *Green tea* may alleviate fatigue [18] and has the effect of antioxidant [19]. These data suggest that this formula composed of the above Chinese herbal medicines may enhance the antistress ability and produce cardioprotection effects.

In this study, we know that the myocardial damage can be relieved in different dose groups, which prove that administration of the granules could improve the blood rheology, decrease st-segment of electrocardiograms, inhibit the activities of LDH and CK, reduce the myocardial infarction size, and alleviate the myocardial histopathologic changes in rat model. The observed effect of the *Yangshen* granules has highcorrelation with the doses of the granules treated for rats. It should be pointed out that administration of the granules at higher dose produces clear and significant effects of cardioprotection. Taken together, the data presented in this paper indicate that the granules have cardioprotection effects similar to other medicines for reinforcing *qi* and nourishing *yin* [1, 2]. These data also provide strong evidence implying that Compound *Yangshen* Granule may be used in the prevention of those patients with ischemic heart disease.

Danshen Dropping Pill has the effects of promoting blood circulation and removing obstruction of the vessels and relieving pain as well. It is an effective traditional Chinese patent medicine in the treatment of acute angina pectoris, and with the syndrome of *qi* and blood stasis in TCM. The *Yangshen* Granule acts to supplement *qi* and nourish *yin*, clear heat, and activate blood. It is designed for healthy population to keep good health under the summer heat or high temperature circumstance. It is especially for those with the syndrome of deficiency of both *qi* and *yin* due to summer heat. The two herbal medicines differ from each other and have their own indications. But the study has shown that both of them may have antimyocardial ischemia effect. It suggests that formulas with different treatments would have some identical pharmacological effects. However, the *Yangsheng* Granule and Danshen Dropping Pill are differed in composition of ingredients, indications, and administration in the light of the knowledge of TCM. It is necessary to have further study and evolution of their effects and characteristics.

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Research Article

The Evaluation of Chinese Therapeutic Food for the Treatment of Moderate Dyslipidemia

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The clinical efficacy of the Chinese therapeutic food (specifically hawthorn fruit and Chinese kiwifruit-extract compound) on dyslipidemia was evaluated in this placebo-controlled, double blind, paired clinical trial conducted in Melbourne, Australia. Forty-three participants diagnosed with moderate dyslipidemia and met the study criteria were randomly assigned to Group A or B, with baseline characteristics matched. Twenty-seven participants completed all the tests, the blood lipid profile including total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and triglycerides (TG) was analysed. The traditional Chinese medicine diagnosis was made based on participants' symptoms and signs. The results indicate that a four-week intake of the compound increased the serum HDL-c levels by 5% ($P = 0.026$) and decreased the ratios of TC/HDL-c and LDL-c/HDL-c ($P = 0.012$ and $P = 0.044$, resp.). The placebo intake did not significantly change the blood lipid profile. In the initial 43 participants with dyslipidemia, 76.7% of them were diagnosed with "*Spleen* deficiency" and 58.1% with "*Liver qi* stagnation." The intake of hawthorn fruit and Chinese kiwifruit extract compound may increase the serum levels of HDL-c and decrease the ratios of TC/HDL-c and LDL-c/HDL-c, therefore, may reduce the risk of cardiovascular disease.

1. Introduction

Atherosclerosis and subsequent cardiovascular disease (CVD) are common and have high mortality. Dyslipidemia is considered to be responsible for the development of atherosclerosis through blood lipid accumulation and oxidation [1, 2]. The atherogenic lipid profile includes increased low-density lipoprotein cholesterol (LDL-c), triglycerides (TG), and decreased high-density lipoprotein cholesterol (HDL-c), which are all recognized as independent risk factors for CVD. Therapeutic lifestyle changes are recommended as the first choice for blood lipid management, and dietary intake is suggested to include reduced intake of saturated fat and increased LDL-c lowering nutrients [3]. HMG-CoA reductase inhibitors (statins), bile acid sequestrants, nicotinic acid, and fibric acids, are conventionally employed to achieve different goals of blood lipid management. However, side effects of the agents, such as myopathy and increased liver enzymes [4], are a concern to users and clinicians.

Chinese therapeutic food may make a valuable contribution to a more balanced diet. They have been used widely with acknowledged safety in Chinese history, and recipes based on herbal dietary therapy are accepted extensively nowadays for the prevention and treatment of heart diseases [5]. Chinese people have traditionally preferred food and herbs to drugs for health care, and many herbs are described as both food and medicine by the Chinese Ministry of Health [6]. In the selected therapeutic food supplement, hawthorn fruit (*Shan Zha*) is known in traditional Chinese medicine (TCM) for its effects on reducing food stagnancy and *blood* stasis. As herbal medicine, it is also used to treat dyslipidemia, angina pectoris, and hypertension [7]. Chinese kiwifruit (*Zhong Hua Mi Hou Tao*) originated from China and its various cultivars have been widely consumed globally. Previous studies have shown that the Chinese kiwifruit cultivar used in

the supplement for this study appears to be antiatherogenic [8, 9].

This study evaluated the efficacy of the selected Chinese therapeutic food supplement for the treatment of dyslipidemia in Australia and compared the outcomes with the previous study conducted in China [10].

2. Methods

2.1. General Description. A placebo-controlled, double-blind, paired research design was used in the present study, based upon a previous study with positive outcomes [10]. Among the 62 applicants, 43 Australian participants who met the inclusive criteria were recruited and assigned into two groups. Two interventions, the therapeutic food compound and the placebo, were applied in this study. The intervention in the two groups was swapped at the middle of the study. Before and after each period of intervention, fasting blood samples were taken and analysed for the serum lipid levels. Participants were also assessed from the TCM perspective.

This study was approved by the Human Research Ethics Committee of Victoria University, Melbourne, Australia. It was registered with the Australian-New Zealand Clinical Trials Registry. The Therapeutic Food Administration (TGA) was notified of this trial.

In this present study, the heavy metal contents in the compound were analysed by the Australian National Measurement Institute and were found to be safe for human consumption. The pesticide concentrations of the supplements used in this study were also analysed at Victoria University and found to be safe for human consumption.

2.2. Participants. All participants met the following criteria before enrollment:

(i) have lived in Australia for the past 10 years,

(ii) diagnosed with moderate dyslipidemia, in which fasting serum LDL-c level within 3.2–4.6 mmol/L (125–180 mg/dL) or TG 2.0–2.9 mmol/L (180–250 mg/dL), according to Adult Treatment Panel III [3],

(iii) aged 40–70 years and with blood pressure lower than 159/99 mmHg (A meta-analysis indicated that older age and increasing blood pressure could attenuate the proportional CVD risk reduction of cholesterol lowering [11]; a similar clinical trial to evaluate the effects of berry consumption on CVD risk factors, using "140–159 mmHg systolic blood pressure or 90–99 mmHg diastolic blood pressure" as inclusive criteria for blood pressure, has shown favourable outcomes [12]),

(iv) absence of other major medical conditions (i.e., established CVD, severe diabetes, thyroid dysfunction, asthma, hepatic or renal disorders) or pregnancy,

(v) not have taken other supplements or medicine which may influence blood lipid levels in the past 3 months,

(vi) not allergic to the extract of the therapeutic food or wheat germ,

(vii) not have fluid retention or severe diarrhoea (according to the TCM theory, the nature of kiwifruit is considered as *cool*, which means that intake of kiwifruit may give rise to or deteriorate fluid retention for people with very weak digestive system and cold nature; hawthorn fruit may cause slight diarrhoea due to its effect on reducing food stagnancy, however, it is unlikely occur in this study because kiwifruit's cool nature can be balanced by hawthorn fruit's warm nature and the nourishing effect of kiwifruit may also balance the hawthorn fruit's effect of reducing stagnancy).

This study purposely did not include participants with an Eastern Asian cultural background as a similar study has already been conducted in China [10]. It is considered the people with an Eastern Asian background, such as Chinese, Korean, and Japanese, who may share similar culture-affected life styles. Therefore, genetic factors, locality, and/or cultural differences were taken into account in comparison with the previous study.

Volunteers participating in this study were recruited from the staff of Victoria University and residents from the local community (Melbourne, Australia). They became aware of this study through public e-mails within Victoria University, posters at community health centers, local newspapers, and word of mouth.

The issues pertaining to this research were reinforced and explained by the researchers at each interview. The treatment and control crossover procedure was clearly described and explained to each participant. The participants were also informed that they could withdraw from this study at any time. Each participant signed the consent form prior to recruitment. All records were kept secure and confidential and can only be accessed by the related researchers.

2.3. Study Design. Participants were assigned into pairs according to the baseline blood lipid levels and demographic characteristics (i.e., age and gender). A random number table was used for grouping the randomly allocated participants. Participants in Group A received the treatment of the supplement for the first four weeks while participants in Group B received the control supplement (placebo) during the same period. For the second four weeks, the interventions were switched, that is, Group B received the supplement and Group A took placebo, to observe long-term treatment effects in Group A. The total duration of participation was eight weeks. Three office visits were organised during the study (at the beginning, mid and the end of the participation, resp.). Treatment effects have been considered within individual Group A and B, and also for the combination of Group A + B.

A practitioner who was familiar with this type of clinical trial was invited as the third party to label the treatment and placebo powder (which were packed in the same container). The powder was assigned to match the participants' codes and groups (i.e., A1, A2…, or B1, B2…; names were kept confidential). This practitioner was asked not to reveal

Table 1: Participants' general information.

[#]Group (No)	Age* Mean ± SD	Male	Female	LDL-c* Mean ± SD	Systolic blood pressure* (mmHg) Mean ± SD	Diastolic blood pressure* (mmHg) Mean ± SD
A ($n = 14$)	56.00 ± 7.09	5	9	4.01 ± 0.60	125.1 ± 13.4	77.9 ± 7.5
B ($n = 13$)	53.62 ± 9.85	8	5	3.95 ± 0.61	125.8 ± 10.0	79.9 ± 7.3

[#] A: Group A—took HFC (treatment) for the first four weeks; then took the placebo for the second four weeks; B: Group B—took the placebo for the first four weeks; then took the HFC for the second four weeks.
* $P > 0.05$.

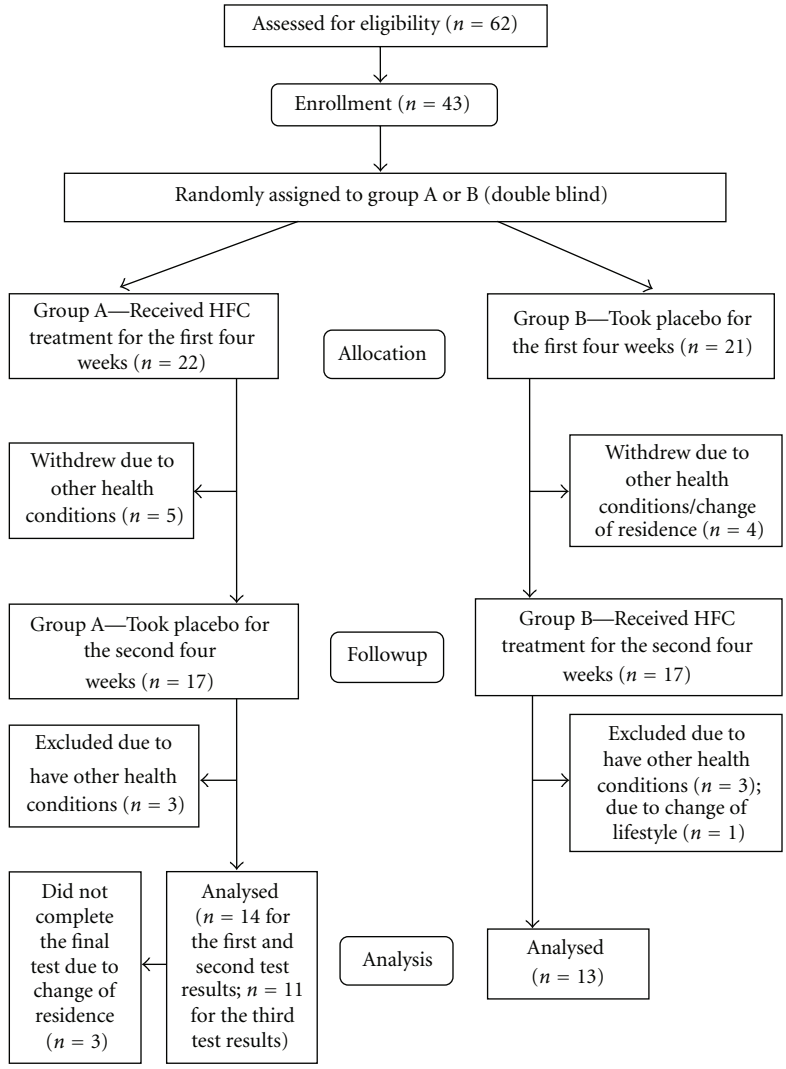

Figure 1: Flow chart of participants through each stage of the trial.

relevant information to either researchers or participants until the final data analysis was completed.

Blood lipid levels can be affected by lifestyle-related factors and can fluctuate over time without treatment. All participants in both the treatment and control groups were advised not to change their normal daily lifestyle during the course of this study. Thus, they were asked to complete a questionnaire as to record their diets and physical activities for the first and last three days in each phase of their participation. The questionnaire allowed the researchers to monitor the participants' diet and physical activity to determine whether there were significant changes. By completing the questionnaire, the participants were also made aware of their habits and were reminded not to make any changes during the study.

2.4. Intervention. Hawthorn fruit compound (HFC) with an established safe and effective dosage of 10 g twice per day

Table 2: Comparison of HDL-c levels (mmol/L) between pre- and post-HFC treatment.

Group (no)	Pretreatment* Mean ± SD	Posttreatment** Mean ± SD
A ($n = 14$)	1.44 ± 0.33	1.53 ± 0.42[#]
B ($n = 13$)	1.50 ± 0.42	1.54 ± 0.48
A + B ($n = 27$)	1.47 ± 0.37	1.54 ± 0.44[##]

[#] $P = 0.09$; [##] $P = 0.026$.

*Pretreatment: Group A—pre-HFC treatment; Group B—completed the first four weeks of the placebo intake and is to start HFC treatment, that is Postplacebo value.

**Posttreatment: Group A completed HFC treatment for the first four weeks, that is Preplacebo value; Group B completed the HFC treatment for the second four weeks.

Table 3: Comparison of HDL-c levels (mmol/L) between pre- and postplacebo intake.

Group (no)	Preplacebo intake* Mean ± SD	Postplacebo intake** Mean ± SD
A ($n = 11$)	1.55 ± 0.39***	1.53 ± 0.34
B ($n = 13$)	1.58 ± 0.44	1.50 ± 0.42

$P > 0.05$.

*Preplacebo intake: Group A completed HFC treatment for the first four weeks and started the placebo intake.

**Postplacebo intake: Group A completed the placebo intake for the second four weeks; Group B completed the placebo intake for the first four weeks.

***This value is different from the value of Group A ($n = 14$) at posttreatment (1.53 ± 0.42, Table 2) because three participants withdrew from the trial. Eleven participants in Group A ($n = 11$) completed their test.

[7, 10] was selected as the treatment supplement. The compound includes Chinese therapeutic food extract hawthorn fruit (*Crataegus pinnatifida Bge.*) and Chinese kiwifruit (*Actinidia chinensis var. deliciosa*). It contains the effective components haw flavone, triterpenoid, rutin, tartaric acid, citric acid, crategeolic acid, ester, glucoside, analytical lipid enzyme, carbohydrate, some saponins, polysaccharides, multiple kinds of organic acids, isoflavones, and adequate amounts of trace elements, for example, zinc (Zn) and strontium (Sr). The placebo consists of wheat germ, food dye, and citric acid, and sweetener was used as a control supplement in the same dose. Both the treatment and placebo drinking powder were provided by the supplier of the previous study in China [7, 10]. Participants were asked to mix the drinking powder (either treatment or placebo) with warm water, and to take the supplement with an interval of minimum one hour after food intake. Each participant was asked to complete a checklist of everyday supplement intake to monitor the progress.

2.5. Blood Lipid Test Parameters. Blood lipid levels including TC, TG, and HDL-c were assayed during pre-, mid- (at the end of 4th week), and posttreatment periods. The LDL-c level was calculated using the Friedewald equation [13]: LDL-c = TC-HDL-c−TG/2.2 (all values in mmol/L). The ratios of TC/HDL-c and LDL-c/HDL-c were also investigated.

Blood samples were taken in the Outpatient Department of the appointed hospitals with Melbourne Health Shared Pathology Service (MHSPS), including Royal Melbourne Hospital, Footscray Western Hospital, and Sunshine Hospital. All blood tests were performed in the Melbourne Health Shared Pathology Laboratory by the appointed staff. Enzymatic and spectrophotometric methods were used for the study assays. Participants were asked to fast 12 hours before the blood sample collection.

2.6. Traditional Chinese Medicine Assessment. Participants' symptom information was collected using the standard TCM diagnostic methods, including inspection, asking questions, listening, smelling, and palpation. The occurrence of certain symptoms and signs indicates certain patterns of disharmony, which was determined at every visit of assessment.

2.7. Statistical Analysis. The data were analysed through the application of paired t-test using a software program SPSS 18.0 to determine the differences between pre- and postinterventions, and $P < 0.05$ was accepted as being significant.

3. Results

3.1. General Information. Twenty-seven of the 43 participants completed all the treatment and placebo intervention applicable to this study in eight weeks (Table 1). There is no significant difference of age, gender, baseline serum LDL-c levels, and blood pressure between the two groups at the baseline level. In Group A, 11 participants completed all the three blood tests; three participants did not complete the third blood test. In Group B, 13 participants completed all the tests. The participants who discontinued were affected by conditions not related to this study, such as moving to places other than Melbourne, other health conditions, and significant lifestyle changes (Figure 1).

3.2. Serum High-Density Lipoprotein Cholesterol (HDL-c) Levels. There is an increase of the HDL-c levels when comparing the pre- and posttherapeutic food treatment in Group A. However, the change is not statistically significant ($P = 0.09$). When combining the treatment results from both groups (Group A + B), it was found that the overall increase of HDL-c is statistically significant ($P = 0.026$, Table 2). This indicates that the HFC intake can improve the HDL-c level. If a larger sample size is used, the result is likely to be more promising.

There was no significant difference of the HDL-c levels between pre- and postplacebo intake in either Group A or Group B (Table 3), although a decreasing trend is observed. This indicates that the placebo intake may not affect the HDL-c level significantly in this study. It is noted that in the participants of Group A, who had completed their HFC treatment prior to placebo intake, the HDL-c levels appear to decrease not as much as compared to the results of the participants of Group B, who meanwhile took placebo

Table 4: Comparison of TC and TG levels (mmol/L) between pre- and post-HFC treatment.

Group (No)	TC Pretreatment* Mean ± SD	TC Posttreatment** Mean ± SD	TG Pretreatment* Mean ± SD	TG Posttreatment** Mean ± SD
A ($n = 14$)	6.23 ± 0.63	6.34 ± 0.57	1.69 ± 0.79	1.76 ± 0.89
B ($n = 13$)	6.41 ± 0.60	6.28 ± 0.70	1.74 ± 0.83	1.55 ± 0.71
A + B ($n = 27$)	6.31 ± 0.61	6.31 ± 0.62	1.71 ± 0.79	1.66 ± 0.80

$P > 0.05$.
*Pretreatment: Group A—pre-HFC treatment; Group B—completed the first four weeks of the placebo intake.
**Posttreatment: Group A completed HFC treatment for the first four weeks; Group B completed HFC treatment for the second four weeks.

Table 5: Comparison of TC and TG levels (mmol/L) between pre- and postplacebo intake.

Group (No)	TC Preplacebo intake* Mean ± SD	TC Postplacebo intake** Mean ± SD	TG Preplacebo intake* Mean ± SD	TG Postplacebo intake** Mean ± SD
A ($n = 11$)	6.40 ± 0.60	6.57 ± 0.65	1.54 ± 0.59	1.59 ± 0.73
B ($n = 13$)	6.35 ± 0.65	6.41 ± 0.60	1.83 ± 1.00	1.74 ± 0.83

$P > 0.05$.
*Preplacebo intake: Group A completed HFC treatment for the first four weeks and started the placebo intake.
**Postplacebo intake: Group A completed the placebo intake for the second four weeks; Group B completed the placebo intake for the first four weeks.

Table 6: Comparison of the TC/HDL-c ratio between pre- and post-HFC treatment.

Group (No)	Pretreatment* Mean ± SD	Posttreatment** Mean ± SD
A ($n = 14$)	4.54 ± 1.12	4.41 ± 1.13
B ($n = 13$)	4.58 ± 1.26	$4.37 \pm 1.20^{\#}$
A + B ($n = 27$)	4.56 ± 1.17	$4.39 \pm 1.14^{\#\#}$

$^{\#}P = 0.032$; $^{\#\#}P = 0.012$.
*Pretreatment: Group A—pre-HFC treatment; Group B—completed the first four weeks of the placebo intake.
**Posttreatment: Group A completed HFC treatment for the first four weeks; Group B completed HFC treatment for the second four weeks.

Table 7: Comparison of the TC/HDL-c ratio between pre- and postplacebo intake.

Group (No)	Preplacebo intake* Mean ± SD	Postplacebo intake** Mean ± SD
A ($n = 11$)	4.29 ± 0.80	$4.44 \pm 0.93^{\#}$
B ($n = 13$)	4.31 ± 1.20	$4.58 \pm 1.26^{\#\#}$

$^{\#}P > 0.05$; $^{\#\#}P = 0.047$.
*Preplacebo intake: Group A completed HFC treatment for the first four weeks and started the placebo intake.
**Postplacebo intake: Group A completed the placebo intake for the second four weeks; Group B completed the placebo intake for the first four weeks.

Table 8: Comparison of LDL-c levels (mmol/L) between pre- and post-HFC treatment.

Group (No)	Pretreatment* Mean ± SD	Posttreatment** Mean ± SD
A ($n = 14$)	4.01 ± 0.60	4.03 ± 0.43
B ($n = 13$)	4.10 ± 0.50	4.02 ± 0.63
A + B ($n = 27$)	4.06 ± 0.55	4.02 ± 0.53

$P > 0.05$.
*Pretreatment: Group A—pre-HFC treatment; Group B—completed the first four weeks of the placebo intake.
**Posttreatment: Group A completed HFC treatment for the first four weeks; Group B completed HFC treatment for the second four weeks.

Table 9: Comparison of LDL-c levels (mmol/L) between pre- and postplacebo intake.

Group (No)	Preplacebo intake* Mean ± SD	Postplacebo intake** Mean ± SD
A ($n = 11$)	4.17 ± 0.33	4.32 ± 0.50
B ($n = 13$)	3.95 ± 0.61	4.10 ± 0.50

$P > 0.05$.
*Preplacebo intake: Group A completed HFC treatment for the first four weeks and started the placebo intake.
**Postplacebo intake: Group A completed the placebo intake for the second four weeks; Group B completed the placebo intake for the first four weeks.

first. Further research with more participants is required to evaluate the long-term effect of HFC on the HDL-c levels.

3.3. Serum Total Cholesterol (TC) and Triglycerides (TG). Tables 4 and 5 show the effects of either HFC or the placebo on TC and TG are not statistically significant ($P > 0.05$).

3.4. The Ratio of Total Cholesterol and High-Density Lipoprotein Cholesterol (TC/HDL-c). Table 6 shows that there is a significant decrease of the TC/HDL-c ratio comparing the pre- and posttreatment in Group B ($P = 0.032$) and in Group A + B ($P = 0.012$). The results indicate that HFC can effectively lower the ratio of TC/HDL-c, an informative marker of atherosclerosis.

There is an insignificant increase of the TC/HDL-c ratio, when comparing preplacebo (post-HFC treatment) and postplacebo in Group A. However, an increase of the TC/HDL-c ratio is statistically significant ($P = 0.047$) in Group B without the impact of HFC treatment (Table 7).

Table 10: Comparison of the LDL-c/HDL-c ratio between pre- and post-HFC treatment.

Group (No)	Pretreatment* Mean ± SD	Posttreatment** Mean ± SD
A ($n = 14$)	2.94 ± 0.82	2.81 ± 0.71
B ($n = 13$)	2.94 ± 0.87	2.83 ± 0.94[#]
A + B ($n = 27$)	2.94 ± 0.83	2.81 ± 0.82[##]

[#] $P = 0.075$; [##] $P = 0.044$.
*Pretreatment: Group A—pre-HFC treatment; Group B—completed the first four weeks of the placebo intake.
**Posttreatment: Group A completed HFC treatment for the first four weeks; Group B completed HFC treatment for the second four weeks.

Table 11: Comparison of the LDL-c/HDL-c ratio between pre- and postplacebo intake.

Group (No)	Preplacebo intake* Mean ± SD	Postplacebo intake** Mean ± SD
A ($n = 11$)	2.80 ± 0.53	2.91 ± 0.60[#]
B ($n = 13$)	2.68 ± 0.80	2.94 ± 0.87[##]

[#] $P > 0.05$; [##] $P = 0.041$.
*Preplacebo intake: Group A completed HFC treatment for the first four weeks and started the placebo intake.
**Postplacebo intake: Group A completed the placebo intake for the second four weeks; Group B completed the placebo intake for the first four weeks.

Table 12: Changes of the blood lipid profile comparing pre- and post-HFC treatment.

Blood Lipid	Group A	Group B	Group A + B
Total cholesterol (TC)	→	→	→
Low-density lipoprotein cholesterol (LDL-c)	→	→	→
High-density lipoprotein cholesterol (HDL-c)	↑	→	↑
The ratio of TC/HDL-c	→	↓	↓
The ratio of LDL-c/HDL-c	→	→	↓
Triglycerides (TG)	→	→	→

Note: ↑ = significant increase.
→ = no significant change.
↓ = significant decrease.

This may indicate a "natural" increase of TC in Group B if no treatment is provided.

3.5. Serum Low-Density Lipoprotein Cholesterol (LDL-c). There is an insignificant difference of LDL-c levels comparing pre- and post-HFC treatment in Group A, Group B and overall Group A + B ($P > 0.05$) (Table 8).

Again there is an insignificant increase of the LDL-c level comparing the pre- and postplacebo intake in both Group A and Group B ($P > 0.05$), which indicates that the placebo intake did not have a significant impact on the LDL-c level in this study (Table 9).

3.6. Ratio of LDL-c/HDL-c. Similar to the ratio of TC/HDL-c, the ratio of LDL-c/HDL-c provides a more informative marker for CVD risk than the individual value of LDL-c and HDL-c. There is a decreasing trend of the LDL-c/HDL-c ratio after the treatment in Group A and Group B (Table 10). When combining the results from both groups, the decrease in the LDL-c/HDL-c ratio is statistically significant ($P = 0.044$).

There is an insignificant increase of LDL-c/HDL-c ratio comparing posttreatment and postplacebo in Group A. However, in Group B, without the impact of HFC treatment, the ratio increased significantly after the placebo intake ($P = 0.04$, Table 11). This may indicate a "natural" increase of LDL-c/HDL-c ratio in Group B if no treatment is provided.

3.7. General Changes of the Blood Lipid Profile. The results of the study show possible benefits of HFC on regulating blood lipid levels. Table 12 summarizes the changes of the blood lipid levels when comparing pre- and posttreatments in the two groups.

3.8. Baseline Characteristics of TCM Assessment-Identification of TCM Patterns. The following subjective symptoms were described by the participants: lower back pain, knee/leg/foot pain, poor appetite, abdominal discomfort, diarrhoea, constipation, tiredness, palpitations, insomnia, irritability/anxiety/stress, headache, and thirst. The main manifestations of the common patterns which may be seen in patients with dyslipidemia are summarized in Table 13. The distributions of patterns, major symptoms, signs of tongue and pulse are summarized in Tables 14, 15, 16, and 17. The most prevalent symptoms of the 43 participants are "abdominal symptoms" (53.5%) "thirst" (51.2%) and "abnormal bowel movement" (46.5%).

Among the 43 participants who were initially recruited, the TCM *Spleen* deficiency was found to be predominant (76.7% of the total). Symptoms and signs which may be relevant to *Spleen* deficiency are prevalent in all the participants. For example, more than half of the participants complained of abdominal symptoms before participation; 37.3% of them had loose stool, diarrhea, or occasional constipation and diarrhea; 39.5% of them prefer a warm condition to a cool one. In addition, 48.8% of the participants had teeth marks on their tongues and more than half had weak pulse. These signs on the tongue and the pulse are usually indicative of *Spleen* deficiency. It was found that 58.1% of the participants were diagnosed of *Liver qi* stagnation. 55.8% of the participants complained of stress and 51.2% of them had wiry pulse, which supports the *Liver qi* stagnation diagnosis.

4. Discussion

4.1. The Effects on HDL-c. In the present study, the four-week intake of HFC appears to effectively increase the serum levels of HDL-c by 5% (Group A + B) for the 27 participants. Compared with the previous clinical study on this compound [10], which has achieved the +7% increase of HDL-c, the increase of 5% in the present study is reasonable. The HDL-c changes are not statistically significant when comparing pre- and postplacebo intakes in both groups,

Table 13: Major TCM patterns of disharmony and clinical manifestations relating to dyslipidemia.

Patterns	Main clinical manifestations
Spleen deficiency	Tiredness, preference of warmth, abdominal distension/discomfort, reduced appetite, indigestion, loose stool/diarrhea (or alternately with constipation), pale or swollen tongue with or without teeth marks, weak (and/or slippery) pulse
Liver qi stagnation	Stress, depression, irritability, anxiety, migraine/dizziness, abdominal distension, oppression in the chest, irregularity of menstruation, wiry/rough pulse
Kidney deficiency	Tiredness, headache/dizziness, insomnia, lower back pain, knee/leg pain, weak pulse in the cubit *(chi)*; *yin* deficiency with feeling of heat/irritability, thirst, night sweating, thin and red tongue with dry/little coating, thin and/or rapid pulse; *qi* and *yang* deficiency with preference of warmth, pale tongue with white coating, weak pulse (especially in the cubit)
Phlegm and *damp* accumulation	Obesity, dizziness, feeling of heavy head and/or body, fullness in the chest and/or abdomen, nausea, bland taste in the mouth, thirst but without intention to have water, greasy tongue coating, slippery pulse
Blood stasis	Dark complexion/lips, encrusted skin, fixed stabbing pain, clots in the menstrual blood with/without irregularity of menstruation, purple tongue, rough/intermittent/bound pulse

Table 14: Frequencies of baseline TCM patterns of the 43 participants.

TCM patterns*	Frequency	Proportion of the total (%)
Spleen deficiency	33	76.7
Liver qi stagnation	25	58.1
Kidney yin deficiency	9	20.9
Kidney qi deficiency	4	9.3
Phlegm and *damp* accumulation	16	37.2
Blood stasis	13	30.2
Food stagnation	6	14.0
Heart heat	14	32.6
Heart qi deficiency	5	11.6
Stomach yin deficiency	15	34.9

* Manifestations of different TCM patterns may coexist in one individual.

Table 15: Frequencies of major baseline symptoms of the 43 participants.

Major symptoms	Frequency	Proportion of the total (%)
Preference of warmth	17	39.5
Preference of coolness	9	20.9
Night sweating	5	11.6
Thirst/Dry mouth	22	51.2
Poor appetite	5	11.6
Abdominal symptoms	23	53.5
Abdominal distension	14	32.6
Other discomfort	13	30.2
Reflux	11	25.6
Abnormal bowel movement	20	46.5
Loose stool/diarrhea	10	23.3
Constipation	4	9.3
Alternate diarrhea and constipation	6	14.0
Feeling of oppression/occasional pain in the chest	6	14.0
Palpitation	7	16.3
Feeling stressful/anxiety/depression	24	55.8
Sleep disorder	23	53.5
Feeling sleepy	13	30.2
Dizziness/feeling light-headed	2	4.7
Headache	11	25.6
Lower back/leg/knee pain	16	37.2

a decreasing trend (5%) has been observed. Because of limited sample size, further research with more participants is required.

Epidemiologic studies have demonstrated a high cardiovascular risk at low levels of HDL-c regardless of the LDL-c levels [14]. Substantial atherosclerotic regression ($\geqslant$5% decrease in atheroma volume) occurred only in patients achieving both low levels of LDL-c (<2.26 mmol/L) and an increase of 7.5% in HDL-c with lipid lowering therapy [15].

According to Ma et al. [16], the component of triterpenoid acid from hawthorn fruit can enhance the activity of HDL receptor, which may be a possible mechanism for the effect on HDL-c. Kiwifruit intake could also contribute to an increase of HDL-c level in the present study. In a previous clinical trial, forty-three participants with dyslipidemia consumed two kiwifruit per day for eight weeks, when comparing both pre- and postintervention, the HDL-c concentration has significantly increased [17].

4.2. The Effects on LDL-c, and Ratios of TC/HDL-c and LDL-c/HDL-c. In this present study, HFC induced a significant decrease of the TC/HDL-c ratio and LDL-c/HDL-c ratio. However, no significant effects on serum TC, LDL-c, and TG were found. Such outcomes are consistent with the previous study [17].

Although the LDL-c is considered as the primary treatment target for lipid management [3, 18], the CVD-predicting power of TC/HDL-c ratio and LDL-c/HDL-c ratio has been investigated extensively. A meta-analysis of 61 observational studies has shown that the TC/HDL-c ratio is substantially more informative as a predictor of CHD

Table 16: Tongue features of the 43 participants.

Tongue feature	Frequency	Proportion of the total (%)
Tongue body color		
Pink	6	14.0
Pale	12	27.9
Red	6	14.0
Purple	14	32.6
Red tip	15	34.9
Tongue body		
Normal	13	34.9
Swollen	6	14.0
Thin	22	51.2
With teeth marks	21	48.8
Without teeth marks	22	51.2
Coating texture		
Thin	7	16.3
Thick/greasy	9	20.9
Dry	6	14.0
Less than normal	3	7.0
Fissure	19	44.2
Coating color		
White	28	65.1
Yellow	15	34.9

Table 17: Pulse features of the 43 participants.

Pulse feature	Frequency	Proportion of the total (%)
Rate		
Normal	33	76.7
Slow	1	2.3
Fast	6	14.0
Uneven	2	4.6
Left pulse		
Slippery	15	34.9
Thin	15	34.9
Soft/weak	23	53.5
Wiry	22	51.2
Right pulse		
Slippery	18	41.9
Thin	19	44.2
Soft/Weak	25	58.1
Wiry	15	34.9

mortality than the individual value of TC, HDL-c, or non-HDL-c [11]. In a post hoc study with 9770 participants, it was indicated that the LDL-c/HDL-c ratio was highly predictive of major cardiovascular events [14].

Another relevant study showed that HFC significantly reduced serum levels of LDL-c, TG, and the LDL-c/HDL-c ratio in atherosclerotic mice, and this effect was comparable with the control of statins therapy [19].

In the previous clinical study conducted by Chen et al. [10], the HFC drink was taken by 60 Chinese participants for 31 days. The results showed that the supplement significantly improved the whole blood lipid profile, including reducing TC (−10%), TG (−12%), LDL-c (−18%), and increasing HDL-c (+7%). Statistically, the design and results of the previous study was used to predict the statistical power of the present study. The power calculation indicated that 62 participants were needed to achieve overall statistically significant outcomes. The insignificant results of LDL-c and TG levels in this study may be due to the low number of participants.

4.3. Impacts of Genetic, Ethnical, Cultural, and Lifestyle-Related Factors. In the present study, the Australian participants originated from more than 10 countries or areas (not including East Asia), which reflects the fact of the composition of Melbourne population, however, this may also affect the research outcomes because of the diversity of genetic factors and culture-related lifestyles. Classification of participants by different lipoprotein subgroups or relevant genotypes was unavailable. It is difficult to determine whether there was a detectable diet-gene interaction involved in the study or not. In the case of disparities of blood lipid level and CVD risk, evidence on their relationships with ethnic features is not convincing.

In Chen et al. study [10], the HFC drink significantly increased the serum levels of apoA—I and superoxide dismutase (SOD), and decreased the levels of apoB and malondialdehyde (MDA) in the Chinese participants. Such results show the additional favorable effects of HFC on cardiovascular health. The serum apoB level represents the total atherogenic property of blood lipids, and the apoA—I level reflects the antiatherogenic property. The MDA and SOD levels indicate the body's oxidative status and antioxidative ability, respectively. Further study with other biomedical parameters is of benefit to investigate the effects of HFC.

4.4. Baseline Characteristics of TCM Assessment. The above TCM findings are consistent with previous research in which *Spleen* deficiency and *Liver qi* stagnation were recognized as major root causes of abnormal blood lipid metabolism [20, 21]. This may indicate that high-level stress and reduced digestion associate with the occurrence of dyslipidemia. The same pattern was found in Australian women with premenstrual syndromes [22]. However, because the data of TCM pattern prevalence in general Australian population are unavailable, it is difficult to determine whether the pattern features observed in this study is specific for the whole cohort with moderate dyslipidemia or not. Studies on TCM pattern prevalence in Australian population are needed and the results can be used as a reference for outcome evaluation of the TCM clinical trials.

Long-term or severe dyslipidemia have also been viewed as a condition or pattern of *blood* and *phlegm* stagnation in TCM. It is noted that 32.6% of the participants have the purple tongue body, a typical sign indicating *blood stasis*.

However, the prevalence of *phlegm* and *blood* stagnation pattern is not as high compared to *Spleen* deficiency in the participants with moderate dyslipidemia. Therefore, the pattern of *blood* and *phlegm* stagnation may need to be identified in severe dyslipidemia cases.

Another finding of this study is that many participants had *heat*-related symptoms and signs, that is, 51.2% of the participants complained of thirst or dry mouth, 34.9% with red tongue tip or yellow tongue coating, and 44.2% with tongue coating fissure. This *heat* pattern was also reported by Fu and Xu [23] in their study on Australian peri-menopausal women. Although *Spleen* deficiency may also result in thirst as the fluid cannot be transformed sufficiently and be transported upward to nourish the mouth, about one third of the participants were diagnosed of *Heart heat* pattern or *Stomach yin* deficiency pattern, respectively. *Heat* patterns in patients with dyslipidemia have not been emphasized in previous TCM research. Possible TCM mechanisms may involve the *qi* stagnation and *phlegm* accumulation which can generate *heat,* resembling the *heat*-generating process of compost; the *heat* may in turn deteriorate lipid metabolism by overheating *fluid* into the thick and pathological *phlegm.* According to the TCM theory, kiwifruit, with the cool nature, can remove *heat* and nourish *yin* [24]. Thus, the consumption of kiwifruit can be beneficial for people with *heat* patterns. Further study is needed to explore the optimized the TCM treatment for people with dyslipidemia and *heat* pattern. The utilization of Chinese therapeutic food according to the TCM differential diagnosis can be explored as a tailored treatment of diet-related conditions.

5. Conclusion

Intake of the Chinese therapeutic food, specifically the hawthorn fruit compound, has shown a positive effect on increasing serum HDL-c levels and decreasing ratios of TC/HDL-c and LDL-c/HDL-c, which indicates an improvement of the blood lipid profile. As a dietary therapy, this compound can be considered for the treatment of hyperlipidemia and the prevention of atherosclerosis, and such effects need to be further explored. The major TCM patterns observed in this moderately dyslipidemic participants' group include "*Spleen* deficiency" and "*Liver qi* stagnation."

Conflict of Interests

The authors have no conflict of interests to declare.

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Research Article

Drug Target Prediction Based on the Herbs Components: The Study on the Multitargets Pharmacological Mechanism of Qishenkeli Acting on the Coronary Heart Disease

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In this paper, we present a case study of Qishenkeli (QSKL) to research TCM's underlying molecular mechanism, based on drug target prediction and analyses of TCM chemical components and following experimental validation. First, after determining the compositive compounds of QSKL, we use drugCIPHER-CS to predict their potential drug targets. These potential targets are significantly enriched with known cardiovascular disease-related drug targets. Then we find these potential drug targets are significantly enriched in the biological processes of neuroactive ligand-receptor interaction, aminoacyl-tRNA biosynthesis, calcium signaling pathway, glycine, serine and threonine metabolism, and renin-angiotensin system (RAAS), and so on. Then, animal model of coronary heart disease (CHD) induced by left anterior descending coronary artery ligation is applied to validate predicted pathway. RAAS pathway is selected as an example, and the results show that QSKL has effect on both rennin and angiotensin II receptor (AT1R), which eventually down regulates the angiotensin II (AngII). Bioinformatics combing with experiment verification can provide a credible and objective method to understand the complicated multitargets mechanism for Chinese herbal formula.

1. Introduction

Coronary heart disease (CHD) remains the single leading cause of death for adults worldwide [1]. Effective prevention and therapy for CHD poses a major challenge to the entire medical community. There exists a strong demand to continue searching for both safe and efficacious products to combat this emerging health epidemic. Traditional Chinese medicine (TCM) has fought against CHD and its related diseases for more than 1000 years and has accumulated thousands of herbal formula as well as clinical literatures, it has been considered to have huge potential as an information source and starting point for the development of CHD products [2]. Meanwhile, more and more patients all over the world take TCM as a complementary and alternative avenue to treat CHD.

However, how herbal formula work and what are their drug targets are still unclear by now. Many studies have focused on active monomer of herbs to explain their therapy mechanism [3], but apparently there are significantly different characteristics between active monomer and herbal formula as whole. Active monomer may have a clear target, such as receptors, enzymes, ion channels, transmembrane signal transduction molecules, mostly acting on single-target, but Chinese herbal formula composed of diverse, complex components, its comprehensive pharmacological effects is accumulated by many active monomers through multichannel and multitargets [4]. How to determine the multitargets from such a complex biological process is a challenge to TCM.

Coronary heart disease (CHD) is now a heavy burden on the society and families in both industrialized and developing countries, and some herbal formula present a definitely clinical effect on it, so it presents a better example and context for investigating the efficacy and the drug targets in TCM.

The ancient TCM Qishenkeli (QSKL), prepared from a basic formula of six Chinese herbs (Radix Astragali Mongolici, salvia miltiorrhiza bunge, Flos Lonicerae, Scrophularia, Radix Aconiti Lateralis Preparata, and Radix Glycyrrhizae, etc.) is widely produced in China in accordance with the China Pharmacopoeia standard of quality control [5] and is commonly used in routine treatment of CHD of clinical practice in China. It contains large-scale epidemiological survey in the randomized controlled clinical trials proved that it has a definite effect on improving heart function [6], while a lot of studies are carried out to investigated in active monomers among them and made great progress, for example, Astragalus Polysaccharide (APS, monomer of Radix Astragali Mongolici) is found has effect on cardiac chymase activities [7], tanshinone IIA (monomer of salvia miltiorrhiza bunge) is found in cardioprotective effects and attenuating myocardial hypertrophy [3], but as mentioned before, monomer pharmacological effects cannot present overall efficacy of the whole formula, studies involved all the compounds are rarely carried out.

In recent years, people develop some bioinformatic methods to infer drug target interactions [8–13]. These methods provide opportunities to reveal the underlying molecular mechanism of TCM. Recent advances on the databases cataloging chemical components of herbs and the interactions between drugs and targets enhance the feasibility of predicting the herbs drug targets.

DrugCIPHER-CS is an efficient drug target prediction method which is recently presented by Zhao and Li [14], and in this paper, we use it to predict the potential targets of QSKL's compositive compounds. This method is based on the principle that (i) drugs with similar chemical structure tend to bind functionally related proteins and (ii) functional relationship between the proteins can be measured by their distance in the protein interaction network. For a query drug, each protein in the protein interaction network will be assigned a score by DrugCIPHER-CS which describes the importance of the protein to the activity of the drug, and proteins with high scores will be hypothesized as this query drug's potential targets.

This paper presents an idea that multi targets for herbs should be investigated by combing bioinformatics and experimental verification to finally determine drug targets. Firstly, herbal components are investigated by data mining from database; secondly, bioinformatics is applied to predict the drug target for all compounds based the principle of that similar structural has similar function, then bioinformatics including GO function analysis are used to look for the pathway that the proteins belong. Finally, experimental verification is taken to confirm how and what the herbs work on the body, thus to provide a credible method to investigate the complicated multitargets mechanism for herbs.

2. Methods

2.1. Drug Targets Prediction. In this paper, we use drugCIPHER-CS to predict drug targets of QSKL's compositive compounds. DrugCIPHER-CS recently presented by Zhao and Li [14] achieves good prediction performance and can infer drug targets in the genome wide scale. This method is based on the hypotheses that (i) drugs with similar chemical structure usually bind functionally related proteins and (ii) functional relationship between the proteins can be measured by their distance in the protein interaction network. Given a set of known drug- (drug-space) target (target-space) interactions, for a query drug and a candidate target gene, drugCIPHER-CS will measure the likelihood of their interaction based on the correlation between the query drug's structure similarity vector with the drug space and the candidate gene's functional similarity vector with the target space. For a query compound, drugCIPHER-CS will prioritize the proteins in the protein interaction network (i.e., candidate proteins) according to the order of the decreasing drug target interaction likelihood, and the candidate proteins with high likelihood will be hypothesized as the potential drug targets (Please refer to paper [14] for more details of DrugCIPHER-CS).

Here, known drug target interactions are obtained from DrugBank database (version: May, 2011) [15]. We only use those drug-target interactions whose drugs are FDA-approved and have InChI identifiers [16] and whose targets are human genes/proteins. In total, we obtain 4299 interactions between 1109 drugs and 1138 targets. The chemical structure similarity is calculated based on compounds' MOLPRINT 2D descriptors and Tanimoto coefficient [17]. The human protein interaction network is constructed by integrating the protein interaction data from HPRD (release 9.0) [18], BioGRID (version: 3.0.66) [19], IntAct (version: 20100628) [20], MINT (version: 20100505) [21], DIP (version: 20100614) [22], and PDB provided by Gibson and Goldberg [23]. In total, there are 102131 interactions between 11654 proteins in the protein interaction network.

2.2. Degree and betweenness Centrality in the Protein Interaction Network. A protein's degree is defined as the number of its direct interaction partners in the protein interaction network. The betweenness centrality of protein n is computed as

$$B(n) = \frac{\left(\sum_{s \neq n \neq t} (\sigma_{st}(n)/\sigma_{st})\right)}{((N-1)(N-2)/2)}, \quad (1)$$

where σ_{st} denotes the number of the shortest paths between protein s and protein t in the protein interaction network, $\sigma_{st}(n)$ denotes the number of the shortest paths across protein n between protein s and protein t, and N is the total number of proteins in the protein interaction network.

Both degree and betweenness centrality can measure a protein's topological importance in the network. The larger a protein's degree/betweenness centrality is, the more important the protein is in the protein interaction network.

2.3. CHD Model Preparation. CHD is induced by direct coronary ligation as described before [24]. Briefly, Sprague-Dawley (SD) rats are anaesthetized with pentobarbital sodium (1%, 50 mg kg^{-1} intraperitoneally). The trachea of each rats is intubated per orally with a plastic tube connected to a respirator (Kent Scientific 325, China) set at a stroke

volume of 3 mL kg^{-1}, respiratory ratio: 2 : 1, and a rate of 80 strokes min^{-1}. After left thoracotomy and exposure of the heart, the left anterior descending coronary artery (LAD) is ligated with a 5–0 polypropylene suture (Surgipro, CT, USA) directly proximal to its main branching point. Control groups are made following an identical procedure but without the actual tying of the polypropylene suture. Thereafter, the thorax is closed and as soon as spontaneous respiration is sufficient, the rats are extubated and are allowed to recover under a heated lamp. They are fed a standard diet and water and are maintained on a 12-hour Light-and-dark cycle. After ECG testing, rats that averaged QT-interval prolongation in three precordial leads are included in the study. The QSKL group is treated for 28 days by daily oral gavage with total daily dosages of 508 mg/kg of the concentrated QSKL (Beijing university of Chinese Medicine, Beijing, China) dissolved in water. The control and model groups receive the same volume water via oral gavage as the QSKL vehicle. At the end of the study, all animals are anaesthetized using pentobarbital sodium following an overnight fast. Blood samples are collected via abdominal aorta puncture, place on ice, and allow to clot. After centrifugation, serum is collected, aliquoted, and stored at −80°C until analysis of each indicator within a short period of time.

2.4. Echocardiographic Assessment of LV Function. Echocardiography is used to detect Left ventricular end-systolic diameter (LVEDs), Left ventricular end-diastolic diameter (LVEDd), ejection fraction (EF), fractional shortening (FS), and other indicators. A PST 65A sector scanner (8-MHz probe) is used, which generates two-dimensional images at a frame rate ranging from 300 to 500 frames/s. LV dimension (LVD) is measured by M model, and fractional shortening (FS%) is calculated by the following equation:

$$\text{FS\%} = \left[\frac{\text{LVEDd} - \text{LVEDs}}{\text{LVEDd}}\right] * 100\%. \quad (2)$$

2.5. Preparation and Dose Consideration of Concentrated QSKL. The QSKL used in this study is manufactured by Beijing university of Chinese medicine (Beijing, China) using the six Chinese herbs at a composition of 460 g Radix Astragali Mongolici, 230 g salvia miltiorrhiza bunge, 160 g Flos Lonicerae, 160 g scrophularia, 140 g Radix Aconiti Lateralis Preparata, and 90 g Radix Glycyrrhizae. Briefly, the residue of Radix Astragali Mongolici is mixed with all salvia miltiorrhiza bunge, Flos Lonicerae, scrophularia, and Radix Glycyrrhizae, follow by extraction with hot water (twice, 2 hr each). The water extract is then concentrated to form a paste, and the ethanol is added for 24 hr, the filtration is collected to form the final product. Based on the recommended daily human dosage of 20 g/d, according to the equivalent conversion between animal and people by body surface area, dosage of 508 mg/kg is chosen in present study.

2.6. Biological Parameter Detection

2.6.1. Measurement of Serum Indicators by Elisa. Levels of serum indicators (appeared in predicting target) are quantified in duplicate using commercial ELISA kits (Abcam Inc., Cambridge, MA, USA). Each assay is performed following the kit instructions. Standards at a series of concentrations are run in parallel with the samples. The concentrations in the samples are calculated in reference to the corresponding standard curves and expressed as ng/mL.

2.6.2. Measurement of Indicators by Western Blot. The serum are homogenised in RIPA buffer (50 mM TrisHCl pH7.4, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.1% SDS) and total protein is extracted from this homogenate. The protein concentration in each sample extract is measured using a protein assay kit (Pierce; Rockford, IL, USA) and then is adjusted to the same value in all samples with 2X 4% SDS sample buffer. The samples are boiled for 5 min followed by loading on a 7.5% SDS-PAGE gel (30 mg protein/10 mL per well) for electrophoresis using a Bio-Rad mini gel apparatus at 100 V for 2 hours. The fractionated protein on the gel is transferred onto a NC membrane (Millipore) and electrophoresed at 300 mA for 90 min. The membrane is first probed with AT1R primary antibody (antiangiotensin II type 1 receptor antibody, ab18801, Abcam, 1 : 500) and secondary antibody (donkey polyclonal secondary antibody to rabbit IgG-HRP, ab97064, Abcam, 1 : 5000), and then treated with ECL (ECL Plus western blotting detection reagent, GE Healthcare) for 1 min at room temperature. The bands in the membrane are visualized and analyzed using UVP BioImaging Systems. After obtaining the AT1R blot density, the membrane is then treated using restore western blot stripping buffer (Thermo Scientific) to remove the AT1R signal, followed by probing with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primary antibodies (GAPDH mouse monoclonal IgG, ab8245, Abcam, 1 : 2000) using the same process as the AT1R antibody to get the AT1R and GAPDH blot densities. The final reported data are the normalized AT1R band densities by GAPDH.

2.6.3. Measurement of Indicators by Immunohistochemistry (IHC). An avidin-biotin-peroxidase complex commercial method (R&D) is used for immunohistochemistry. Briefly, 4-mm-thick paraffin wax sections are mounted on slides, which are dried for 30 minutes in an oven (60–70°C) and deparaffinized in xylene. The slides are then placed in changes of ethanol for 2 minutes each. Washing in buffer solution is performed between steps. The slides are then placed in 3% hydrogen peroxide for 15 minutes. And then are subsequently incubated in avidin block for 15 minutes, biotin block for 15 minutes, primary antibody (Ang II antibody, Phoenix Pharmaceuticals Inc. or Anti angiotensin II type 1 receptor antibody, ab18801, Abcam) for 12 hours at 4°C, and biotinylated secondary antibody for 1 hours. The reagent incubation is performed with streptavidin peroxidase for 15 minutes. A 1-minute Mayer's hematoxylin counterstain is used. The slides are dehydrated, cleared with xylene, and mounted with permanent mounting medium. Finally, integral optical density (IOD) of pictures is analyzed by IPP6.0 software.

2.7. Statistical Analysis. Data analyses are performed by one-way ANOVA using SAS 9.2 statistical software (SAS Institute,

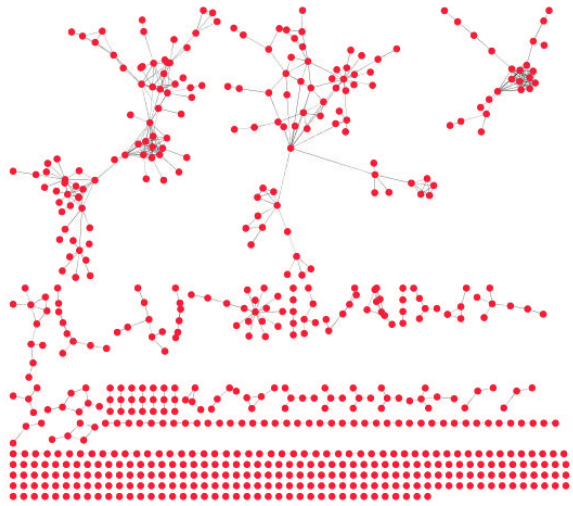


Figure 1: The protein interaction network consists of top 0.1% candidate target genes.

NC, USA). $P < 0.05$ was considered statistically significant. Results are presented as mean values with their standard deviation.

3. Results

3.1. Drug Target Prediction and Analyses. In order to reveal the underlying molecular mechanism of QSKL, we firstly use bioinformatic method to infer the targets of its chemical components.

By use of literature curation, we determine QSKL's 231 compositive compounds. Then we use drugCIPHER-CS method [14] to infer their potential targets (Supplementary Table 1 avaliable online at doi: 10.1155/2012/698531). drugCIPHER-CS published recently by Zhao and Li achieves good performance for predicting the targets of drugs and can infer targets in the genome-wide scale [14]. For each compositive compound, drugCIPHER-CS prioritizes its candidate targets according to the order of the decreasing possibility being targeted by the compound. When we choose top 1% candidate targets, we obtain 3725 candidate target genes for 207 compositive compounds which have clear chemical structures. Average, one target gene is shared by 6.5 compounds. When we choose top 0.1% predicted targets, we obtain 639 target genes. Average, one gene is targeted by 3.6 compounds. As shown in Figure 1, there are 510 protein interactions between these 639 top 0.1% candidate targets (Figure 1).

By comparing with the known cardiovascular disease-related drug targets (i.e., the known targets of drugs whose ACT code uses "C" as the first level) in DrugBank [15], we find both top 0.1% and top 1% candidate targets are significantly enriched with known cardiovascular disease-related targets (upper-tailed P value of hypergeometric cumulative distribution is $2.03E-10$ for top 0.1% and $2.05E-08$ for top 1% candidate targets). And the corresponding enrichment extent of top 0.1% candidate targets is higher than that of top 1% targets.

After obtaining the potential targets for the QSKL's chemical components, we analyze the enriched KEGG biological pathways [25] (version: 2009.11) among these potential targets. In total we find 16 significantly enriched pathways among top 0.1% candidate targets (Table 1), including the pathways of neuroactive ligand-receptor interaction, ami-noacyl-tRNA biosynthesis, calcium signaling pathway, glycine, serine and threonine metabolism, Renin-angiotensin system, and so on. The importance of Neuroactive ligand-receptor interaction in the development and progress of cardiovascular disease processes such as CHD is well known, The key protein in this pathway such as Adrenergic receptor, Angiotensin receptor, Calcitonin receptor-like, Neurotensin receptor are closely related to the cardiac function. The pathway of Aminoacyl-tRNA biosynthesis plays a important roles in cardiovascular angiogenesis [26], The relationship between calcium signaling pathway and CHD is confirmed, and calcium antagonists have been widely used in clinical to inhibit extracellular calcium influx, reducing the concentration of intracellular calcium and lower myocardial contractility [27]. Glycine, serine, and threonine metabolism mainly provide the ATP for myocardial contractility [28]. Renin-angiotensin system plays a central role in the deterioration of cardiovascular function [29].

Also, we research the functional distribution of these candidate targets (Table 2). The significantly enriched gene ontology (GO) functional annotations [30] (version: 20111103) of these targets include cellular amino acid metabolic process, biosynthetic process, small molecule metabolic process, cellular nitrogen compound metabolic process and circulatory system process, indicating the QSKL intervening in these pathological progresses. These enriched pathways and GO functional annotations provide important clues for understanding the molecular mechanism of QSKL.

In addition, by checking the degree and betweenness centrality of these candidate target genes in the protein interaction network, we find these candidate targets are significantly depleted with the proteins with the highest degree or betweenness centrality (Table 3). And the depletion extent for top 0.1% candidate targets is larger than that for top 1% candidate targets. That is, these QSKL's candidate target genes do not tend to be topologically the most important in the protein interaction network. This result is consistent with Hase et al.'s conclusion that known human drug targets tend to be less connected nodes in the network [31]. The TCM with multiple chemical components targets multiple less-connected nodes, which may produce greater synergetic efficacy and fewer side effects.

3.2. Experimental Validation

3.2.1. Model Evaluation. 28 days after surgery, echocardiography showed that EF and FS values in the model group were significantly different ($P < 0.05$). EF value of ligation rats in model group dropped down to 49.03% compared with

Table 1: Significantly enriched KEGG biological pathways among top 0.1% candidate target genes of QSKL compositive compounds.

KEGG pathway number and name	P value[a]	Coverage[b]
hsa04080 neuroactive ligand-receptor interaction	$1.17E-10$	0.1358
hsa00970 aminoacyl-tRNA biosynthesis	$1.54E-08$	0.3171
hsa04020 calcium signaling pathway	$1.34E-06$	0.1348
hsa00260 glycine, serine, and threonine metabolism	$3.90E-04$	0.2258
hsa04614 renin-angiotensin system	$7.51E-04$	0.2941
hsa00290 valine, leucine, and isoleucine biosynthesis	$1.09E-03$	0.3636
hsa00590 arachidonic acid metabolism	$1.12E-03$	0.1552
hsa00350 tyrosine metabolism	$4.42E-03$	0.1522
hsa04260 cardiac muscle contraction	$1.02E-02$	0.1125
hsa00330 arginine and proline metabolism	$1.07E-02$	0.1296
hsa04270 vascular smooth muscle contraction	$1.13E-02$	0.0960
hsa00250 alanine, aspartate, and glutamate metabolism	$1.22E-02$	0.1613
hsa04144 endocytosis	$2.32E-02$	0.0802
hsa04115 p53 signaling pathway	$3.69E-02$	0.1014
hsa00071 fatty acid metabolism	$4.09E-02$	0.1190
hsa00591 linoleic acid metabolism	$4.11E-02$	0.1379

[a]A pathway is significantly enriched with candidate target genes when its corresponding upper-tailed P value of hypergeometric cumulative distribution is smaller than 0.05. The pathways are ranked according to the order of the increasing P values. [b]The coverage for each pathway is referred to as the fraction of candidate target genes among all the pathway member genes.

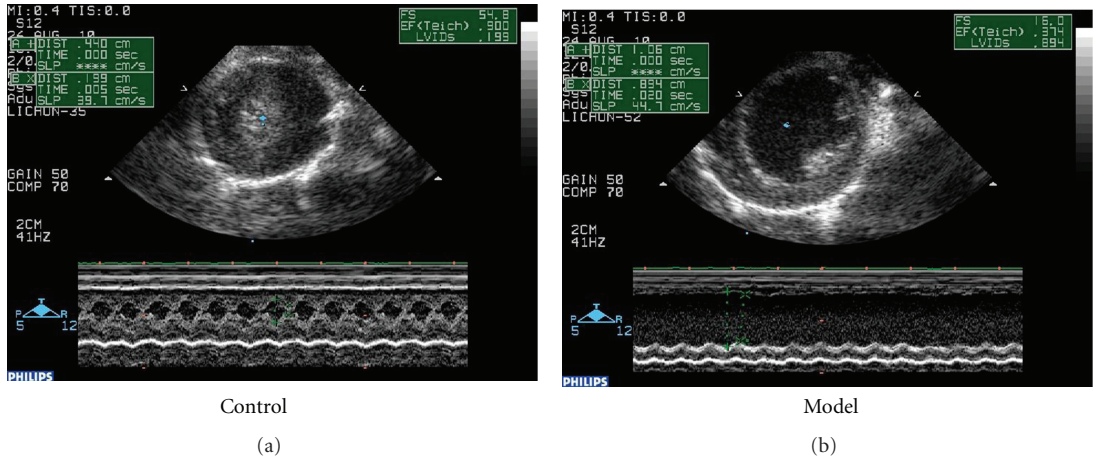
Table 2: Significantly enriched GO term among top 0.1% candidate target genes of QSKL compositive compounds.

GO term ID	GO term name	P value[a]
GO:0006520	Cellular amino acid metabolic process	$1.99E-13$
GO:0009058	Biosynthetic process	$4.32E-09$
GO:0044281	Small molecule metabolic process	$2.55E-08$
GO:0034641	Cellular nitrogen compound metabolic process	$2.27E-07$
GO:0003013	Circulatory system process	$1.40E-06$
GO:0006399	tRNA metabolic process	$6.43E-06$
GO:0007267	Cell-cell signaling	$1.60E-04$
GO:0006950	Response to stress	$1.88E-04$
GO:0006412	Translation	$3.70E-04$
GO:0042592	Homeostatic process	$4.40E-04$
GO:0055085	Transmembrane transport	$4.90E-04$
GO:0071941	Nitrogen cycle metabolic process	$7.37E-04$
GO:0007568	Aging	$7.90E-04$
GO:0006810	Transport	$9.10E-04$
GO:0050877	Neurological system process	$2.35E-03$
GO:0006461	Protein complex assembly	$1.75E-02$
GO:0019748	Secondary metabolic process	$1.99E-02$
GO:0065003	Macromolecular complex assembly	$4.30E-02$

[a]The top 0.1% candidate target genes are significantly enriched with genes annotated with a GO term when its corresponding upper-tailed P value of hypergeometric cumulative distribution is smaller than 0.05. These GO terms are ranked according to the order of the increasing P values.

control group, suggesting a steady CHD model is established. After treated by QSKL for 28 days, the EF value recovers by 37.62% compared with model group (Figure 2).

3.2.2. Predicting Pathway Validation. The importance of neurohormonal activation in the development and progress of cardiovascular disease processes such as CHD is well known, and the renin-angiotensin system plays a central role in this [32].The chronically activated renin-angiotensin aldosterone system (RAAS) is believed to contribute significantly to the deterioration of cardiovascular function, Inhibitors of it have been routinely used to treat patients with CHD [29]. In this paper, RAAS are selected as example and context to validate predicting pathway. Critical indicators in RAAS pathway are



Control

(a)

Model

(b)

QSKL

(c)

Figure 2: The cardiac function in different groups. Control groups showed a high EF value, while abnormal ventricular wall movement in model group is seen, in QSKL group, EF value recovers in some extent.

Table 3: The depletion analyses of proteins with the highest degree/betweenness centrality among top 0.1% and top 1% candidate target-genes.

	Lower-tailed P values of hypergeometric cumulative distribution	
	Top 0.1% candidate targets	Top 1% candidate targets
The proteins with the highest degree[a]	$4.97E-07$	$3.89E-06$
The proteins with the highest betweenness centrality[a]	$4.97E-07$	$3.24E-05$

[a]The proteins with the highest degree/betweenness centrality are referred to as those with top 5% degree/betweenness centrality in the protein interaction network.

detected to test the accuracy of the predicting pathway, we carry out series experiments to validate them including Elisa, IHC, and westernrblot.

The western blot of renin shows that at the end of the study, the serum renin in model group increases by 45% ($P < 0.05$) compared with control, after treated by QSKL for 28 days, the level of renin shows a 22.76% reduction compared with model group ($P < 0.05$), which had no statistical significance when compared to the control (Figure 3(a)).

Both Elisa and IHC results show that the levels of Ang II in model group upregulated by 27.88% compared with control ($P < 0.05$), after treated by QSKL for 28 days, a 16.59% reduction are detected in QSKL group compared with model ($P < 0.05$),which almost return to the level of the control (Figures 4 and 5, Table 4).

AT1R is thought to be a better target to cure the CHD. The AT1R in model group up regulated by 59.00% compared with control. In QSKL group, its level decreases by 42.12% compared with model, which has no significant difference with control (Figures 3(b), 4, and 5). The level of serum aldosterone (ALD) in each group does not show any significant difference.

4. Discussion

At present, monomer in herbs is usually applied to explain the pharmacological efficacy of a whole Chinese herbal

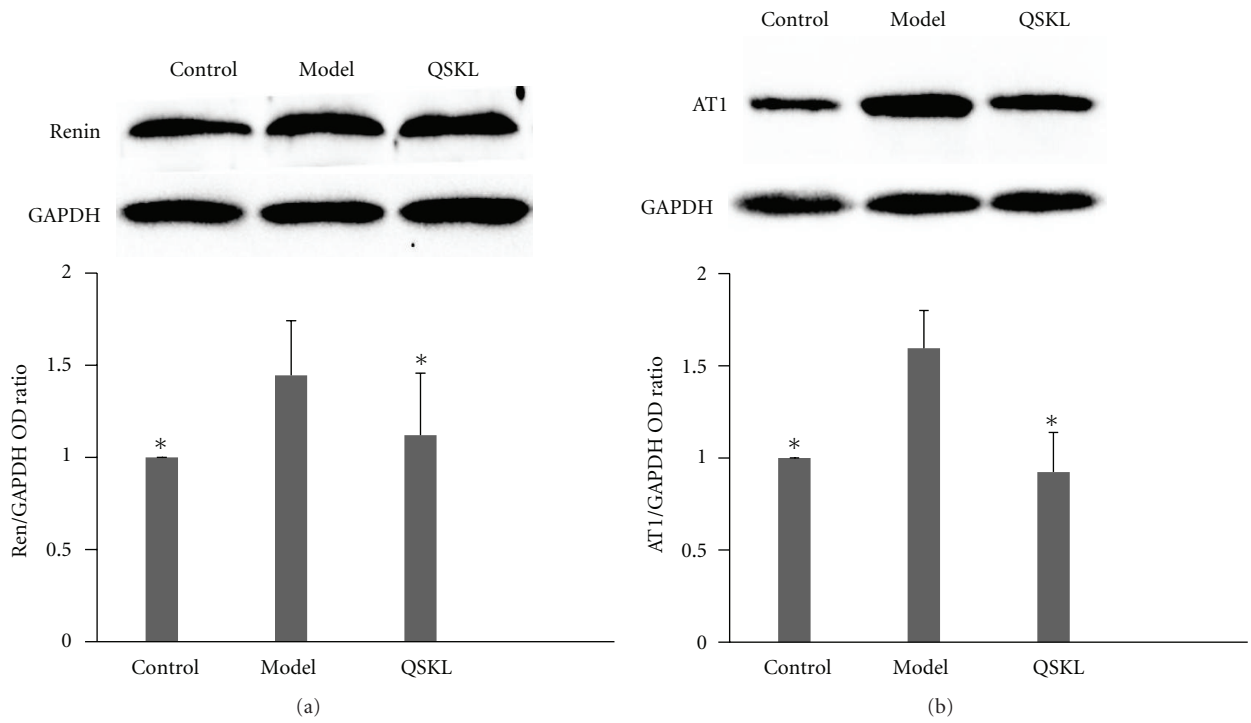


Figure 3: QSKL significantly lowers rennin and AT1R in CHD rats. (a) Rennin levels in different group; (b) AT1 levels in different group. Data are analyzed by one-way ANOVA. $P < 0.05$ indicates statistical significance. Results are presented as mean values with their standard deviation ($n = 20$). *Differed significantly from model ($P < 0.05$).

Table 4: The change in indicators related to renin-angiotensin-aldosterone system (mean values with their standard deviation, $n = 20$).

	Control		Model		QSKL	
	Mean	SD	Mean	SD	Mean	SD
Ang II ($\times 10^{-6}$ μg/mL)	165.59*	21.352	211.28	19.853	176.71*	17.661
ALD ($\times 10^{-3}$ μg/mL)	208.85	47.953	220.32	20.608	236.49	32.965

QSKL: Qishenkeli; *mean values are significantly different from model (*$P < 0.05$).

formulation. In fact, it did not present the multitarget characteristic of the multi component Chinese herbal formulation. If the multi targets can be predicted according to chemical structure of its composition through the bioinformatics, and experiments to verify the results, things will be go easy and concise to confirm herbs pharmacological mechanisms.

With the development of high-throughput drug screening and structural analysis technology, the chemical compositions of formulation are gradually revealed, mature database of the chemical composition of Chinese herbs are gradually established, and the identification of the chemical structure makes it possible to predict drug targets by investigating the relations between the drug and the biomarkers proteins. As the development of system biology, bio formations technique becomes more and more mature. Its advantages are very applicable to the complex correlativity study of compound in herbs and the drug targets.

In this paper, we take drugCIPHER-CS to predict the target of QSKL which has been used for treating CHD effectively for thousand years. Five pathways were predicted as a main way that the QSKL may act on. RAAS was selected to elaborate the pharmacological mechanism of QSKL. After experimental verification, more than one target was verified including renin, Ang II, AT1, which can elaborate the characteristic of the milt-target of Chinese herbal formulation.

The chronically activated renin-angiotensin-aldosterone system (RAAS) is believed to contribute significantly to the deterioration of cardiovascular function. In the pathway, angiotensin II has critical roles including the regulation of blood pressure, vasoconstriction, increasing aldosterone secretion, amplifying sympathetic activity, increasing sodium retention, as well as lots of other actions. It is considered a factor in virtually every form of CHD, and it is applied as a therapeutic target in hypertension and chronic heart failure. Numerous researches focus on its inhibitors to provide clinical drug for CHD. Among them, Antagonists to AT1R and angiotensin-converting enzyme inhibitors (ACEI) have been routinely used to treat patients with CHD [33, 34]. Both experimental and clinical studies have shown that ACEI, besides inhibiting the concentration of Ang II, could have desirable effects by down regulating

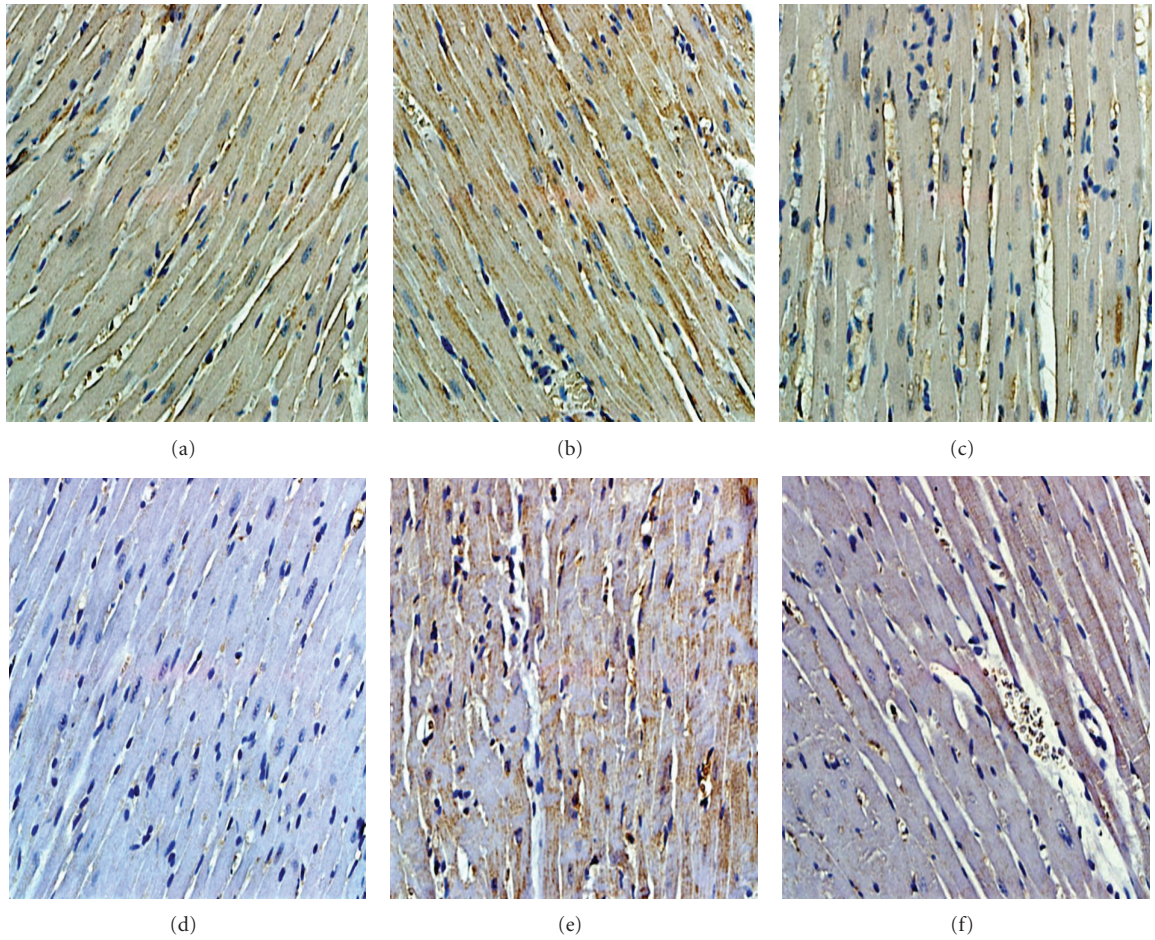


Figure 4: IHC results of Ang II and AT1R in control, model and QSKL group. (a) Cardiac Ang II expression in control group. (b) Upregulating cardiac Ang II expression in model group. (c) QSKL can reduce the level of the Ang II. (d) Cardiac AT1R expression in control group. (e) Disorders in myocardial cells, upregulating cardiac AT1 expression in model group. (f) QSKL can decrease the level of the AT1.

the bradykinin [35], moreover, patients levels of angiotensin II have a tendency to return to pre treatment levels after long-term ACEI treatment [36]. Since ACEI do not seem to have complete protective effects against the detrimental effects of Ang II, AT1-receptor blockers may offer advantages relative to ACEI by effectively blocking the AT1-receptor, which mediates all known detrimental effects of Ang II. The AT1R mediates the majority of classical biological functions of Ang II [37] and plays a critical role in the control of regulation of blood pressure, vasoconstriction, increasing aldosterone secretion, amplifying sympathetic activity, and so forth. All the AT1-receptor antagonists in routinely clinical use are extremely well tolerated. Since AT1R blockers for the treatment of cardiovascular disease seem very promising, indeed, the AT1R has been regarded as an important target for cardiovascular treatment. In our research, the QSKL can significantly down regulated the level both Ang II and AT1R, indicating a same efficacy as AT1 agonists. Besides, the QSKL can lower the RAAS activation form the very beginning-the renin. Renin is an aspartyl-protease enzyme produced and activated within the juxtaglomerular (JG) cells of the afferent arteriole in the kidney. Through Angiotensin I, it can activate Ang II which is the primary biologically active hormone of the renin-angiotensin system as referring before. Renin secretion is the critical rate-limiting step in the activity of the entire system [38]. Because of this, QSKL regulating renin secretion are of particular interest and importance in understanding its collaboration effect with Ang II as well as understanding therapeutic targets for CHD. ALD seems not to change, which is consistent with the published papers [39]. "ALD breakthrough" is thought to be its important mechanism.

To sum up, this paper presents an idea that the study of multi target for Chinese herbal formula are carried out based on the known chemical composition of herbs both by bioinformatics and experimental verification. We take the research of QSKL effect on CHD as an example. And the results show it can act on CHD in multi targets, especially in renin and AT1, eventually decrease the level of the Ang II, which can treat CHD efficiently. From this, a credible and objective method can be provided to understand and confirm the complicated multi targets mechanism for Chinese herbal formulation.

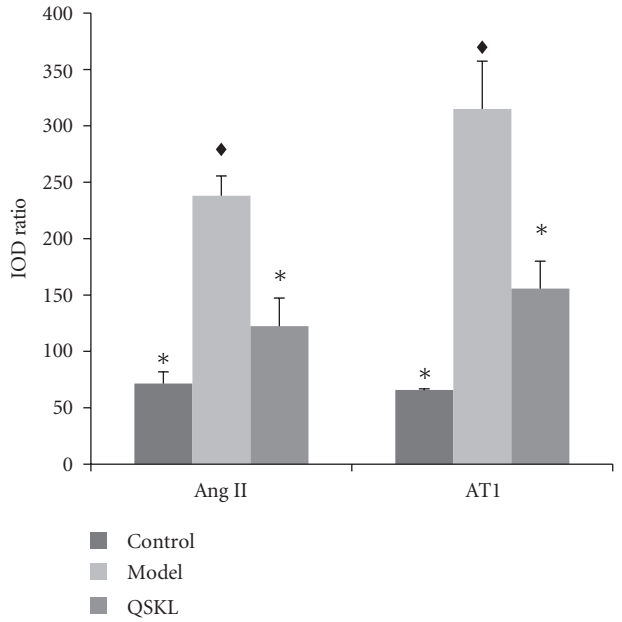


Figure 5: Semiquantitative determination of Ang II and AT1R expression with IHC in different groups. *Differed significantly from model ($P < 0.05$).

But some problems still exist. For example, in predicting drug targets, the distribution and metabolism of herbal formulation in the body are not taken into consideration in our research; we presume all components of herbal formulation compounds are absorbed and utilized; improvement should be made in our future work.

Author's Contribution

Y. Wang, Z. Liu and C. Li contributed equally to this work.

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Research Article

Berberine Improves Insulin Sensitivity by Inhibiting Fat Store and Adjusting Adipokines Profile in Human Preadipocytes and Metabolic Syndrome Patients

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Berberine is known to inhibit the differentiation of 3T3-L1 cells *in vitro,* improve glycemic control, and attenuate dyslipidemia in clinical study. The aim of this study was to investigate the effects of berberine on preadipocytes isolated from human omental fat and in metabolic syndrome patients treated with berberine for 3 months. We have shown that treatment with 10 μM berberine resulted in a major inhibition of human preadipocyte differentiation and leptin and adiponectin secretion accompanied by downregulation of PPARγ2, C/EBPα, adiponectin, and leptin mRNA expression. After 3 months of treatment, metabolic syndrome patients showed decrease in their BMI (31.5 ± 3.6 versus 27.4 ± 2.4 kg/m^2) and leptin levels (8.01 versus 5.12 μg/L), as well as leptin/adiponectin ratio and HOMA-IR. These results suggest that berberine improves insulin sensitivity by inhibiting fat store and adjusting adipokine profile in human preadipocytes and metabolic syndrome patients.

1. Introduction

The metabolic syndrome is a cluster of multiple metabolic diseases based on obesity and insulin resistance. Obesity leads to insulin resistance and a proatherogenic state. Therefore, the role of obesity, especially visceral (or central or abdominal) obesity, is believed to be the main physiological force resulting in disorders of glucose and lipid metabolism in metabolic syndrome [1]. However, because of the effects of insulin in fat cell differentiation and metabolism of glucose and lipids, patients who are treated by insulin, sulphonylureas, and thiazolidinediones may suffer from varying degrees of weight gain. Effects of metformin on body weight may be based on calorie intake reduction rather than energy consumption increased. The statins for regulating lipid metabolism are generally expensive and some of them have liver toxic side effects. Therefore, the search for a cost/effective drug that can not only lower blood glucose and lipids but also reduce weight for metabolic syndrome treatment has a significant importance.

Berberine is an isoquinoline derivative alkaloid isolated from many kinds of medicinal herbs, such as *Hydrastis canadensis* (goldenseal), Cortex Phellodendri (Huangbai), and Rhizoma Coptidis (Huanglian). It is safe and cheap and has been extensively used as an antibacterial drug [2]. Berberine has been proven to have many other pharmacological effects including antimicrobial [3], antitumor [4], anti-inflammation [5], blood glucose lowering [6], and even inhibiting chronic cocaine-induced sensitization [7]. In one recent single-blind clinical observation, the study showed that diet supplementation of some natural substances including berberine was beneficial for correcting lipid metabolism disorders and reducing cardiovascular risk factors [8]. However, the body weight reduction effect is poorly characterized in clinical study.

Pharmacokinetics of berberine indicates that adipose tissue is its main target [9]. Adipose tissue is a huge energy reserve organ. The excessive proliferation and differentiation of fat cells can lead to excessive fat accumulation in adipose tissue, resulting in obesity [10]. At the same time, fat cells can

secrete a variety of hormones, named adipokines, through endocrine, paracrine, and autocrine mechanisms that affect energy metabolism of the body [11]. It is assumed that unfavorable changes in the secretion of adipokines, considered as an early symptom of impaired adipose tissue function, are the potential link between obesity and insulin resistance, influencing the development of metabolic syndrome [12]. Leptin and adiponectin are the key biomarkers of adipose tissue. Hyperleptinemia and hypoadiponectinemia are common in obesity. They reflect increased adiposity and may contribute to hypertension, dyslipidemia, impaired glucose metabolism, and proatherogenic state in obesity and metabolic syndrome [13, 14]. Many studies have been published on the mechanism of berberine's effect on adipose tissue. Zhou et al. found that berberine significantly inhibited differentiation of mouse 3T3-L1 preadipocytes into fat cells [15]. In addition, it has also been shown to reduce leptin and resist secretion [16] and increase the mRNA expression of adiponectin [17]. Members in our research team, Zhang et al. also found berberine-moderated glucose and lipid metabolism through a multipathway mechanism that includes AMP-activated protein kinase- (AMPK-) p38 MAPK-GLUT4, JNK pathway, and PPARα pathway in KKAy mice [18]. These results showed that berberine may have excellent potential as an agent to prevent metabolic syndrome. However, these studies were performed in rodent models or murine cell lines. The effects of berberine on human adipose tissue are rarely reported. Due to lack of well-established human adipocyte model, human primarily cultured preadipocytes have been particularly useful for verifying the results obtained from the preadipocyte cell lines. Thus, in this paper, we presented evidence obtained from human primarily cultured omental preadipocytes as well as from metabolic syndrome patients and demonstrated that berberine improves insulin sensitivity by inhibiting fat store and adjusting the profile of adipokines.

2. Materials and Methods

2.1. Materials. Berberine used *in vitro* study was purchased from Sigma Aldrich Co, St. Luis, MO, USA. Oral medication berberine used *in vivo* study has Chinese Drug Approval Number: H.M.L.N., H11022584.

2.2. Adipose Biopsies. Omental adipose tissue biopsies were obtained from nine patients (3 females, 6 males, age range 22~47 years) who underwent elective inguinal hernia repair surgery. None of these patients suffered from endocrine malignant or chronic inflammatory diseases or severe systemic illnesses or any recent weight change. None were taking medications known to affect adipose tissue mass or metabolism. The study was approved by the local ethical committee. All patients gave their informed consent. On the day of surgery, all patients fasted for at least 6 h preoperatively, and all underwent general anesthesia. Adipose tissue specimens from the omental adipose tissue regions were obtained within 30–45 min after the onset of surgery. In general, 10–15 g of adipose tissue was obtained and transported to the laboratory in normal saline (transport time with 10 min). Specimens from three patients were used for cell proliferation experiments and six for cell differentiation studies.

2.3. Cell Culture. The isolation and culture of preadipocytes was performed according to the method described elsewhere by ourselves [19]. Briefly, Adipose tissue was cut into 1 mm $\times$ 1 mm pieces with ophthalmic scissors. Collagenase digestion was performed at 37°C on a shaking platform (200 rpm) for 1 to 3 hours. Next, digest was transferred to filter by 74 μm sieve size filter. This procedure was repeated until the complete digestion was filtered. The cell suspension was centrifuged at 480 g for 5 min, and the preadipocyte fraction was resuspended in growth medium (PromoCell, Germany). Then cells were counted and cultured in different mediums at 37°C in a humidified 5% CO_2 atmosphere.

2.4. Analysis of Cell Proliferation. 4×10^3 preadipocytes/well were inoculated into 96-well plates and cultured in growth medium supplemented with varying concentrations of berberine (0 μM, 0.1 μM, 1 μM, and 10 μM). Proliferation was determined by MTT assay after 1, 2, and 3 days of culture. Briefly, after culture medium was removed, MTT (0.5 mg/mL, 50 μL/well) was added into the plates and incubated at 37°C for 4 h, followed by the addition of DMSO (150 μL/well), and incubated at 37°C for 1 hour. The proliferation values were obtained from the optical density (OD) measured at 570 nm with 650 nm as background. Data are presented as percentage of the untreated controls (0 μM berberine) at each time point.

2.5. Analysis of Cell Differentiation. 5×10^4 cells/well cells were inoculated in 24-well plates. After 48 hours, cells were induced to differentiate in differentiation medium (PromoCell, Germany) containing varying concentrations of berberine (0 μM, 0.1 μM, 1 μM, and 10 μM). After 16 days, the degree of differentiation was determined by Oil-Red-O staining performed as previously reported [20]. In brief, medium was removed and cells were washed with PBS twice, fixed with 3.7% formalin at room temperature for 30 min, added 60% 2-propanol and incubated for 5 min, then moved out 2-propanol and stained cells with Oil-Red-O solution (Sigma, USA) at room temperature for 10 min. Images were obtained using an Olympus IX70 inverted phase-contrast microscopy (Olympus, Japan). After staining, the cells were washed twice with 70% ethanol and dissolved in 2-propanol containing 4% Nonidet-P40. OD values were measured at an absorbance of 490 nm using a standard microtiter reader (Bio-Rad, Canada).

2.6. RT-PCR Analysis. 5×10^5 cells/well cells were seeded in 6-well plates. 8 days after differentiation, 10 μM berberine was added in differentiation medium. Cells were harvested for 24 hours afterwards and mRNAs were extracted with Trizol reagent (Invitrogen, USA). RNA recovery and quality were checked by measuring the 260/280 nm optical density ratio and by electrophoresis on 1.5% agarose gel. 1 μg of total RNA from each sample was used for reverse transcription reaction using the TaqMan reverse transcription

reagents (Applied Biosystems, USA). The expression levels of peroxisome proliferator-activated receptor $\gamma 2$ (PPAR$\gamma 2$), CCAAT enhancer-binding protein α (C/EBPα), lipoprotein lipase, leptin, and adiponectin were measured using the following oligonucleotides (Shanghai Biotechnology Engineering Service Co. Ltd., China): 5′-GTG/GGG/CGC/CCC-/AGG/CAC/CA-3′ and 3′-CTT/TAG/CAC/GCA/CTG/T-AA/TTC/CTT/C-5′ primers for β-actin; 5′-ACC/CTG/T-GC/GGA/TTC/TTG/TGG/CTC/TGT-3′ and 3′-CGA/AG-T/CCG/ATG/AGG/TGT/CTC-5′ primers for leptin; 5′-CTG/GGA/GCT/GTT/CTA/CTG/C-3′ and 3′-AGT/CAC/C-CT/AAC/CTC/GT-5′ primers for adiponectin; 5′-GCG-/ATT/CCT/TCA/CTG/ATA/CAC-3′ and 3′-CGG/ACG/T-AG/AGG/TGG/AAT/AAT-5′ primers for PPAR$\gamma 2$; 5′-GCA-/AGG/CCA/AGA/AGT/CGG/TGG/AC-3′ and 3′-GAG/GA-A/CCA/GTT/CCG/GTA/CCC/GT-5′ primers for C/EBPα; 5′-ACA/CAG/CTG/AGG/ACA/CTT/GC-3′ and 3′-GAG-/TCC/TCG/TAA/TGG/GTC/AC-5′ primers for lipoprotein lipase. The basic reaction conditions are as follows: DNA denaturation at 94°C for 5 min; PCR amplification: 94°C denaturation for 50 sec, specific annealing temperature for 50 sec, 72°C extension for 1 min, and final extension also at 72°C for 8 min. To ensure that amplification of these products was within the exponential range, different numbers of PCR cycles (25–40 cycles) were run. PCR products were sent to Shanghai Biotechnology Engineering Service Co. Ltd. for sequence verification. PCR products were analyzed on a 2% agarose gel, and semiquantitative analysis was performed (quantification with Bio-1D software, France).

2.7. Effects of Berberine on Secreted Proteins in the Human Preadipocyte Differentiation Process. 10^5 cells/well cells were inoculated in 12-well plates. Cells were induced with differentiation medium. Beginning on the third day, supernatants were collected every 2 days and the final collections were done after 21 days of differentiation. Leptin and adiponectin proteins were measured using commercial ELISA kits (Quantikine, R&D Systems, Germany). The intra- and interassay CVs for leptin were <3.3% and <5.4%, respectively; the intra- and interassay CVs for adiponectin were <5.0% and <7.9%, respectively.

2.8. Clinical Intervention Study. 41 patients (age ranged 32∼68 years) with newly diagnosed metabolic syndrome enrolled in this study. 3 of them were initiative to withdraw from the study on medication 1, 3, and 6 days, and 1 person lost contact. In the end, 37 people (17 males/20 females) finished the clinical trials. Metabolic syndrome was defined according to Chinese Diabetes Society definition set in 2004 [21]. The study had the approval of the local ethical committee, and informed consent was obtained from all patients. Patients were treated with berberine 0.3 g three times a day for 12 weeks, and the following indicators before and after treatment were measured: height, weight, waist circumference, fasting plasma glucose, fasting insulin, hemoglobin A1C (HbA1c), triglyceride, cholesterol, LDL cholesterol, high-density lipoprotein, adiponectin, and leptin. The following were calculated: BMI, Leptin/Adiponectin ratio, and homeostasis model of assessment insulin resistance index [HOMA-IR = fasting insulin (mIU/L) × fasting glucose (mmol/L) /22.5].

2.9. Statistical Analysis. Descriptive statistics and analysis were performed in SPSS 13.0 for Windows (SPSS Inc. Chicago, IL). t-tests of two independent samples were done to determine the mean comparison in cell study (test of homogeneity of variance, such as $P < 0.10$, line t-test), and t-test of paired measurement data was done in clinical study before and after medication. Data of normal distribution were expressed as means ± the standard deviation. Data of nonnormal distribution were expressed as median (M) and quartile (Q1/4). The α level was set at 0.05.

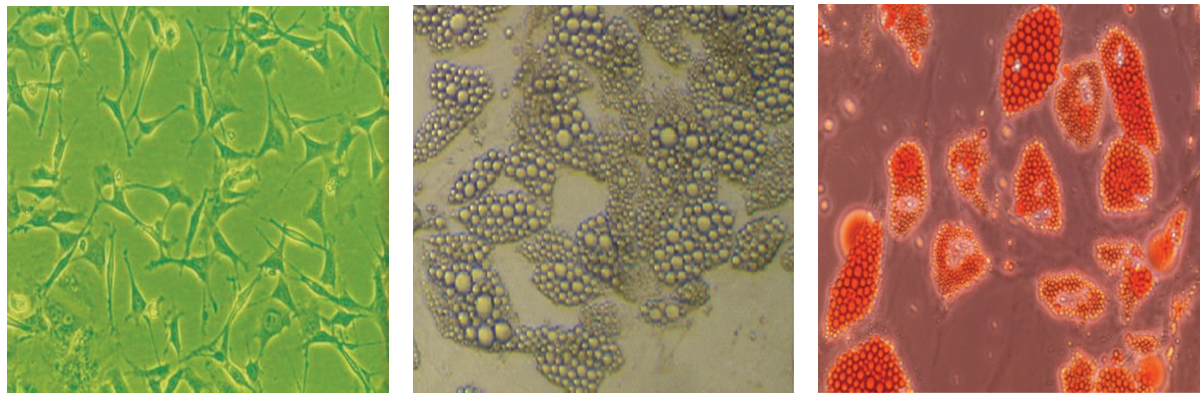
3. Results

3.1. Omental Preadipocytes and Induced Mature Adipocytes. Human omental preadipocytes were isolated and primarily cultured in growth medium. After 3-4 days, these cells began to show the typical long spindle shape (Figure 1(a)) and started to proliferate. Preadipocytes were induced to differentiate, and morphological changes can be observed after 15 days. When preadipocytes gradually mature, their cytoplasm was filled with lipid droplets, and small lipid droplets were integrated into big lipid droplets (Figure 1(b)). Fat droplets in adipocytes can be observed in the cytoplasm by Oil-Red-O staining (Figure 1(c)).

3.2. Effect of Berberine on Human Preadipocyte Proliferation. Primary human omental preadipocytes were treated with different concentrations of berberine and OD values were measured at day 1, day 2, or day 3. Results show that the relative OD values are significantly higher when berberine was added at 0.1 μM, 1 μM, and 10 μM concentrations compared with the control group ($P < 0.05$) (Figure 2). No significant difference was found among berberine-treated groups ($P > 0.05$).

3.3. Effect of Berberine on Human Preadipocyte Differentiation. Different concentrations of berberine were used during the cell differentiation process, and cells' morphological changes through Oil-Red-O staining were observed at day 16. Cell density was reduced judging by the stained color per increasing drug concentrations at low magnification field of vision. This suggests that berberine inhibits the process of cell differentiation and hypertrophy (Figure 3(a)). The staining intensity was measured. OD values of the berberine groups at concentrations of 1 μM and 10 μM were significantly lower than of the control groups, and the decreases determined were dose dependent ($P < 0.05$) (Figure 3(b)).

3.4. Effect of Berberine on PPAR$\gamma 2$, Lipoprotein Lipase, C/EBPα, Leptin, and Adiponectin mRNA Expression. Preadipocytes were induced to differentiate over 8 days, then 10 μM berberine was added and cells were harvested for 24 hours. The expression levels of PPAR$\gamma 2$, lipoprotein lipase, C/EBPα, leptin, and adiponectin were measured by RT-PCR. For quality control, the resulting PCR products were sequenced in duplicate and showed >85% homology with



(a) (b) (c)

Figure 1: Representative phase-contrast images of human omental preadipocytes in primary culture and differentiated preadipocytes. (a) Human omental preadipocytes in primary culture, (b) mature adipocytes induced from preadipocyte differentiation, and (c) mature adipocytes stained with Oil-Red-O, ×200.

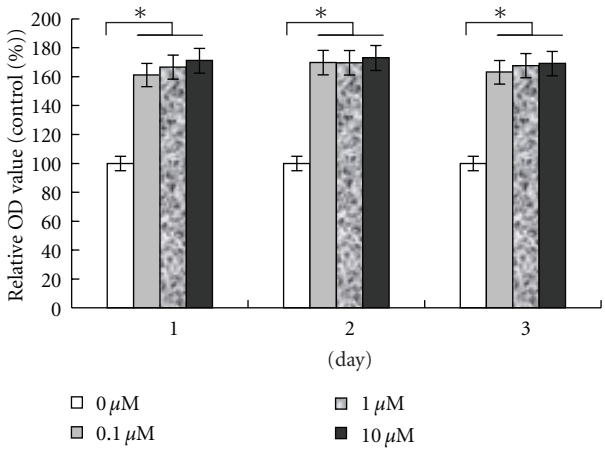


Figure 2: Effect of berberine on human preadipocyte proliferation. Cells were cultured in growth medium with different concentrations of berberine for 1, 2, and 3 days. At each culture time point, proliferation capacity was determined by MTT assay. Values are expressed as percentage of the untreated controls and represent the mean ± SEM of the three separate experiments in eight replicates. $^*P < 0.05$, compared to control at each time point.

GenBank registration sequences. Comparison of the ratio of the gray degree between the specific gene band and internal reference β-actin band showed that 10 μM of berberine inhibits PPARγ2, lipoprotein lipase, C/EBPα, adiponectin, and leptin mRNA expression (Figures 4(a) and 4(b)).

3.5. Effect of Berberine on Leptin and Adiponectin Secretion during the Process of Preadipocyte Differentiation. When preadipocytes were cultured with growth medium, a very low level of leptin protein was detected. However, the levels did not change with time. No adiponectin secretion can be detected. Once preadipocytes were cultured in differentiation medium, the secretion of leptin increased gradually with time. It increased much more rapidly after day 9 and reached the peak at day 17~19. After that, it maintained a high level of secretion. The secretion of adiponectin was also differentiation induced, and at day 7, low levels of secretion can be detected. This was the time when fat cells containing lipid droplets can be seen under microscope. After 15~17 days, adiponectin secretion reached its peak; however, it began to decrease significantly at day 21 ($P < 0.05$). For the 10 μM berberine-treated differentiation medium group, the levels of leptin and adiponectin secretion were significantly reduced starting from day 9 and remained low until day 21 (from mid- to terminal stages of differentiation) (Figure 5).

3.6. Effects of Berberine on the Impact Clinical Markers. Patients with newly diagnosed metabolic syndrome were treated with berberine for 12 weeks. Their BMI, waist circumference, fasting plasma glucose, fasting insulin, HbA1c, triglyceride, total cholesterol, LDL cholesterol, leptin, the ratio of leptin and adiponectin, and HOMA-IR were measured before and after the treatment. All of those indexes showed a decreasing trend, with a significant statistical difference ($P < 0.05$–0.01). However, the levels of adiponectin did not change significantly (Table 1).

3.7. Safety. None of the patients experienced severe gastrointestinal adverse events when berberine was used. Incidence of gastrointestinal adverse events was mainly constipation ($n = 1$; percentage, 5%). The adverse effect disappeared when berberine dosage was decreased from 0.3 g three times a day to 0.2 g three times a day. Liver and kidney functions were monitored in this study. No significant changes of plasma ALT, γ-GT, and creatinine were observed during the 12 weeks of berberine treatment (Table 1). None of the patients were observed with hypoglycemia.

4. Discussion

Proliferation and differentiation are two important aspects in fat tissue development. In our experiment, we have shown that berberine has a growth-factor-like role on human preadipocytes, promoting cell proliferation. Our result is

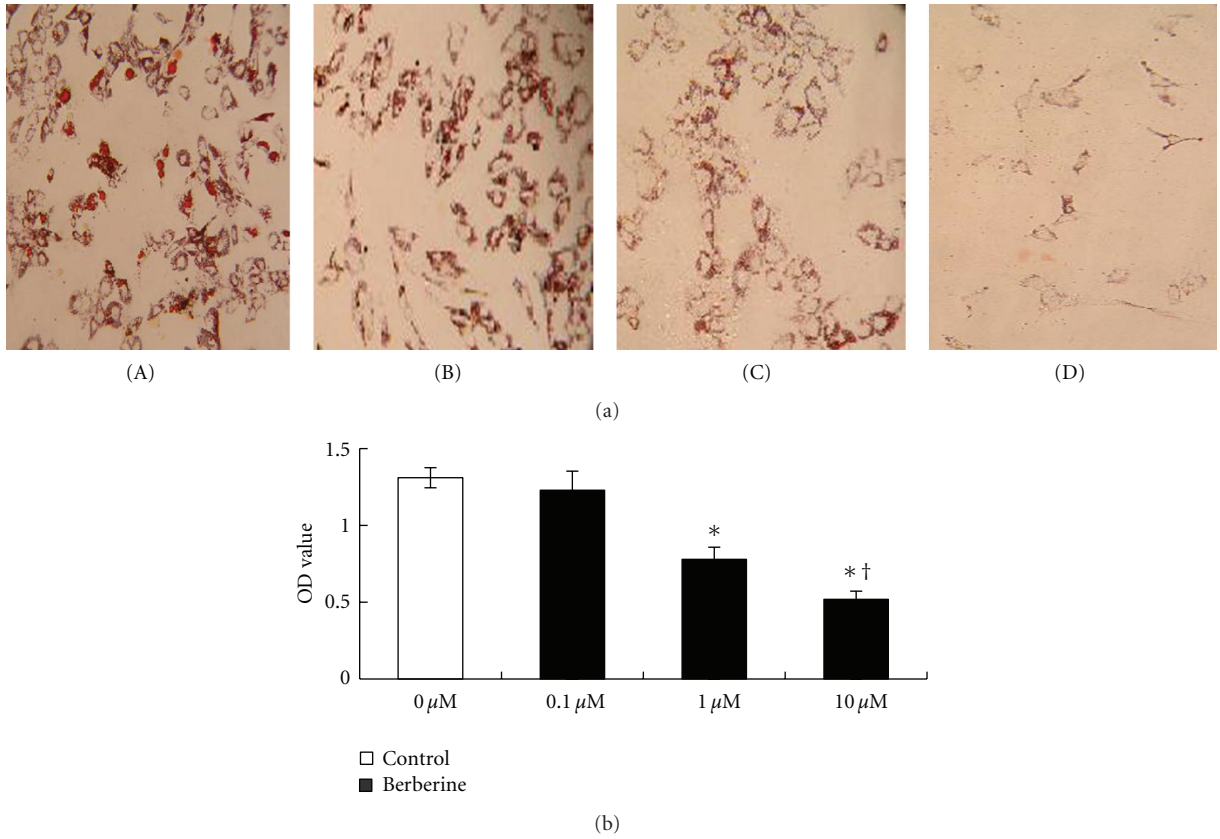


Figure 3: Effect of berberine on human preadipocyte differentiation. Cells differentiated in the absence or presence of different concentrations of berberine over 16 days. The degree of differentiation was determined by Oil-Red-O staining. (a) Photomicrographs representing cells maintained in different concentrations of berberine: (A) control, (B) 0.1 μM berberine, (C) 1 μM berberine, (D) 10 μM berberine, $\times$100. (b) Absorbance value representing the mean $\pm$ SEM of the three separate experiments in six replicates. $^{*}P < 0.01$, comparison between berberine-treated groups and control group, $^{\dagger}P < 0.05$, comparison among berberine-treated groups.

consistent with previous studies showing that berberine can promote the proliferation of mouse 3T3-L1 preadipocyte [22]. Adult obesity is mainly due to the increased volume of fat cells which are abnormally hypertrophic [23]. Our *in vitro* experiments showed that berberine significantly inhibited the omental preadipocytes to become mature adipocytes judging by their morphology or lipid-specific Oil-Red-O staining. Therefore, berberine has potential clinical application in reducing visceral fat and controlling central obesity. It has been reported that fat tissue composed of a higher amount of small fat cells is more sensitive to insulin compared with fat tissue with the same lipid content composed of a small number of large fat cells, and also the former has very little inflammatory responses [24]. Our results showed that berberine can promote human fat cell proliferation and inhibit fat cell enlargement, indicating that it may be able to reduce inflammation responses, improve insulin sensitivity of visceral adipose tissue, and reduce or eliminate the visceral adipose tissue. Moreover, our *in vivo* study also showed that, after taking berberine for three month, patients with metabolic syndrome were found to reduce their waist circumferences and BMI to varying degrees. This positive result therefore seems in good agreement with the *in vitro* study.

The nuclear receptor PPARγ and members of the C/EBP family take important roles in adipogenesis [25], and the major players are PPARγ2 [26] and C/EBPα [27]. Many studies have showed that berberine inhibited the mRNA and protein levels of adipogenesis-related transcription factors PPARγ2 and C/EBPα [28, 29]. We studied the effect and transcriptional impact of berberine on human preadipocyte differentiation. Our result showed that the berberine can inhibit PPARγ2 and C/EBPα mRNA expression simultaneously during the human preadipocyte differentiation process. Recently, the transcription factors GATA binding protein 2 and 3 (GATA-2 and GATA-3) have been shown to be important gate keepers of the differentiation process [30, 31]. Studies from HU et al. showed that berberine increases expression of GATA-2 and GATA-3 during inhibition of adipocyte differentiation in both murine cell lines 3T3-L1 and human white preadipocytes cell line. But contradictorily, they also found that the differentiation inhibition mechanisms of berberine appeared to be independent of PPARγ2 and C/EBPα in that human white preadipocyte cell line while being dependent on decreasing of PPARγ2 and C/EBPα gene expression in 3T3-L1 lines [32, 33]. Those results also seem to contrast with our findings from human primarily cultured preadipocytes. Since there is still lack

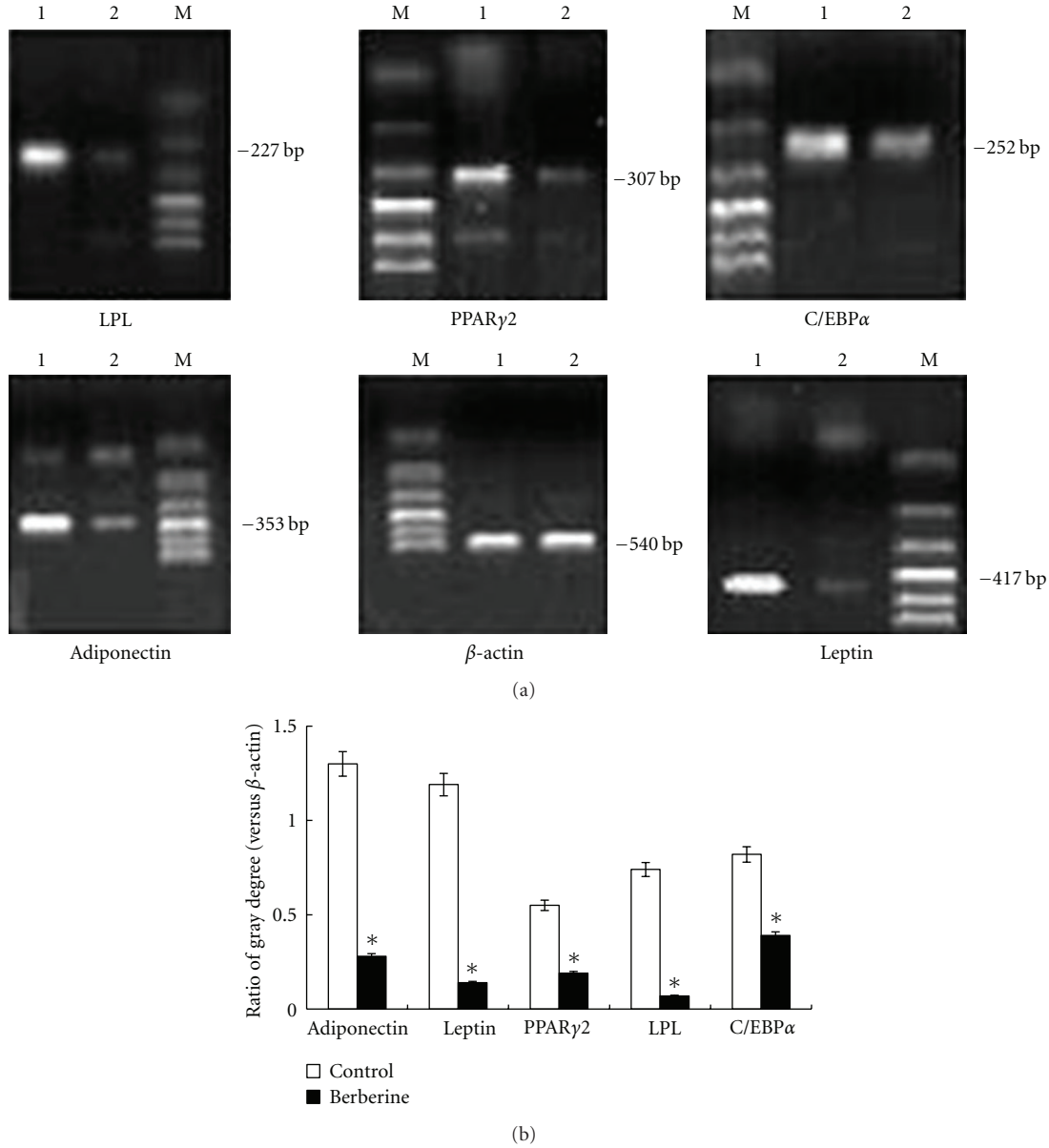


Figure 4: Effect of berberine on PPARγ2, lipoprotein lipase, C/EBPα, leptin, and adiponectin mRNA expression in differentiated preadipocytes analyzed using RT-PCR. (a) Digital photos of PCR products in agarose gel. Lane 1: control group, lane 2: 10 μM berberine, lane M: DNA markers. (b) Results are expressed as the ratio between the intensity of band corresponding to target gene versus that to β-actin, representing the mean ± SEM of the three separate experiments in triplicate. $^*P < 0.05$, compared to control.

of well-characterized human preadipocyte cell lines, further studies in human primarily cultures are particularly needed to clarify the results from those cell lines.

Lipoprotein lipase is a kind of glycoprotein synthesized and secreted by fat cells. Current understandings of lipoprotein lipase's physiological functions are to decompose chylomicrons and triglycerides into very low-density lipoproteins and to promote the lipoprotein transfer between triglyceride phospholipids and apolipoproteins and so forth. We found that berberine reduced lipoprotein lipase mRNA expression in human fat cells which is consistent with the recent study by Choi et al. showing that berberine reduces mouse 3T3-L1 lipoprotein lipase mRNA expression [9]. As discussed by Kong et al. [34], oral administration of berberine in 32 hypercholesterolemia patients for 3 months reduced serum cholesterol by 29%, triglycerides by 35%, and LDL cholesterol by 25%. Our clinical study of the effects of berberine on total cholesterol, triglycerides, and LDL cholesterol along with analysis of liver and kidney adverse reactions also indicates that berberine could be a cheap, efficient, and safe lipid-lowering drug in metabolic syndrome patients.

Leptin and adiponectin have been shown to play an important role in insulin resistance. During the process of

Table 1: The general information and laboratory data of the new diagnosed metabolic syndrome patients at the baseline and 12 weeks after the therapy.

	Before medication	After medication	*P* value
Participants (M/F)	37 (17/20)	37 (17/20)	
Age (years)	41.1 ± 7.3	41.1 ± 7.3	
Body mass index (kg/m^2)	31.5 ± 3.6	27.4 ± 2.4	<0.01
Waist circumference (cm)	97.3 ± 10.5	92.1 ± 9.10	0.04
Systolic pressure (mmHg)	146.1 ± 14.9	134.0 ± 13.3	0.32
Diastolic pressure (mmHg)	95.5 ± 8.7	88.5 ± 10.3	0.24
Total cholesterol (mM)	6.69 ± 1.04	5.74 ± 0.84	0.03
High-density lipoprotein cholesterol (mM)	1.09 ± 0.36	0.92 ± 0.36	0.06
Low-density lipoprotein cholesterol (mM)	3.68 ± 0.85	2.86 ± 0.57	0.03
Triglyceride (mM)	3.03 ± 2.05	1.86 ± 0.90	<0.01
Fasting plasma glucose (mM)	7.37 ± 0.72	6.13 ± 0.85	0.03
HbA1c (%)	7.10 ± 0.64	6.04 ± 0.62	0.02
Leptin (ug/L)	8.01 (2.04~15.17)	5.12 (1.88~12.89)	0.04
Adiponectin (mg/L)	8.42 (4.75~14.81)	10.02 (5.13~15.59)	0.14
Leptin/adiponectin	0.76 (0.29~2.85)	0.58 (0.14~1.22)	0.02
Fasting insulin (mIU/L)	16.90 (11.6~20.1)	12.50 (9.7~14.8)	0.04
HOMA-IR	5.46 (3.62~6.69)	3.25 (2.50~4.58)	0.03
Glutamic-pyruvic transaminase (U/L)	37.28 ± 4.12	39.89 ± 7.08	0.62
γ-Glutamyl transpeptidase (U/L)	48.71 ± 8.12	41.79 ± 7.11	0.74
Creatinine (mM)	87.45 ± 4.71	89.11 ± 8.07	0.77

t-test of paired measurement data comparisons between two comparing lines before and after medication. Data of nonnormal distribution were described using the median (M) and quartile (Q1~Q4).

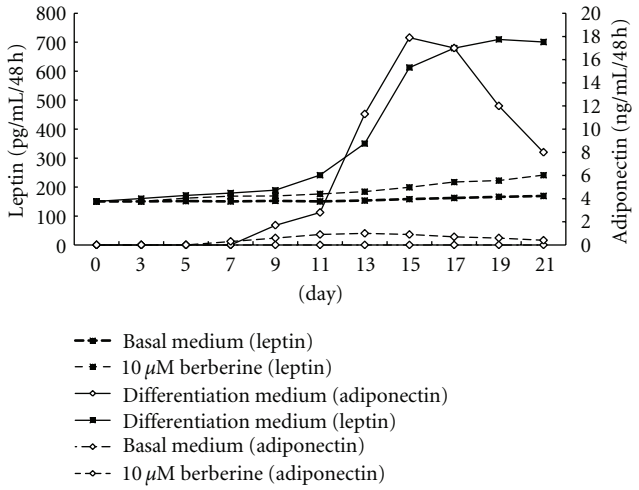


Figure 5: Effect of berberine on leptin and adiponectin secretion during the process of preadipocyte differentiation. Results represent the mean values of the three separate experiments in triplicate.

preadipocytes differentiation, secretion of leptin and adiponectin showed different kinetics: in the first phase low levels of leptin secretion can be detected in fat cells, but no adiponectin secretion can be detected. The amount of leptin secretion continued to increase in a synchronized fashion with the differentiation process, while the secretion of adiponectin was only observed when preadipocytes gradually mature and their cytoplasm began to be filled with lipid droplets. This result suggests that only mature fat cells can secrete adiponectin which can be used as a specific marker to determine the maturity of fat cells. In the midstages of differentiation, secretion of leptin and adiponectin increased with an increased number of fat cells; however, the rate of increase in adiponectin was more obvious. In the late stages of differentiation (at day 17–21), a morphological change of the cells showed that the majority of cells differentiated into fat cells with their cytoplasm filled with large lipid droplets. Oil-Red-O staining showed that lipid content in the cytoplasm remained at a steady high level. At this point, leptin secretion remained at high levels, while adiponectin secretion was seen to show a clearly downward trend. This difference suggests that fat cells in different fat-storing states secrete leptin and adiponectin differently, which may also reflect on their functional differences. The differentiation stages of fat cells from clinically obese patients may be different from normal people. In obese patients, the fat cells may show a high-leptin and low-adiponectin secretion pattern. In our differentiation experiment, berberine not only inhibited the differentiation and maturation of preadipocytes but also suppressed leptin and adiponectin secretion. This suggests to us that, in the visceral adipose tissue accumulation process, berberine has a role in endocrine function regulation and can promote the reversion of the initial process of fat storing. Our clinical observation showed that in patients with newly diagnosed metabolic syndrome the level of leptin dropped

significantly with berberine treatment after 3 months. This is different from results shown in rat studies and may reflect species differences. This may suggest that berberine has the potential for anticentral obesity and regulating obesity-related endocrine dysfunctions, thus achieving a balance between fat cell factors. Studies have reported that the ratio of leptin and adiponectin reflects the body's insulin resistance [35]. We have confirmed that berberine reduces HOMA-IR and the ratio of leptin and adiponectin. As it inhibits PPARγ2 mRNA expression and has more effects on weight loss and reducing leptin levels, berberine regulates insulin sensitivity with a mechanism different from the insulin sensitizer, thiazolidinediones. Thus, berberine provides an additional way for clinical treatment of metabolic syndrome and obesity-related diseases. However, our experiment is only a preliminary study on the mechanisms of effects of berberine on serum adipokines. A large-scale clinical study is ideally required to include different population groups and more experiments concerning the mechanism details.

In conclusion, in order to explore the mechanism of berberine's role in improving insulin sensitivity, we used human adipose tissue as material, focusing on the proliferation, differentiation, and adipokine secretion of human preadipocytes. We tried to find clues from the *in vitro* experiments and then verified them in clinical trial. Our clinical study has some limitations relative to the randomized, placebo-controlled clinical design. However, our result is in agreement with the findings from previous large sample, well-designed clinical studies [23, 34], indicating that berberine improves glucose and lipid metabolism disorders. More particularly, we find that berberine can improve insulin sensitivity by adjusting adipokine secretion both in primarily cultured preadipocytes as well as in metabolic syndrome patients, and this was not well characterized in previous human studies.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgments

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Review Article

Chinese Herb and Formulas for Promoting Blood Circulation and Removing Blood Stasis and Antiplatelet Therapies

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Atherothrombosis, which directly threatens people's health and lives, is the main cause of morbidity and mortality all over the world. Platelets play a key role in the development of acute coronary syndromes (ACSs) and contribute to cardiovascular events. Oral antiplatelet drugs are a milestone in the therapy of cardiovascular atherothrombotic diseases. In recent years, many reports have shown the possibility that "resistance" to oral anti-platelet drugs and many adverse reactions, such as serious bleeding risk, which provides an impetus for developing new anti-platelet drugs possesses highly efficiency and fewer adverse effects. Study on the blood stasis syndrome and promoting blood circulation and removing blood stasis is the most active field of research of integration of traditional and western medicine in China. Blood-stasis syndrome and platelet activation have close relationship, many Chinese herb and formulas for promoting blood circulation and removing blood stasis possess definite anti-platelet effect. This paper covers the progress of anti-platelet mechanism of Chinese herb and formulas for promoting blood circulation and removing blood stasis and is to be deeply discussed in further research.

1. Introduction

Cardiovascular and cerebrovascular events have become the major killer of people's health and life all over the world. Rupture of atherosclerotic plaque in an artery wall and the ensuing thrombotic events are the triggers for acute ischemic injury. Activated platelets play a pivotal role in the formation of pathogenic thrombi underlying acute clinical manifestations of vascular atherothrombotic disease. Oral antiplatelet drugs are a milestone in the therapy of cardiovascular atherothrombotic diseases and provide the primary and secondary prevention strategy to combat these diseases. Efficient antiplatelet therapy can make the death rates of heart disease and stroke decline by about 25% [1, 2]. Commonly used oral antiplatelet drugs include cyclooxygenase inhibitor aspirin, the glycoprotein IIb/IIIa inhibitor ReoPro, and the P_2Y_{12} inhibitor clopidogrel, et al. Many clinical studies show that dual antiplatelet therapy with aspirin and clopidogrel is currently the standard of drugs for prevention of adverse cardiovascular events in most patients at high risk owing to acute coronary syndromes or recent placement of a stent.

But along with prolonging of treatment by dual or triple antiplatelet drugs, the effectiveness and security have garnered particular attention in clinic. Despite their proven benefit, recurrent cardiovascular events still occur in those taking antiplatelet drugs. This has led to the concept of antiplatelet resistance [3], most commonly aspirin resistance as this drug is the cornerstone of most regimens. Although there are some debates on definition and mechanism of antiplatelet resistance [4, 5], it cannot be denied that it has important clinical significance. At the same time, numerous adverse reactions including serious bleeding risk (digestive and nervous systems) and combination with PPIs and statin [6, 7], which limit the clinical practice of antiplatelet drugs. So developed novel classes of antiplatelet agents possess high efficiency, and fewer adverse effects have been always the research focus for prevention of cardiovascular disease. Modern medicine and pharmacology has done a lot of valuable exploration, newer agents are in development recent years that include prasugrel, cangrelor, ticagrelor, and vorapaxar, et al. [8].

Study on the *blood stasis syndrome* (BSS) and *promoting blood circulation and removing blood stasis* (PBCRBS) is the most active field of research of integration of traditional and western medicine in China. During the past 50 years, much

Table 1: The ingredient of frequently used formulas for promoting blood circulation and removing blood stasis.

Names of formulas	Ingredients of formulas	Label
Xiongshao capsule	*Szechuan Lovage Rhizome, Red Paeony Root*	Chinese patent drug
Compound danshen dripping pills	*The root of red-rooted salvia, Panax notoginseng, Borneol*	Chinese patent drug
Buyanghuanwu decoction	*Radix Astragali Bunge, Peach Seed, Safflower, Szechuan Lovage Rhizome, Angelica sinensis, Red Paeony Root, earthworm*	
Xuesaitong capsule	*Panax Notoginsenosides*	Chinese patent drug
Tongxinluo capsule	*Sanguisuge, Scorpio, centipede, ground beeltle, cicada slough,* et al.	Chinese patent drug
Danhong injection	*The root of red-rooted salvia, safflower*	Chinese patent drug
Taohongsiwu decoction	*Peach Seed, Safflower, Szechuan Lovage Rhizome, Angelica sinensis, white paeony root, Radix Rehmanniae Praeparata*	
Xue Fu Zhu Yu decoction	*hovenia dulcis, radix achyranthis bidentatae, peach seed, safflower, Szechuan Lovage Rhizome, Angelica sinensis, white paeony root, Radix Rehmanniae Praeparata, radix bupleuri, Platycodon grandiflorum,* et al.	

significant progress has been made from theory, experiments to clinic fields based on the inherit, and innovation of thoughts in traditional Chinese medicine [9], to clarify the treatment regulations and principles of PBCRBS, which has already got consensus in medical community in China. A lot of formulas for PBCRBS (see Table 1) have showed great antiplatelet effect in clinic, and most of them are the Chinese patent drugs. On the prevention of atherosclerosis or vulnerable plaque, Chinese and Western medicine have the consensus that stabling plaque and promoting blood circulation. Based on the agreed thoughts of the Eastern and Western worlds, the application of Chinese herb and formulas for PBCRBS has valuable significance in the exploration of reducing the risk of cardiovascular event [10].

Blood-stasis syndrome has the status of platelet activation, and it has high correlation [11, 12]. As early as the last century of 1970s, there were scholars who had made pilot study to observe the mechanism of Chinese herb and formulas for PBCRBS on platelet function [13]. BSS has the definite diagnostic criteria [14] from 1991 in China, and during the past 5 years, diagnosis criteria have improved by scholars [15] and keep pace with the development of TCM. There is a special focus on natural compounds present in dietary and medicinal plants exhibiting antiplatelet/thrombotic properties. Now we know that platelet mainly was regulated by three kinds of substance, one kind is generated out of platelet such as catecholamine, collagen, thrombin, and prostacyclin; the second kind is generated from platelet and acts on the platelet membrane glycoproteins such as ADP, PGD_2, PGE_2, and 5-HT; the last kind is generated from platelet and acts on the platelet such as TXA_2, cAMP, cGMP, and Ca^{2+}, et al. Some of these substances have been identified as effective target of antiplatelet. Owing to the many problems of effectiveness and security of current antiplatelet drugs, a great need now arises to develop both efficacious and pharmaceutical medicines to combat these diseases. Screening the highly efficiency and fewer adverse effects of antiplatelet drugs from Chinese herb and formulas for PBCRBS attracts great attention of researchers, and the study of target or mechanism of Chinese herb and formulas for PBCRBS to be the hot topic of research and development of antiplatelet drugs. It had been approved that antiplatelet mechanism of Chinese herb and formulas for PBCRBS involves the following aspects.

2. Antiplatelet Mechanism of Chinese Herb and Formulas of Promoting Blood Circulation and Removing Blood Stasis

2.1. Inhibition of Platelet Aggregation. Platelet aggregation means the clumping together of platelets in the blood, which is the main function of platelet and has key role in the physiological hemostasia and pathogenesis of atherothrombosis. Platelet activates when it adheres to breakage of vessel or has been induced by activator. Activated platelet membrane glycoprotein (GP) IIb/IIIa exposes its fibrinogen receptor with the participation of Ca^{2+}, one fibrinogen can bind to at least two GP IIb/IIIa at the same time, and platelet clump together with fibrinogen by GP IIb/IIIa. The typical aggregation is induced by different activators, which included the following two aspects, one is chemical agents such as ADP, collagen, thrombin, AA, and PAF, et al.; the other is shear stress. It is now taken that platelet aggregation rate (PAR) is the evaluation criterion of the intensity. Born [16] designed the platelet aggregation analyzer in 1962 by the turbidimetry principle which to accelerate the understanding of platelet aggregation. Now PAR was considered as the marker of antiplatelet efficacy evaluation and was used intensively in medical research of platelet. Studies show that the vast majority of Chinese herb and formulas for PBCRBS such as *Xiongshao Capsule* [17], *Compound Danshen dripping pills* [18], *Buyanghuanwu Decoction* [19], *Xuesaitong Capsule* [20], *Da Huang Zhe Chong pill* [21], and *Tongxingluo Capsule* [22], et al. can reduce the PAR of patients or animal model of thromboembolic diseases significantly. Active principles such as *ferulic acid* [23], *ligustrazine* [24], *propyl gallate* [25], *resveratrol* [26], *curdione* [27], *Total flavone in Sanguis Draconis* [28], *Salvianolic acid B* [29], *Hirulog* [30], and *Safflower flavin* [31] et al. can inhibit the platelet aggregation induced by AA, ADP, PAF, collagen, and thrombin to some extent, bringing out the superior antiplatelet effect.

2.2. Inhibition of Platelet Release Reaction. Platelet release reaction means that many substances stored in α-granules, dense granule, and lysosome in platelet are released out of platelet upon different activator. These substances including CD62p (P-selection), GPIIb/IIIa compound, PKC, β-TG, PF-4, and Ca^{2+}, which has been considered as the usual evaluation indicator of screening the effective antiplatelet drug from Chinese herb and formulas for PBCRBS.

2.2.1. CD62P. CD62p (P-selection) is a 140 kD glycoprotein which is present in the granules of platelets and translocates rapidly to the cell surface after platelet activation and is generally considered to be the gold marker of platelet activation [32, 33]. Clinical research indicates that the expression of CD62p increases markedly in the different types of cardiovascular patients (including patients with stable angina and ACS) [34–36] and has found high positive correlation between CD62p level and blood stasis syndrome (BSS) [37]. So making the increased expression of CD62p after platelet activation dropped is taken for the one of the antiplatelet mechanisms and scientific measurements of Chinese herb and formulas for PBCRBS. According to the current studies, *Danhong injection* [38], *Ligustrazine injection* [39], *Compound Danshen dripping pills* [40], *Taohongsiwu Decoction* [41], and *Tongxinluo capsule* [42] can reduce the CD62p expression after platelet activation significantly and inhibit platelet activation *in vivo*, to show satisfactory effect of antiplatelet.

2.2.2. GPII b/III a Compound. The detection of PAC-1 is considered as the sensitive and important marker of platelet activation [43], PAC-1 is the specific monoclonal IgMK, which only binds to activating platelet GPIIb/IIIa compound, while it has no recognition capability for resting one. The activation of GPIIb/IIIa depends on the platelet activation which makes the former change its configuration to have strong affinity with receptors. Using the flow cytometry to detect PAC-1 which has the characteristic of specific fast sensitive, and has splendid future in the study on screening antiplatelet drugs from Chinese herb and formulas for PBCRBS.

Da Huang Zhe Chong pill is the earliest formula of PBCRBS and is widely used for atherothrombotic disease treatment. Research shows that it has better antiplatelet aggregation ability than aspirin [44], the further study indicates that it can reduce the level of PAC-1 after ADP-induced platelet activation and of patients with coronary heart disease and cerebral infarction in clinic, which also has superior antiplatelet activation than aspirin [21] and is an ideal antithrombotic drug. Other study [45] found that *Xue Fu Zhu Yu decoction* can inhibit the ADP-induced expression of GPIIb/IIIa compound significantly and restrain the ADP-induced platelet activation, which provides experimental evidence to long-term treatment of coronary heart disease, and no symptoms of myocardial ischemia, et al.

2.2.3. PKC. protein kinase C (PKC), a ubiquitous protein kinase found in a variety of animal tissues, has been implicated in the regulation of many cellular processes and plays a central role in signal transduction. In platelets, the PKC is an important signaling mediator required for activation, secretion of granule contents, and aggregation [46]. During the process of platelet activation, close relationship between translocation of PKC in platelet and platelet function has been found. PKC has both cytosolic and plasma membrane-bound forms, and the former is the most abundant under resting conditions. The cytosolic form can translocate to the plasma membrane upon cell stimulation and elevation of cellular Ca^{2+}, one particular and important aspect of PKC activation is the intracellular redistribution of the enzyme from the cytosol to the cell membrane [44]. Now translocation or redistribution of PKC from the cytosolic form to the plasma membrane can be taken for an indicator of PKC activation [47].

Resveratrol (*RESV*), a well-known polyphenolic compound of, was extracted from *Polygonum Cuspidatum*, which was a Chinese herb for PBCRBS and has been efficaciously used in traditional Chinese medicine to treat several diseases, including thromboembolic diseases for over hundreds of years. In recent years, pharmacological studies have found that *RESV* possesses multifaceted cardiovascular benefits, but the mechanism is not clear. Recent research [48] shows that PKC distributed mostly across the cytosol of platelets in resting platelets and redistributed to the membrane later to be activated by ADP. If pretreated by *RESV*, PKC translocation to the membrane was partially inhibited in the platelets activated by ADP. These results suggested that *RESV* inhibited the PKC-mediated signal transduction pathway in platelets, and it might act as an inhibitor on PKC activity in platelets and serve as a novel antithrombotic agent.

2.2.4. PF-4 and β-TG. It is thought that PF-4 and β-TG are the specific indicators of platelet release reaction [49]. Both increases of PF-4 and β-TG indicate the height of platelet release reaction, which is common in thromboembolic disease and prethrombotic state. On the contrary, both decreases of PF-4 and β-TG indicate the suppression of platelet release reaction. β-TG can make the PGI_2 concentration and adenylate cyclase activity reduction, and then make cAMP decrease which bring about weak inhibition and enhance the aggregation of platelet [50, 51]. PF-4 can reduce the anticoagulation of heparan sulphate in endothelial cell and enhance the metabolism of membrane phospholipid and AA, to produce TXA_2, also PF-4 can promote precipitation and polymerization of fibrin monomer and accelerate platelet aggregation [52]. Research has found that *Salvia miltiorrhiza Bunge* injection [53] can reduce the PF-4 and β-TG concentration markedly to inhibit platelet aggregation.

2.2.5. Ca^{2+}. Calcium ion plays a vital role in the development of platelet activation. The transformation, aggregation, and release reaction of platelet are triggered by the increase of free calcium ion concentration of platelet ($[Ca^{2+}]_i$), which is the essential mechanism of thrombosis [54]. Studies have found that the increase of $[Ca^{2+}]_i$ in patient with CHD, meanwhile calcium antagonist can reduce $[Ca^{2+}]_i$ of platelet accompanied by inhibiting platelet aggregation [55].

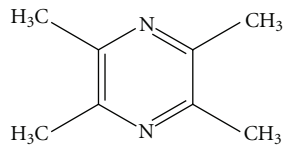

Figure 1: Chemical structures of ligustrazine.

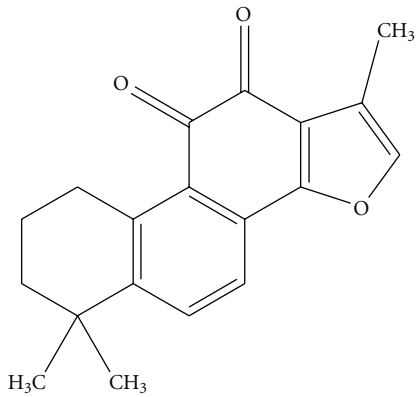

Figure 2: Chemical structures of Tanshinone IIA.

Studies [56] have indicated that some Chinese herbs, such as *Salvia Miltiorrhiza, Ligusticum wallichii Franch, Carthamus tinctorius, Radix Paeoniae Rubra,* and some active constituents as *Ligustrazine* (see Figure 1), Tanshinone IIA (see Figure 2), et al. have the certain effect of calcium channel antagonists and have good results of inhibit platelet aggregation and activation. Another research [57] shows that *Safflor yellow* (a kind of soluble natural pigment of *Carthamus tinctorius*) can inhibit platelet release of 5-HT and Ca^{2+}, which has similar effect to *Ginkgolides* (admitted PAF receptor antagonist), which means that *Safflor yellow* might suppress the platelet activation via inhibition of PAF and calcium influx.

2.3. Influence of the Process of Platelet Metabolism

2.3.1. Influence of the Metabolic System of Arachidonic Acid (AA). TXA_2 and PGI_2 are the metabolites of AA, which have the strong bioactivity of PG, and have a short half-life, quickly degrad to the TXB_2 and 6-keto-PGF1α, the latter make further metabolizes to the 6-keto-PGE.

It is now thought that many cardiovascular diseases such as atherosclerosis, thrombosis, coronary spasm, acute myocardial infarction, and hypertension have close relationship with the disequilibrium of TXA_2/PGI_2 [58]. TXA_2, which is synthesized and released by platelet microsome and has the function of promoting platelet aggregation and thrombosis, is one of the strong inducers of platelet aggregation and vasoconstrictor. TXA_2 promotes the Ca^{2+} of density tube system free to make dense bodies contracting and releasing ADP and 5-HT, which result in platelet aggregation. PGI_2 is the main metabolite of AA and is the strong endogenous inhibitor of platelet aggregation; it has the function of antiplatelet aggregation and vasorelaxant and is considered as the vascular protection factor. Under normal physiological state, TXA_2 and PGI_2 have the balance condition and keep the platelet internal environment stable. Out of balance of TXA_2 and PGI_2 in plasma or tissue is one of the reasons of platelet aggregation, vasospasm, and thrombosis. Studies show that influence of TXA_2/PGI_2 has been closely related to antiplatelet mechanism of Chinese herb and formulas for PBCRBS, such as *Total saponins of paeonia* [59] can reduce the ADP-induced platelet maximum aggregation rate and plasma TXB_2 concentration, meanwhile, increase the plasma 6-keto-PGF1α concentration, which means it can promote the release of PGI_2, inhibit the produce of TXA_2, improve the balance of TXA_2/PGI_2, and reach the aim of antithrombotic therapy. The same results have been found in the following drugs: *Guanxin II* [60], *Taohongsiwu Decoction* [61], *Notoginsenoside* [62], *salvianolic acid A* [63], *Honghua injection* [64], et al.

2.3.2. Influence of the Metabolic System of cAMP and cGMP. cAMP and cGMP in platelet are the second messengers of signal transmission, which make the different platelet activators acting on the specific receptor, then resulting in platelet aggregation and activation. Studies [65, 66] show that drugs which make the level of cAMP and cGMP increase can inhibit platelet aggregation owing to promoting the intake of calcium ions, lowering the level of Ca^{2+}, and having close relation with the phosphorylation of myglobulin. So whether can affect the metabolic system of cAMP and cGMP has been taken as the main point of antiplatelet mechanism of Chinese herb and formulas for PBCRBS. Research [19] shows that *BuYangHuanWu decoction* can inhibit the ADP-induced platelet aggregation and the decrease of cAMP and cGMP after the platelet aggregation, which suggested that its antiplatelet aggregation may be related to inhibiting the decrease of cyclic nucleotide in platelets after the aggregation. *Compound Danshen dripping pills* [67] and *pseudoginseng* [68] have the same mechanism of antiplatelet.

2.4. Influence of the Signal Transduction in Platelet. There is a series of signal transductions in platelet, which has close relationship with platelet activation. Upon agonist stimulation, specific receptor of membrane binds to the ligand to make the conformational changes and to activate the key enzymes action, which produces or releases the signal molecules and led to adhesion, aggregation, and reaction release to form thrombus at last. The mechanism of transmembrane signal transduction in platelet is unclear owing to more than one receptor bound by platelet agonist and the activated platelet release α-granules as secondary agonist to bring about amplification effect [69]. Platelet signal transduction pathway usually includes several aspects [70]: PI3-K pathway, PLC-β pathway, PTK pathway, MARK pathway, cAMP-PKA pathway, and PLA_2 pathway. At present, most researches are about Phosphoinositide 3-kinase (PI3K). PI3K is a critical transmitter of intracellular signaling during platelet activation. The PI3K family is divided into three classes (I, II, and III). Depending on differences in the heterodimerization of catalytic subunits and regulatory subunits, class I is further

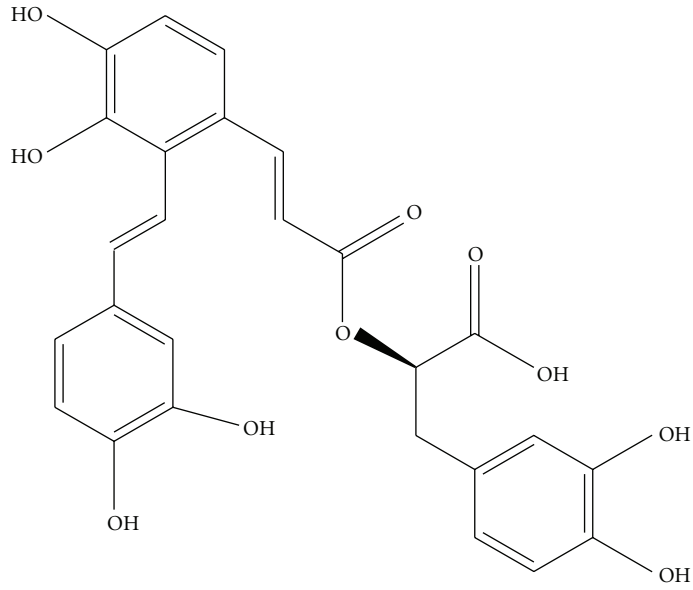

Figure 3: Chemical structures of salvianolic acid A.

divided into IA (PI3Kα, PI3Kβ, and PI3Kγ) and IB (PI3Kδ), PI3Kβ and PI3Kγ are crucial in platelet signaling [71]. Akt phosphorylation can be used as an indicator of PI3K pathway activation [72, 73].

In recent years, with the further study of antiplatelet mechanism of Chinese herb and formulas for PBCRBS, there are studies involving signal transduction in platelet to investigate the mechanism. *Salvianolic acid A* (SAA, Figure 3) is a water-soluble component from the root of *Salvia miltiorrhiza Bunge*, a herb that is widely used for atherothrombotic disease treatment in China. New study [74] shows that SAA could inhibit platelet spreading on fibrinogen, a process mediated by outside-in signaling. Western blot analysis showed that SAA, like the PI3K inhibitors LY294002 and TGX-221, potently inhibited PI3K, as shown by reduced akt phosphorylation, which indicates that the target spot may be the PI3Kβ. The *in vitro* findings were further evaluated in the mouse model of arterial thrombosis, in which SAA prolonged the mesenteric arterial occlusion time in wild-type mice. Interestingly, SAA could even counteract the shortened arterial occlusion time in $Ldlr^{tmlHer}$ mutant mice. And for the first defined the fact [74] that SAA inhibits platelet activation via the inhibition of PI3K and attenuates arterial thrombus formation *in vivo*. The results suggest that SAA may be developed as a novel therapeutic agent for the prevention of thrombotic disorders.

3. Discussion and Perspective

From above mentioned, during the past 30 years, research of antiplatelet and antithrombotic therapy of Chinese herb and formulas for PBCRBS has made rapid progress, but there are still some problems existing. In the clinical research, at present many studies limited to small sample of curative effects, lack of multicenter, prospective, large sample, and control study which made the clinical practice of Chinese herb and formulas for PBCRBS be short of definite clinical evidence. And Chinese scholars has begun to attempt to study like above and got to some good results [75]. But those which deserve attention are, in the practical use of clinical medicine, we should comply with the principle of differentiation of symptoms and signs, minimize the potential abuse, and improve on the clinical practical effects. In the experimental research, many studies mainly focused on the mechanism on one aspect of a certain Chinese herb and formulas for PBCRBS, the experimental design owes rigor, and only a few studies were equipped with *in vitro* and *in vivo* at the same design. It is generally known that platelet activation is a complex, multifactor process, which involves adhesion, aggregation, and reaction release, for example, there are different platelet activation stimulators, which have the different mechanism of platelet aggregation and signal transduction, it is necessary to take a systematic study on the mechanism of Chinese herb and formulas for PBCRBS inhibiting platelet aggregation by different stimulators in the future and making further study on the signal transduction in platelet, now Chinese scholars [74] have made good study and publish the paper on the well-famous journal.

Proteomics technology has been successfully applied to platelet research, contributing to the emerging field of platelet proteomics which led to the identification of a considerable amount of novel platelet proteins, many of which have been further studied at functional level [76]. During the last 3 years, a rapid development of two-dimensional gel electrophoresis and mass spectrometry-based proteomic approaches has been used to profile alterations in platelet proteins [77–79]. Using differential proteomics of platelet, our previous studies found many different platelet proteins [37, 80] between CHD patients of blood stasis syndrome (BSS) and non-BSS patients, and healthy controls, which indicate that the platelet cytoskeleton may play an important role in the development in BSS of CHD. Based on

the Chinese medicine principle of "prescription and syndrome are corresponding", these platelet differential proteins may be the new target spots or target group. Getting intensive study on it, we believe that we can develop many new antiplatelet and antithrombolytic drugs possess definite curative effect and target, clear mechanism.

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Research Article

Systematic Review of Chinese Herbal Medicines for Preventing in-Stent Coronary Restenosis after Percutaneous Coronary Intervention

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Percutaneous coronary intervention (PCI) with stent placement is a standard treatment for coronary artery disease (CAD). In-stent restenosis after PCI remains a challenging clinical problem. In China, Chinese herbal medicines (CHMs) are widely used for preventing restenosis. This paper systematically reviewed the literature on the effectiveness and safety of CHMs in preventing restenosis after PCI in patients with CAD. Electronic databases were searched for randomized controlled trials that compared CHMs plus RWM with the same RWM plus placebo in preventing restenosis after PCI. A total of 52 trials (4905 patients) on 34 CHMs met the inclusion criteria and were analyzed. Ten trials had low risk of bias. Methodological quality of included trials was generally poor. Meta-analysis showed that at the end of at least 3 months' followup, CHMs plus RWM could significantly reduce restenosis rate, cardiac mortality, recurrence rate of angina, acute myocardial infarction, numbers of repeat PCI, and numbers of coronary artery bypass graft. Reported adverse events included gastrointestinal upset, granulocytopenia, and increased alanine transaminase (ALT). CHMs may help prevent restenosis, thus reducing cardiac mortality after PCI. Caution should be exercised in drawing a definitive conclusion due to the poor methodological quality of the trials reviewed.

1. Introduction

Coronary artery disease (CAD) is the single leading cause of death and disability in the world [1–3]. Percutaneous coronary intervention (PCI) with stent placement is the standard nonsurgical treatment for CAD, effective in relieving the symptoms of coronary ischemia [4]. But the main limitation of coronary stenting, in particular with bare-metal stents, is in-stent restenosis (ISR) [5]. This is the formation of scar tissue over the stent, which can cause the opened artery to narrow again. The risks of ISR include symptoms of coronary ischemia, often warranting repeat revascularization [6]. With the development and the universal application of the drug-eluting stent (DES), restenosis has been reduced from 10% to 50% for bare-metal stents [7] to <10% for DESs [8]. Despite this improvement, a major drawback of DESs has come to light involving late (after 30 days) and very late (after 1 year) stent thrombosis [9, 10]. In addition, studies have shown that DESs do not reduce late cardiac-related death and the incidence of myocardial infarction (MI) [11–14]. Antithrombotic therapy after PCI, consisting of lifelong aspirin and clopidogrel, is effective in reducing cardiac-related death, MI, and stroke [15]. But dual antiplatelet therapy also has limitations, as stopping prematurely significantly increases the risk of stent thrombosis, MI, and death [16]. Thus, treatment of ISR remains a challenging clinical issue.

In China, Chinese herbal medicines CHMs have a long history of integration with routine Western medical (conventional) interventions. Advancements in such interventions have spurred concomitant application of CHMs in attempts to enhance outcomes. In the past decade, CHMs have been tested in clinical trials as an adjunct therapy for preventing

ISR after PCI. CHMs appear to ameliorate ISR after PCI when used alone or combined with routine western medicine (RWM) [16, 17]. Pharmacologic studies have found that some (CHMs) can be administered to dilate coronary vessels, improve circulation, and remove blood stasis (Chinese medicine concept of blood circulation disturbance, resulting in slowing of blood flow, thrombosis, retained blood). Additionally, these CHMs possess antiinflammatory, immune response inhibition, anti-platelet aggregation, and antiperoxidative properties, as well as functions that inhibit proliferation and migration of vascular smooth muscle cells [18–21]. Reviews on the efficacy of CHMs in preventing restenosis after PCI have been published in Chinese [22–24]. But the evidence supporting or disproving the benefits of CHMs is not robust because of methodological deficiencies of those reviews. Our study presents a more vigorous attempt to examine the existing studies to draw more useful conclusions about the safety and efficacy of CHMs in preventing restenosis post-PCI.

2. Methods

2.1. Inclusion Criteria. Only randomized controlled trials (RCTs) were included regardless of being published or unpublished. We focused on trials with participants diagnosed with major angiographic criteria-documented [25] coronary artery disease who were eligible for PCI regardless of gender, age, and ethnic origin.

The treatment group in the trials was treated with any CHM, or CHM plus RWM with at least 1 month of therapy regardless of dosage. The control group was treated with the same RWM based on the Chinese Society of Cardiology guidelines for percutaneous transluminal coronary intervention [25], or the same RWM plus placebo. "Chinese herbal medicines" include extracts from mixtures of herbs, single herbs, Chinese prepared medicines, or a compound of herbs that is prescribed by a Chinese medicine practitioner [26].

Primary outcome measures were restenosis, cardiac death, and adverse events occurring in at least 3 months of followup. Secondary outcome measures included recurrent angina, acute myocardial infarction (AMI), revascularization, repeat PCI, coronary artery bypass graft (CABG), minimal luminal diameter (MLD), late loss of lumen (LLL), net gain in lumen diameter (NG), and lesion area net gain (LANG) after PCI, and quality of life during at least 1 month of followup.

2.2. Study Identification and Assessment in Included Studies. Two authors (G.-H. Zheng, H.-Y. Chen) independently identified studies through searches of the Cochrane Library (Issue 4, 2010), PubMed (January1966 to December 2010), Embase (January 1980 to December 2010), the China Biological Medicine Database (CBM, January 1980 to December 2010), Chinese Scientific Journal Database (VIP, January1989 to December 2010), China National Knowledge Information database (CNKI, January 1994 to December 2010) and Chinese Medical Citation Index (CMCI, January 1999 to December 2010) with free terms related to heart disease and CHM (e.g., "coronary heart disease" OR "coronary artery disease" OR "cardiovascular disease" OR "percutaneous coronary intervention" OR "stent" OR "stenosis" OR "restenosis" OR "CHD" OR "CVD" OR "CAD" OR "MI" "Chinese" OR "herbal" OR for English databases. The Chinese counterpart terms were used for Chinese databases. The reference list of each relevant article was searched for further studies. Unpublished literature was searched using Chinese Master's Theses Full-text Database (CMFD), China Doctor Dissertation Full-text Database (CDFD) and China Proceedings of Conference Full-text Database (CPCD).

Risk of bias in included studies was assessed using The Cochrane Collaboration's tool for assessing risk of bias. Six criteria were applied: adequate sequence generation, concealment of allocation, blinded of primary outcomes, adequately addressed incomplete outcome data, free from selective reporting, and free of other risk of bias [27]. In addition, we assessed the baseline characteristics between the comparison groups.

2.3. Data Extraction. Two authors (G.-H. Zheng, J.-F. Chu) independently selected those trials that met the inclusion criteria and extracted details on randomization, allocation concealment, blinding, intent to treat analysis, numbers lost to followup, patient demographics, methods, interventions, outcomes, and results. Missing data were obtained from the original authors when possible.

2.4. Data Analysis. Heterogeneity across studies was tested using a standard χ^2 test [28] and Higgins I^2 [29]. When heterogeneity was not significant ($P \geq 0.1$), the results were pooled using a fixed effect model and the Mantel-Haenszel test. Otherwise, a random effect model and the Dersimonian and Laird method were applied [30]. The results were reported as risk ratio (RR) with corresponding 95% confidence interval (CI) for dichotomous data. If continuous data were available, weighted mean difference or standardized mean difference was calculated [31]. All data were analyzed using the statistical software RevMan 5.0.1 (Oxford, England) of The Cochrane Collaboration, and all P values were two sided.

3. Results

3.1. Study Identification. Eligible literature was screened and identified (Figure 1). A total of 806 records were retrieved. Of these, full-text evaluation was conducted on 154 studies. This was followed by elimination of 102 studies: irrelevant to CHM ($n = 61$); irrelevant to the primary or secondary outcomes ($n = 10$); control group combined with another CHM ($n = 23$); duplicate publication ($n = 6$) [32–37]; primary outcomes <3 months' followup ($n = 1$)[38]; CHM treatment <1 month treatment ($n = 1$) [39]. Finally, 52 RCTs with a total of 4905 patients in treatment and control groups, fulfilled the inclusion criteria [40–91]. All studies were conducted in China from 1979 to 2010.

3.2. Characteristics of Included RCTs. Table 1 summarizes the characteristics of included studies. Average age of patients in the included studies ranged from 51.2 to 72 years. Each

Table 1: Characteristics of baseline, diagnosis, intervention, and outcomes in included studies.

Author, year	No. of patients (T/C)	Baseline characteristics of treatment and control groups	Intervention		CAD diagnostic guidelines	PCI type	Treatment course (mos)	Followup (mos)	Outcomes
			Control	Treatment					
An et al. 2009 [41]	100 (60/40)	Similar in age and sex between the two groups (narrative only)	RWM	RWM plus Yi qi hua yu fang	AG	Stent	3	3	Restenosis
Bao et al. 2005 [42]	76 (32/44)	Age, sex, hypertension, diabetes, hyperlipidemia, smoking, lesion distribution, stent number	RWM	RWM plus Ge gen shu tablet	AG	Stent	3	6	Restenosis
Chen et al. 2005 [43]	80 (40/40)	Age, sex, hypertension, diabetes, hyperlipidemia, smoking, lesion number	RWM	RWM plus Lei gong teng glycosides	AG	Stent	6	6	Restenosis, repeat intervention, MLD
Chen et al. 2005 [44]	86 (43/43)	Age, sex, CAD classification, results and characteristics of AG before PCI	RWM	RWM plus Dan shen injection	AG	Stent	1	6	Restenosis, death, recurrent angina, AMI
Chu et al. 2009 [45]	57 (28/29)	Age, sex, hypertension, diabetes, smoking, course of CAD, BMI, blockage number	RWM plus placebo	RWM plus Xue fu zhu yu capsule	AG	Stent	1	1	Quality of life
Cui et al. 2010 [46]	97 (51/46)	Age, sex, CAD classification, comorbidities, lesion number, stent number	RWM	RWM plus Liang xue shen ji fang	AG	Stent	2	6	Restenosis, death, recurrent angina, CABG, repeat PCI
Dan 2009 [47]	120 (60/60)	Age, sex, hypertension, diabetes, CAD classification, stent type	RWM	RWM plus Wen yang huo xue fang	AG	PTCA or stent	3	12	Recurrent angina and accompanying symptoms
Ding et al. 2007 [48]	55 (26/29)	Average age, sex, lesions, stent (narrative only)	RWM	RWM plus Xue zhi kang capsule	AG	Stent	6	6	Restenosis
Feng et al. 2006 [49]	61 (30/31)	Similar in baseline information between the two groups (narrative only)	RWM	RWM plus Tong xin fang	AG	PTCA	6	6	Restenosis, MLD
Fu 2009 [50]	80 (40/40)	Lesion characteristics and degree, stent type (narrative only)	RWM	RWM plus Fu fang dan shen pill	AG	Stent	6	6	Restenosis, recurrent angina, AMI, repeat intervention, CABG
Gu et al. 2007 [51]	60 (30/30)	Age, sex, complications, lesion number and type, stent type	RWM	RWM plus Xue fu zhu yu pill	AG	Stent	6	6	Restenosis, recurrent angina

Table 1: Continued.

Author, year	No. of patients (T/C)	Baseline characteristics of treatment and control groups	Intervention		CAD diagnostic guidelines	PCI type	Treatment course (mos)	Followup (mos)	Outcomes
			Control	Treatment					
Guo et al. 2009 [52]	80 (40/40)	Age, sex, complications, lesion number	RWM	RWM plus Dan shen tablet	AG	DES	6	6	Restenosis, death, CABG, repeat PCI
Han 2008 [53]	60 (30/30)	Age, sex, patient characteristics, hypertension, diabetes, smoking, lesion characteristics	RWM	RWM plus Xue sai tong capsule	AG	Stent	3	3	Recurrent angina
He et al. 2010 [54]	120 (60/60)	Age, sex, hypertension, diabetes, course of CAD, lesion characteristics	RWM	RWM plus Guan tong fang	AG	BMS	5	6	Restenosis, recurrent angina, MLD, LLS, NG, LANG
Kai et al. 2008 [55]	72 (36/36)	Age, sex, CAD characteristics, lesion characteristics (narrative only)	RWM	RWM plus Tong xin luo capsule	AG	Stent	6	6	Restenosis, LLS, NG, LANG
Li et al. 2004 [56]	57 (37/20)	Age, sex, hyperlipidemia, diabetes, smoking, lesion distribution and characteristics	RWM	RWM plus Shu xue tong injection	AG	Stent	1	6	Recurrent angina, MLD, LLS, NG
Li et al. 2010 [57]	80 (42/38)	Age, sex, lesion characteristics and degree, stent type (narrative only)	RWM	RWM plus Fu fang dan shen pill	AG	Stent	6	6	Recurrent angina, MI, repeat PCI, CABG
Li et al. 2005 [58]	52 (26/26)	Age, sex, hypertension, diabetes, smoking, lesion number	RWM	RWM plus Tong guan capsule	AG	PTCA or stent	3	6	Clinical effect of AP
Li et al. 2006 [59]	80 (40/40)	Age, hypertension, diabetes, lesion number and site	RWM	RWM plus Shi ni decoction	AG	Stent	6	6	Restenosis, repeat intervention, MLD
Li et al. 2008 [60]	121 (61/60)	Age, sex, hypertension, diabetes, smoking, lesion number and lsite	RWM	RWM plus Tong mai yi xin pill	AG	PTCA	6	6	Restenosis, recurrent angina
Li et al. 2004 [61]	70 (36/34)	Age, sex, complications, lesion number (narrative only)	RWM	RWM plus Xing mai fu tong decoction	Meeting guide	Stent	3	6	Restenosis
Li 2009 [62]	151 (76/75)	Age, sex, hypertension, diabetes, smoking, lesion characteristics	RWM	RWM plus Tong mai yi xin pill	AG	Stent	6	6	Restenosis, recurrent angina
Li and Niu 2008 [63]	59 (36/33)	Age, sex, hypertension, diabetes, smoking, lesion number and degree of blockage	RWM	RWM plus Jiang lian he ji	AG	PTCA	3	6	Restenosis, recurrent angina

Table 1: Continued.

Author, year	No. of patients (T/C)	Baseline characteristics of treatment and control groups	Intervention		CAD diagnostic guidelines	PCI type	Treatment course (mos)	Followup (mos)	Outcomes
			Control	Treatment					
Liu et al.2002 [64]	75 (30/45)	Age, sex, hypertension, diabetes, smoking, lesion number and degree of blockage	RWM	RWM plus Lei gong teng glycosides	AG	Stent	6	6	Restenosis, recurrent angina, MACE, MLD, LLS, NG, LANG
Liu et al. 2004 [65]	165 (63/102)	Age, sex, hyperlipidemia, diabetes,, lesion number and characteristics	RWM	RWM plus Chuan qiong qing	AG	PTCA or stent	6	6	Restenosis, death
Liu et al. 2007 [66]	60 (30/30)	Sex, hypertension, diabetes	RWM	RWM plus Yi qi huo xue fang	AG	PTCA	6	6	Recurrent angina
Lu et al. 2006 [67]	124 (62/62)	Age, sex, hypertension, diabetes, hyperlipidemia, lesion number, location and degree of stenosis, stent number	RWM	RWM plus Xiong shao capsule	AG	Stent	6	6	Restenosis, recurrent angina, AMI, CABG, MLD
Ma et al. 2009 [68]	92 (50/42)	Age, sex, complications, lesion number (narrative only)	RWM	RWM plus Guan mai zai tong	Meeting guide	Stent	3	3	Restenosis
Niu et al. 2003 [69]	36 (18/18)	Baseline patient characteristics (narrative only), hypertension, diabetes, smoking, characteristics and degree of stenosis	RWM	RWM plus Xue yu tong he ji	AG	PTCA or stent	6	6	Restenosis, recurrent angina
Qi et al. 2003 [70]	50 (30/20)	Baseline patient characteristics, lesion number and site (narrative only)	RWM	RWM plus Tong guan capsule	AG	PTCA	6	6	Restenosis, recurrent angina
Qiao et al. 2006 [71]	59 (30/29)	Baseline patient characteristics, hypertension, diabetes, smoking, lesion number and degree of stenosis	RWM plus placebo	RWM plus Tong guan capsule	AG	PTCA	1	1	Quality of life
Shi et al. 1997 [72]	73 (35/38)	Age, sex, hypertension, diabetes, lesion characteristics	RWM	RWM plus Xue fu zhu yu pill	AG	PTCA	6	6	Restenosis, recurrent angina
Wang et al. 2009 [73]	101 (54/47)	Age, sex, course of CAD, degree of blockage, complications	RWM	RWM plus Yi qi huo xue formula	AG	PTCA or stent	3	3	Angina symptoms
Wang et al. 2006 [74]	92 (62/30)	Age, sex, course of CAD (narrative only)	RWM	RWM plus Shan shen tong mai ji	AG	PTCA or stent	1	1	Angina symptoms
Wang et al. 2002 [75]	44 (20/24)	Baseline patient characteristics, CAD course, AG characteristics (narrative only)	RWM	RWM plus Shu xin yin	AG	Stent	6	6	Restenosis, recurrent angina, MI, MACE

Table 1: Continued.

Author, year	No. of patients (T/C)	Baseline characteristics of treatment and control groups	Intervention		CAD diagnostic guidelines	PCI type	Treatment course (mos)	Followup (mos)	Outcomes
			Control	Treatment					
Wang et al. 2003 [76]	44 (20/24)	Age, sex, hypertension, diabetes, smoking, AG characteristics	RWM	RWM plus Bu xin yin	AG	Stent	6	6	Restenosis, recurrent angina, AMI, revascularization
Wang and Gao 2004 [77]	94 (41/53)	Age, sex, hypertension, diabetes, smoking, lesion number and type	RWM	RWM plus Yi xin capsule	AG	Stent	6	6	Restenosis, recurrent angina, AMI, revascularization,
Wang et al. 2010 [78]	40 (20/20)	Baseline characteristics similar (narrative only)	RWM	RWM plus Ku lie zhi injection	AG	Stent	6	6	Restenosis, death, AMI
Xiao et al. 2007 [79]	132 (62/70)	Age, sex, hypertension, diabetes, arrhythmia, stent number	RWM	RWM plus Tong xin luo	AG	BMS	6	6	Restenosis, recurrent angina, death, AMI, MACE
Xu et al. 2000 [80]	65 (28/37)	Age, sex, hypertension, diabetes, lesion number and site	RWM	RWM plus Xiong shao capsule	AG	PTCA or stent	6	6	Restenosis, recurrent angina, AMI
Xu et al. 2002 [40]	108 (53/55)	Age, sex, CAD classification	RWM	RWM plus Xiong shao capsule	AG	PTCA or stent	6	6	Restenosis, recurrent angina, AMI, CABG, revascularization
Chen et al. 2006 [81]	314 (157/157)	Age, sex, hypertension, diabetes, lesion number and site	RWM plus placebo	RWM plus Xiong shao capsule	AG	PTCA or stent	6	6	Restenosis, recurrent angina, death, CABG
Yao et al. 2006 [82]	76 (38/38)	Age, sex, lesion number and site	RWM	RWM plus Tong xin luo	AG	PTCA	6	6	Restenosis, recurrent angina, LLS, NG, LANG
Yu et al. 1998 [84]	84 (43/41)	Baseline patient characteristics, hypertension, diabetes, smoking, lesion number and degree of blockage	RWM	RWM plus Xue fu zhu yu pill	AG	Stent	6	6	Restenosis, recurrent angina and accompanying symptoms
Z. A. Yu and S. Y. Yu2006 [85]	82 (42/40)	Age, sex, course of CAD, lesion number and site and degree of blockage	RWM	RWM plus Dan shen tablets	AG	Stent	6	6	Restenosis, recurrent angina, AMI, death

Table 1: Continued.

Author, year	No. of patients (T/C)	Baseline characteristics of treatment and control groups	Intervention		CAD diagnostic guidelines	PCI type	Treatment course (mos)	Followup (mos)	Outcomes
			Control	Treatment					
Yi et al. 2005 [83]	40 (20/20)	Age, sex, lesion numbers and sites, history of hypertension and diabetes	RWM	RWM plus Guan tong jian ji	AG	PTCA or stent	6	6	Restenosis, recurrent angina
Zhang et al. 2007 [88]	63 (33/30)	Age, sex, hypertension, diabetes, smoking, lesion site, angina,	RWM	RWM plus Shen mai gua lu shi xiao san	AG	Stent	1	4	Angina symptoms
Zhang et al. 2006 [87]	500 (250/250)	Age, sex, hypertension, diabetes, hyperlipidemia, BMI, degree of blockage (narrative only)	RWM	RWM plus Wen tong jian	AG	PTCA or stent	6	6	Restenosis, recurrent angina
Zhao et al. 2009 [89]	68 (35/33)	Age, sex, hypertension, diabetes, stenosis and lesion diameter of vascular lesion	RWM	RWM plus Shen qi tang	AG	Stent	6	6	Restenosis
Zheng et al. 2004 [86]	66 (36/30)	Baseline patient characteristics, lesion number and characteristics (narrative only)	RWM	RWM plus Fu fang dan shen pill	AG	Stent	6	6	Restenosis, recurrent angina, LLS, NG, LANG
Zhou and Guo 2007 [90]	136 (70/66)	Baseline patient characteristics, lesion characteristics and degree of blockage, stent type (narrative only)	RWM	RWM plus Tong xin luo	AG	Stent	6	6	Restenosis, recurrent angina, AMI, CABG, repeat PCI
Zhu et al. 2009 [91]	138 (70/68)	Age, sex, hypertension, diabetes, hyperlidemia, smoking, lesion number and degree of blockage, stent number	RWM	RWM plus Dan hong tong mai capsule	AG	DES	6	6	Restenosis, recurrent angina, death, AMI, MACE, CABG, repeat PCI

AG = angiography; BMI = body mass index; BMS = bare metal stent; CABG = coronary artery bypass graft; CAD = coronary artery disease; DES = drug eluting stent; LANG = lesion area net gain; LLL = late loss of lumen; MLD = minimum lumen diameter; NG = net gain in lumen diameter; PCI = percutaneous coronary intervention; PTCA = percutaneous transluminal coronary angioplasty; RWM = routine Western medicine; T/C = treatment/control; TCM = traditional Chinese medicine.

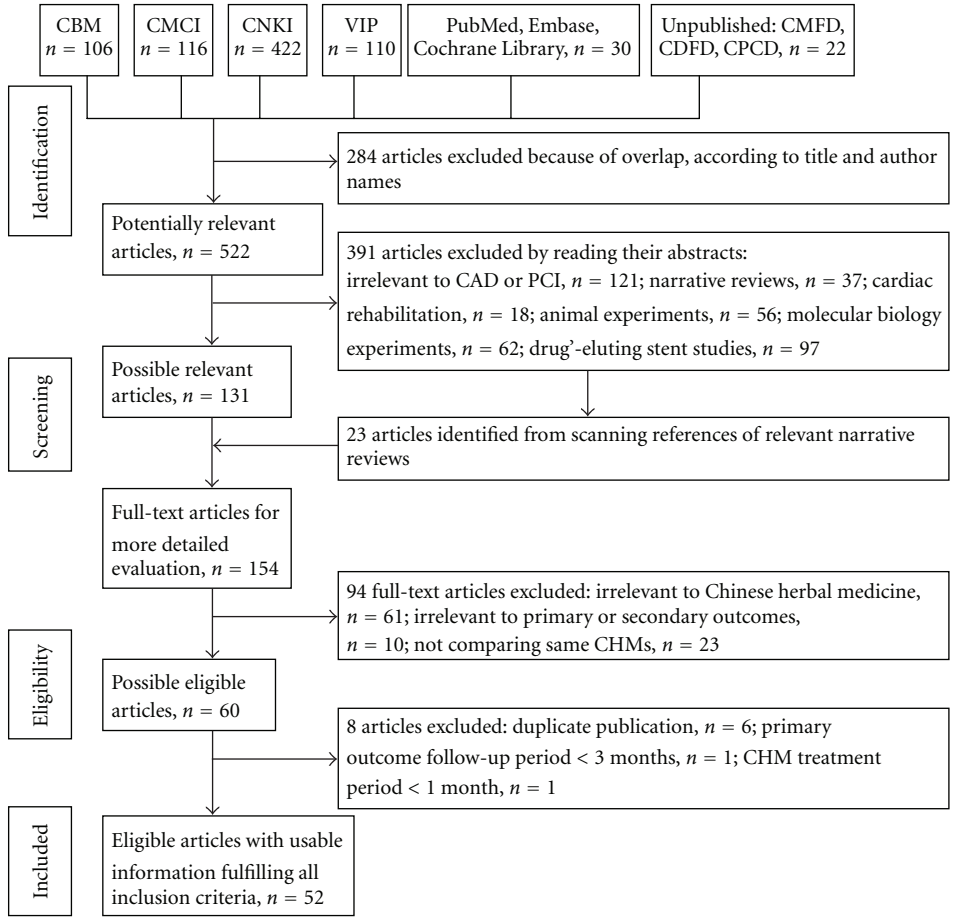

Figure 1: Literature search and selection. Abbreviations: CBM: China Biological Medicine Database; CDFD: China Doctor Dissertation Full-Text Database; CMCI: Chinese Medical Citation Index; CMFD: Chinese Master's Theses Full-Text Databases; CNKI: China National Knowledge Infrastructure; CPCD: China Proceedings of Conference Full-text Database; VIP: VIP Database.

trial had more males than females. The diagnostic criteria of CAD were mainly based on coronary angiography criteria. All patients successfully underwent PCI.

Four trials [45, 67, 71, 81] were randomized double blind, and placebo controlled comparing RWM plus CHM *versus* the same RWM plus placebo. The remaining trials were designed comparing CHM plus RWM *versus* the same RWM alone. The dosage and types of RWM were prescribed according to Chinese Society of Cardiology guideline recommendations [25]. In the trials, 34 kinds of CHMs were used, and the period of treatment with a CHM was at least 1 month. Followup after PCI ranged from 3 to 12 months, with 6 months in the majority of studies.

Restenosis was assessed using angiography in 40 trials. Of these, 10 studies reported angiography assessments at the end of at least 3 months after PCI. Adverse events caused by CHMs were reported in 21 studies, but none of the studies underwent statistical analysis. Recurrent angina was reported in 33 studies. Major cardiac events were reported in 16 trials, and 10 trials reported cardiac death after followup of at least 6 months. Only 2 studies assessed quality of life using the Short-Form 36 (SF-36) Health Survey and Seattle Angina Questionnaire (SAQ) at the end of 1 month after PCI.

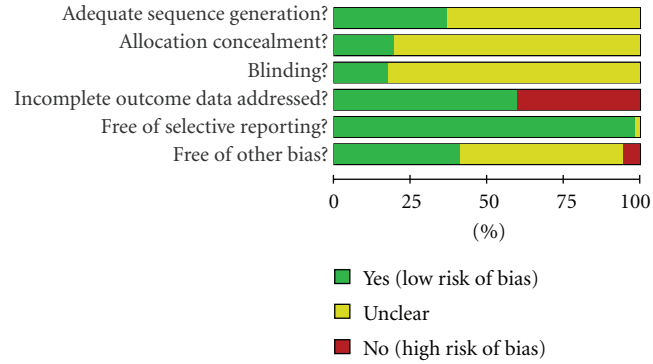

Figure 2: Risk of bias graph depicting proportions of studies with each judgment.

3.3. Methodological Quality of Included RCTs. Risk of bias in the studies is shown in Figure 2. Of the 52 studies, 19 studies reported randomization using random number tables or computer random number generator such as SAS. The remaining 33 studies reported "randomly allocating" participants, but the method of randomization was not

Table 2: Risk ratios of Chinese herbal medicines administered to prevent restenosis after PCI.

Chinese herbal medicine	Number of studies	Number of patients	Risk ratio (RR)	95% CI of RR
Bu xin yin plus RWM *versus* RWM	1 [76]	44	0.69	0.23~2.01
Chuan qiong qing plus RWM *versus* RWM	1 [65]	165	0.51	0.19~1.31
Dan hong tong mai capsule plus RWM *versus* RWM	1 [91]	103	0.35	0.10~1.23
Dan shen plus RWM *versus* RWM	5 [44, 50, 52, 85, 86]	388	0.27	0.14~0.52
Ge gen shu plus RWM *versus* RWM	1 [42]	76	0.23	0.06~0.95
Guan mai zhai tong decoction plus RWM *versus* RWM	1 [68]	92	0.32	0.09~1.11
Guan tong formula plus RWM *versus* RWM	1 [54]	120	0.43	0.18~1.04
Jia wei xue fu zhu yu particle plus RWM *versus* RWM	1 [51]	40	0.75	0.27~2.07
Jiang lian he ji plus RWM *versus* RWM	1 [63]	26	0.57	0.11~2.87
Ku lie zhi injection plus RWM *versus* RWM	1 [78]	40	1.00	0.07~14.9
Lei gong teng plus RWM *versus* RWM	2 [43, 64]	133	0.75	0.40~1.40
Liang xue shen ji formula plus RWM *versus* RWM	1 [46]	97	0.45	0.12~1.70
Shen qi decoction plus RWM *versus* RWM	1 [89]	68	0.47	0.09~2.40
Shu xin yin plus RWM *versus* RWM	1 [75]	44	0.69	0.23~2.01
Si ni tang plus RWM *versus* RWM	1 [59]	63	0.55	0.14~2.09
Tong guan capsule plus RWM *versus* RWM	1 [70]	25	0.67	0.26~1.72
Tong mai yu xin plus RWM *versus* RWM	2 [60, 62]	272	0.39	0.21~0.70
Tong xin fang plus RWM *versus* RWM	1 [49]	61	1.03	0.23~4.72
Tong xin luo plus RWM *versus* RWM	4 [39, 79, 82, 90]	328	0.34	0.20~0.59
Wen tong jian plus RWM *versus* RWM	1 [87]	467	0.03	0.00~0.49
Xue fu zhu yu pin plus RWM *versus* RWM	2 [72, 84]	157	0.61	0.32~1.16
Xue yu tong he ji plus RWM *versus* RWM	1 [69]	36	1.0	0.16~6.35
Xue zhi kang plus RWM *versus* RWM	1 [48]	55	0.30	0.09~0.99
Xing mai fu tong decoction plus RWM *versus* RWM	1 [61]	70	0.71	0.17~2.94
Xiong shao capsule plus RWM *versus* RWM	2 [40, 80]	173	0.43	0.23~0.81
Yi qi hua yu formula plus RWM *versus* RWM	1 [41]	100	0.13	0.02~1.10
Yi xin capsule plus RWM *versus* RWM	1 [77]	94	0.50	0.27~0.91
Self-prepared guantong decoction plus RWM *versus* RWM	1 [83]	30	0.40	0.16~1.00
Overall (CHMs plus RWM versus RWM)	**38**	**3367**	**0.43**	**0.36~0.51**
Xiong shao capsule plus RWM *versus* RWM plus placebo	2 [67, 81]	438	0.46	0.27~0.76
Overall (CHMs plus RWM versus RWM plus placebo)	**2**	**438**	**0.46**	**0.27~0.76**

CHM = Chinese herbal medicine; CI = confidence interval; RWM = routine Western medicine.

described. Allocation concealment in 10 of the 52 studies was by sealed, opaque envelopes. In 9 trials, participants and/or outcome assessors were blinded. In most studies, data collection was clearly described and reported, so we judged them as free of selective reporting outcomes. We graded 28 of the 52 studies as "unclear" in terms of free of other bias because there was no evidence of statistical testing of baseline characteristics between comparison groups or the outcome data were incomplete. As a whole, 10 studies [44–46, 53, 67, 71, 75, 76, 81, 85] had low risk of bias with high methodological quality. Most studies were found to be high risk of bias with low methodological quality.

3.4. Measures of Effect

3.4.1. Restenosis. In 40 studies on 29 Chinese herbal medicines (CHMs) comprised of 3805 patients, restenosis was assessed using coronary angiography after PCI with at least 3 months' followup (Table 2). Compared to RWM alone, the rate of restenosis was clearly low in patients administered the same RWM plus CHMs. Of 29 CHMs, 9 CHMs (including *Xiong shao capsule, Dan shen, Ge gen shu, Tong mai yu xin pill, Tong xin luo, Wen tong jian, Xue zhi kang, Yi xin capsule,* and *self-prepared guan tong decoction*) showed significant ability in reducing restenosis. In particular, two studies [67, 81] on *Xiongshao* capsule (438 patients) appeared to have reliable results because their designs were randomized, double blinded, and placebo controlled. The overall risk ratio (RR) of restenosis was 0.46 with 95%CI = 0.27 to 0.76 compared to placebo.

3.4.2. Cardiac Mortality. In 10 studies on 9 CHMs, comprised of 628 patients in the treatment group and 667 patients in the control group, cardiac death was included as a measure of effect at the end of 6 months of follow-up after PCI. A pooled analysis of CHM plus RWM versus the same RWM found a statistically significant decrease in risk of cardiac death associated with CHM, though we did not

Table 3: Risk ratios of Chinese herbal medicines administered to prevent cardiac mortality after PCI.

Chinese herbal medicine	Number of studies	Number of patients	Risk ratio (RR)	95% CI of RR
Bu xin yin plus RWM *versus* RWM	1 [76]	44	0.4	0.02~9.24
Chuan qiong qing plus RWM *versus* RWM	1 [65]	165	0.23	0.01~4.38
Dan hong tong mai capsule plus RWM *versus* RWM	1 [91]	138	2.92	0.12~70.35
Dan shen plus RWM *versus* RWM	2 [44, 85]	168	0.33	0.03~3.07
Ku lie zhi injection plus RWM *versus* RWM	1 [78]	40	0.33	0.01~7.72
Liang xue shen ji formula plus RWM *versus* RWM	1 [46]	97	0.15	0.02~1.2
Shu xin yin plus RWM *versus* RWM	1 [75]	44	0.40	0.02~9.24
Tong xin luo plus RWM *versus* RWM	1 [79]	132	0.38	0.02~9.06
Wen tong jian plus RWM *versus* RWM	1 [87]	467	0.07	0.00~1.32
Overall (CHMs plus RWM versus RWM)	**10**	**1295**	**0.27**	**0.11~0.63**

CHM = Chinese herbal medicine; CI = confidence interval; RWM = routine Western medicine.

find a significant difference upon subgroup analysis based on different CHMs (Table 3).

3.4.3. Adverse Effects. Adverse effects due to CHMs were not mentioned in 30 studies. In 14 studies, the authors reported that there were no noteworthy adverse events. The remaining 8 studies on 7 CHMs reported adverse events, including gastrointestinal upset, granulocytopenia, elevated alanine transaminase (ALT), aphthous stomatitis, skin pruritus, papular urticaria. Most adverse events were not severe and disappeared without special treatment. With the CHM *Lei gong teng,* adverse events in the treatment group were higher than those in the control group (RR = 37.02, 95%CI = 2.29 to 597.88). With CHM *Si ni tang,* adverse events in the treatment group were lower than that of the control group (RR = 0.38, 95%CI = 0.21 to 0.69). There was no statistically significant difference between the two groups in the remaining 5 CHMs. Pooled analysis could not be done in 8 studies on 7 CHMs because of heterogeneity among the studies.

3.4.4. Recurrent Angina. Recurrent angina 6 months post-PCI was reported as a measure of effect in 33 studies comprised of 3375 patients (Table 4). The rate of recurrent angina in the RWM plus CHM group was lower than that of the same RWM alone group for the CHMs *Dan hong tong mai capsule, Dan shen, Guan tong formula, Lei gong teng, Tong mai yi xin pill, Tong xin luo, Wen tong jian, Xiong shao capsule, Xue fu zhu yu pill, Xue zhi kang, Wen yang huo xue formula*. A statistically significant difference was not observed for each of the remaining CHMs. Pooled results of these trials showed recurrent angina was significantly decreased. For *Xiong shao capsule,* risk of recurrent angina was clearly decreased in the CHM plus RWM group than in the same RWM plus placebo control group.

3.4.5. Major Adverse Cardiac Effects

Acute Myocardial Infarction (AMI). In 15 studies on 9 CHMs, involving 1551 patients, acute myocardial infarction was reported as a measure of effect 6 months after PCI (Table 5). No statistically significant difference was found in each study, but meta-analysis did show decreasing incidence of AMI in the CHM plus RWM group (RR = 0.22, 95%CI = 0.1 to 0.49).

Revascularization. Revascularization after the index PCI was reported as a measure of effect in 6 studies on 4 CHMs (*Shu xin yin, Bu xin yin, Yi xin capsule*, *and Xiong shao capsule*) comprised of 716 patients (Table 5). Numbers of revascularization in the CHM plus RWM group was not significantly lower than that in RWM alone group (RR = 0.64, 95% CI = 0.38 to 1.08). However, compared to RWM plus placebo, administering CHM *Xiong shao capsule* plus the same RWM (2 studies with 426 patients) clearly reduced the numbers of revascularization (RR = 0.48, 95%CI = 0.30 to 0.78).

Repeat PCI. Repeat PCI was reported as a measure of effect in 9 studies, which involved 8 CHMs with 807 patients (Table 5). Numbers of repeat PCI in the CHM plus RWM group was lower than that of the RWM alone group for *Fu fang dan shen pill* (RR = 0.14, 95%CI = 0.03 to 0.58) and *Tong xin luo* (RR = 0.16, 95%CI = 0.04 to 0.68). The remaining CHMs showed no statistically significant difference between the CHM plus RWM group and RWM alone group. But the overall result of their meta-analyses showed an obvious statistical difference between CHMs plus RWM *versus* RWM groups (RR = 0.37, 95%CI = 0.23 to 0.59).

Coronary Artery Bypass Graft. Coronary artery bypass graft (CABG) after index PCI was reported as a measure in 9 studies on 7 CHMs with 1141 patients (Table 5). CHM plus RWM was found to markedly reduce the risk of CABG over the same RWM alone (RR = 0.29, 95%CI = 0.09 to 0.96). But compared to RWM plus placebo, CHM *Xiong shao capsule* plus RWM could not reduce risk of CABG (RR = 0.20, 95%CI = 0.02 to 1.68).

3.4.6. Effect on Angina. Effect on angina was reported as a measure in 6 studies with 522 patients (Table 6). Two studies reported a followup period of 1 month, 2 studies reported a followup of 3 months, and 2 studies reported a followup of 6 months after index PCI. Angina improvement

Table 4: Risk ratios of Chinese herbal medicines administered to prevent recurrent angina after PCI.

Chinese herbal medicine	Number of studies	Number of patients	Risk ratio (RR)	95% CI of RR
Bu xin yin plus RWM *versus* RWM	1 [76]	44	0.60	0.33~1.10
Dan hong tong mai capsule plus RWM *versus* RWM	1 [91]	138	0.24	0.07~0.82
Dan shen plus RWM *versus* RWM	5 [44, 50, 57, 85, 86]	390	0.24	0.15~0.41
Guan tong formula plus RWM *versus* RWM	1 [54]	120	0.27	0.08~0.93
Jia wei xue fu zhu yu particle plus RWM *versus* RWM	1 [51]	60	0.45	0.18~1.15
Jiang lian he ji plus RWM *versus* RWM	1 [63]	69	0.71	0.30~1.70
Lei gong teng plus RWM *versus* RWM	1 [64]	75	0.39	0.17~0.94
Liang xue shen ji formula plus RWM *versus* RWM	1 [46]	97	0.52	0.16~1.65
Shu xue tong injection plus RWM *versus* RWM	1 [56]	43	0.37	0.11~1.25
Shu xin yin plus RWM *versus* RWM	1 [75]	44	0.60	0.33~1.10
Tong guan capsule plus RWM *versus* RWM	1 [70]	50	0.67	0.22~2.01
Tong mai yi xin plus RWM *versus* RWM	2 [54]	272	0.41	0.26~0.65
Tong xin luo plus RWM *versus* RWM	3 [79, 82, 90]	344	0.27	0.13~0.54
Wen tong jian plus RWM *versus* RWM	1 [87]	467	0.30	0.17~0.52
Wen yang huo xue formula plus RWM *versus* RWM	1 [47]	120	0.53	0.38~0.75
Xue fu zhu yu pin plus RWM *versus* RWM	2 [72, 84]	157	0.44	0.25~0.78
Xue yu tong he ji plus RWM *versus* RWM	1 [69]	36	0.67	0.23~1.97
Xue zhi kang plus RWM *versus* RWM	1 [48]	60	0.38	0.16~0.94
Xiong shao capsule plus RWM *versus* RWM	2 [40, 80]	173	0.48	0.31~0.75
Yi qi hua yu formula plus RWM *versus* RWM	1 [41]	60	0.44	0.15~1.29
Yi xin capsule plus RWM *versus* RWM	1 [77]	94	0.46	0.20~1.05
Self-prepared guan tong decoction plus RWM *versus* RWM	1 [83]	40	0.33	0.11~1.05
Overall (CHM plus RWM versus RWM)	**31**	**2953**	**0.40**	**0.35~0.47**
Xiong shao capsule plus RWM *versus* RWM plus placebo	2 [67, 81]	422	0.25	0.17~0.37
CHM plus RWM versus RWM plus placebo	**2**	**422**	**0.25**	**0.17~0.37**

CHM = Chinese herbal medicine; CI = confidence interval; RWM = routine Western medicine.

in these studies was defined as "significant improvement," "improvement," and "no improvement" based on Chinese herbal medicine clinical research guidelines [92]. To permit overall analysis, we converted these outcomes into dichotomous data. We grouped together "significant improvement" and "improvement" as "effective," and "no improvement" as "ineffective." There was no statistically significant difference between CHM plus RWM *versus* RWM groups for every CHM except for *Shen mai gua lou shi xiao powder* (RR = 1.55, 95%CI = 1.11 to 2.17). Furthermore, the results were unsuitable for meta-analysis pooling due to heterogeneity (I^2 = 60%).

3.4.7. Angiographic Measurements. Follow-up angiography was done on diffuse ISR 6 months after index PCI in 10 studies on 8 CHMs with 811 patients. Baseline information shows that the mean minimal luminal diameter (MLD) before and immediately after index PCI and gain in luminal diameter following stent placement were comparable between the comparison groups.

Minimum Lumen Diameter (MLD). Minimum lumen diameter is defined as smallest diameter of the lesion area being treated [93]. MLD was measured in 7 CHM studies (Table 7). The CHM plus RWM groups showed significant MLD improvement over the same RWM alone or plus placebo for *Fu fang dan shen capsule* (MD (mean difference) = 0.05 mm, 95%CI = 0.04 to 0.26 mm), *Guan tong formula* (MD = 0.21 mm, 95%CI = 0.06 to 0.36 mm) and *Xiong shao capsule* (placebo control; MD = 0.49 mm, 95%CI = 0.12 to 0.86 mm). No significant difference was evident in the studies on *Lei gong teng, Tong xin fang, Si ni tang*. But in pooling the results of those trials, there was a statistically significant difference (MD = 0.15 mm, 95%CI = 0.05 to 0.24 mm), with patients receiving CHMs more likely to have increased MLD than those in the control group.

Late Loss of Lumen (LLL). Late loss of lumen is defined as the decreased amount in lumen diameter after PCI, which is calculated by subtracting MLD at followup from MLD immediately post-procedure [93]. LLL was measured in 6 studies on 5 CHMs after 6 months' followup (Table 7). Five studies on 4 CHMs (*Guan tong formula, Tong xin luo, Shu xue tong injection,* and *Lei gong teng*) reported significantly better results for reducing late loss of lumen in the treatment group over the control group. There was no significant difference in the study on *Fu fang dan shen capsule.* The pooled results showed significant difference (MD = −0.24 mm, 95%CI = −0.34 to −0.15 mm).

Net Gain in Lumen Diameter (NG). Net gain in lumen diameter is defined as the net increase in MLD after PCI [93].

Table 5: Risk ratios of Chinese herbal medicines administered to prevent MACE after PCI.

Chinese herbal medicine	Number of studies	Number of patients	Risk ratio (RR)	95% CI of RR
Acute myocardial infarction				
Bu xin yin plus RWM *versus* RWM	1 [76]	44	0.24	0.01~4.69
Dan hong tong mai capsule plus RWM *versus* RWM	1 [91]	138	0.14	0.01~2.64
Dan shen plus RWM *versus* RWM	2 [44, 85]	168	0.14	0.02~1.11
Fu fang dan shen pill plus RWM *versus* RWM	2 [50, 57]	156	0.32	0.03~2.99
Ku lie zi injection plus RWM *versus* RWM	1 [78]	40	0.33	0.01~7.72
Shu xin yin plus RWM *versus* RWM	1 [75]	44	0.24	0.01~4.69
Tong xin luo plus RWM *versus* RWM	2 [79, 90]	268	0.25	0.03~2.22
Xiong shao capsule plus RWM *versus* RWM	2 [40, 80]	173	0.14	0.02~1.06
Yi xin capsule plus RWM *versus* RWM	1 [77]	94	0.65	0.06~6.88
Overall (CHMs plus RWM versus RWM)	**13**	**1125**	**0.22**	**0.10~0.49**
Xiong shao capsule plus RWM *versus* RWM plus placebo	2 [73]	426	0.59	0.08~4.41
Overall (CHMs plus RWM versus RWM plus placebo)	**2**	**426**	**0.59**	**0.08~4.41**
Revascularization				
Shu xin yin plus RWM *versus* RWM	1 [75]	44	0.69	0.23~2.01
Bu xin yin plus RWM *versus* RWM	1 [76]	44	0.69	0.23~2.01
Yi xin capsule plus RWM *versus* RWM	1 [77]	94	0.43	0.02~10.26
Xiong shao capsule plus RWM *versus* RWM	1 [40]	108	0.62	0.30~1.30
Overall (CHMs plus RWM versus RWM)	**4**	**290**	**0.64**	**0.38~1.08**
Xiong shao capsule plus RWM *versus* RWM plus placebo	2 [67, 81]	426	0.48	0.30~0.78
Overall (CHMs plus RWM versus RWM plus placebo)	**2**	**426**	**0.48**	**0.30~0.78**
Repeat PCI				
Dan hong tong mai capsule plus RWM *versus* RWM	1 [91]	138	0.39	0.08~1.94
Dan shen plus RWM *versus* RWM	1 [52]	80	1.0	0.21~4.66
Fu fang dan shen pill plus RWM *versus* RWM	2 [50, 57]	156	0.14	0.03~0.58
Ku lie zi injection plus RWM *versus* RWM	1 [78]	40	0.33	0.01~7.72
Lei gong teng plus RWM *versus* RWM	1 [43]	80	0.56	0.20~1.51
Liang xue shen ji formula plus RWM *versus* RWM	1 [46]	97	0.54	0.14~2.14
Si ni tang plus RWM *versus* RWM	1 [59]	80	0.56	0.20~1.51
Tong xin luo plus RWM *versus* RWM	1 [90]	136	0.16	0.04~0.68
Overall (CHMs plus RWM versus RWM)	**9**	**807**	**0.37**	**0.23~0.59**
CABG				
Dan hong tong mai capsule plus RWM *versus* RWM	1 [91]	138	0.14	0.01~2.64
Dan shen plus RWM *versus* RWM	1 [52]	80	Not	estimable
Fu fang dan shen pill plus RWM *versus* RWM	2 [50, 57]	156	0.32	0.03~2.99
Liang xue sheng ji formula plus RWM *versus* RWM	1 [46]	97	0.30	0.01~7.22
Tong xin luo plus RWM *versus* RWM	1 [90]	136	0.19	0.01~3.86
Xiong shao capsule plus RWM *versus* RWM	1 [40]	108	1.04	0.07~16.2
Overall (CHMs plus RWM versus RWM)	**7**	**715**	**0.29**	**0.09~0.96**
Xiong shao capsule plus RWM *versus* RWM plus placebo	2 [67, 81]	426	0.20	0.02~1.68
Overall (CHMs plus RWM versus RWM plus placebo)	**2**	**426**	**0.20**	**0.02~1.68**

CABG = coronary artery bypass graft; CHM = Chinese herbal medicine; CI = confidence interval; PCI = percutaneous coronary intervention; RWM = routine Western medicine; MACE = major adverse cardiac event.

NG after 6 months' followup was measured in 4 studies on 4 CHMs (Table 7). Significant difference was found between the treatment group plus RWM and the control group with the same RWM alone for *Guan tong formula* (MD = 0.21 mm, 95%CI = 0.08 to 0.34 mm) and *Shu xue tong* injection (MD = 0.25 mm, 95%CI = 0.09 to 0.41 mm). The pooled results of meta-analyses, suggested that CHMs plus RWM increased net gain of lumen compared to using RWM alone (MD = 0.2 mm, 95%CI = 0.11 to 0.29 mm). These results are displayed in Table 7.

Lesion Area Net Gain (LANG) . Lesion area net gain is defined as the net increase in lumen area before PCI and at followup after PCI [53, 55, 64, 82, 86]. Five studies on

Table 6: Risk ratios of Chinese herbal medicines administered to prevent angina after PCI.

Chinese herbal medicine	Number of studies	Number of patients	Risk ratio (RR)	95% CI of RR
Followup 1 month				
Shan shen tong mai he ji plus RWM *versus* RWM	1 [74]	102	1.18	0.95~1.45
Shen mai gua lou shi xiao power plus RWM *versus* RWM	1 [88]	63	1.55	1.11~2.17
*Overall (CHMs plus RWM versus RWM)**	2	165	1.31	1.00~1.72
Followup 3 months				
Tong guan capsule plus RWM *versus* RWM	1 [58]	52	1.26	0.98~1.64
Yi qu huo xue formula plus RWM *versus* RWM	1 [73]	101	1.07	0.98~1.16
*Overall (CHMs plus RWM versus RWM)**	2	153	1.13	0.93~1.36
Followup 6 months				
Xue fu zhu yu pill plus RWM *versus* RWM	1 [84]	84	1.02	0.57~1.84
Wen yang huo xue formula plus RWM *versus* RWM	1 [47]	120	1.05	0.99~1.12
Overall (CHMs plus RWM *versus* RWM)	2	204	1.05	0.99~1.12
*Total overall (CHMs plus RWM versus RWM)**	**6**	**522**	**1.13**	**1.02~1.26**

* Random effect model
CHM = Chinese herbal medicine; CI = confidence interval; PCI = percutaneous coronary intervention; RWM = routine Western medicine.

Table 7: Effect of Chinese herbal medicines on angiographic characteristics after PCI.

Chinese herbal medicine	Number of studies	Number of patients	Mean difference	95% CI of mean difference
Minimum lumen diameter (mm)				
Fu fang dan shen plus RWM *versus* RWM	1 [86]	47	0.05	0.04~0.26
Guan tong formula plus RWM *versus* RWM	1 [54]	120	0.21	0.06~0.36
Lei gong teng plus RWM *versus* RWM	2 [43, 64]	119	0.11	−0.13~0.36
Si ni tang plus RWM *versus* RWM	1 [59]	63	0.26	−0.03~0.55
Tong xin fang plus RWM *versus* RWM	1 [49]	61	−0.10	−0.44~0.24
Overall (CHMs plus RWM versus RWM)	**6**	**410**	**0.15**	**0.05~0.24**
Xiong shao capsule plus RWM *versus* RWM plus placebo	1 [67]	97	0.49	0.12~0.86
CHMs plus RWM versus RWM plus placebo	**1**	**97**	**0.49**	**0.12~0.86**
Late loss of lumen (mm)				
Fu fang dan shen plus RWM *versus* RWM	1 [86]	47	0.00	−0.22~0.22
Guan tong formula plus RWM *versus* RWM	1 [54]	120	−0.16	−0.28~ −0.04
Lei gong ten plus RWM *versus* RWM	1 [64]	53	−0.30	−0.48~ −0.12
Shu xue tong injection plus RWM *versus* RWM	1 [56]	64	−0.29	−0.44~ −0.14
Tong xin luo plus RWM *versus* RWM	2 [55, 82]	148	−0.33	−0.46~ −0.21
*Overall (CHMs plus RWM versus RWM)**	**6**	**432**	**−0.24**	**−0.34~ −0.15**
Net gain in lumen diameter (mm)				
Fu fang dan shen plus RWM *versus* RWM	1 [86]	47	0.04	−0.18~0.26
Guan tong formula plus RWM *versus* RWM	1 [54]	120	0.21	0.08~0.34
Lei gong tang plus RWM *versus* RWM	1 [64]	53	0.28	−0.03~0.59
Shu xue tong injection plus RWM *versus* RWM	1 [56]	64	0.25	0.09~0.41
Overall (CHMs plus RWM versus RWM)	**4**	**284**	**0.20**	**0.11~0.29**
Lesion area net gain (mm^2)				
Fu fang dan shen plus RWM *versus* RWM	1 [86]	47	0.06	−0.54~0.66
Guan tong formula plus RWM *versus* RWM	1 [54]	120	0.66	0.25~1.07
Lei gong teng plus RWM *versus* RWM	1 [64]	53	0.66	0.41~0.91
Tong xin luo plus RWM *versus* RWM	2 [55, 82]	148	0.99	0.64~1.33
Overall (CHMs plus RWM versus RWM)	**5**	**368**	**0.69**	**0.52~0.87**

* Random effect model
CHM = Chinese herbal medicine; CI = confidence interval; PCI = percutaneous coronary intervention; RWM = routine Western medicine.

4 CHMs measured LANG at 6 months' followup after PCI (Table 7).The CHMs *Guan tong formula, Tong xin luo,* and *Lei gong teng* demonstrated a significant improvement in LANG. The same result was not found with the fourth CHM, *Fu fang dan shen* capsule. The pooled results of meta-analysis were statistically significant (MD = 0.69 mm^2, 95%CI = 0.52 to 0.87 mm^2).

3.4.8. Quality of Life. Two studies [45, 71] with randomized, double-blind and placebo-control design reported quality of life 1 month after PCI using the Chinese versions of the Seattle Angina Questionnaire (SAQ) and Short-Form 36 (SF-36) Health Survey. The SAQ is a 19-item self-administered questionnaire that assesses how patients with CHD are fairing in five dimensions: physical limitation (PL), angina stability (AS), angina frequency (AF), treatment satisfaction (TS), and disease perception (DP). The results of comparing *Xue fu zhu yu capsule* plus RWM with the same RWM plus placebo suggested that receiving CHM had a positive effect on AS (MD = 12.13,95%CI = 4.61 to 19.65) and TS (MD = 12.13, 95%CI = 6.19 to 17.87). However, in the study with *Tong guan capsule*, the results were contrary to that of *Xue fu zhu yu capsule:* AS (MD = −36.2, 95%CI = −48.97 to −23.43), PL (MD = −11.32, 95%CI = −19.38 to −3.26), AF (MD = −35.68, 95%CI = −50.16 to −20.6), TS (MD = −25.03, 95%CI = −33.93 to −16.13), and DP (MD = −9.79, 95%CI = −35.95 to −16.38). Pooled results were not available because of heterogeneity and the $I^2(\%)$ ranging from 88% to 98%.

SF-36 is a survey of patient health comprised of eight multiple-item scales measuring these dimensions: physical function (PF), role-physical (RP), bodily pain (BP), general health (GH), vitality (VT), social function (SF), role-emotional (RE), and mental health (MH). There is also a single-item measure that assesses health transition (HT). Patients receiving *Tong guan capsule* had low scores on all dimensions except for RP when compared to placebo control. Patients administered *Xue fu zhu yu capsule* scored high only on the RE dimension when compared to placebo control. We did not find any statistically significant difference between the treatment and placebo groups in the remaining dimensions. As with the SAQ, pooled results were not available because of heterogeneity with $I^2(\%)$ ranging from 85% to 98%.

4. Discussion

In this systematic review, 52 studies accounting for 4905 CAD patients who underwent PCI were identified. Definitive randomization was found in 19 studies and 33 studies were found to be lacking definitive randomization. For the latter, we attempted to contact authors by telephone or e-mail for further information. But most replies were unsatisfactory and did not resolve our questions; other authors did not reply. Therefore, as a whole, the included studies were of low quality. Of all studies, only four were designed to compare CHMs plus RWM versus the same RWM plus placebo [45, 67, 71, 81]. The remaining studies were designed to compare CHMs plus RWM versus the same RWM alone.

In the primary outcomes, 40 studies with 3805 patients assessed in-stent restenosis after PCI. Twenty of these studies involving 10 CHMs showed clear evidence of decrease in restenosis rate. Furthermore, 13 of these studies were of low or moderate risk of bias over a minimum 6 months' followup (*Xiong shao capsule* [67, 80, 81], *Dan shen* [44, 52, 85], *Guan tong formula* [54], *Bu xin yin* [76], *Jiang lian he ji* [63] *Shu xin yin* [75], *Tong guan capsule* [70], *self-prepared guan tong decoction* [83], and *Xue yu tong he ji* [69],). Therefore, a moderate definitive conclusion can be drawn that CHMs are beneficial for preventing coronary restenosis after PCI. In particular, the CHM *Xiong shao capsule*, which was studied in 4 trials with 613 patients showed strong evidence with low risk of bias in preventing restenosis [40, 67, 80, 81]. Other CHMs including *Dan shen* [44, 50, 52, 85, 86] and *Tong xin luo capsule* [55, 79, 82, 90] showed the same significant result. But a definitive conclusion cannot be drawn because of limited numbers of patients in these trials as well as their low methodological quality.

We were unable to conclude whether CHMs decrease cardiac mortality during 6 months' followup after PCI. Although the result of meta-analysis showed a statistically significant difference between comparison groups, we could not assess a similar result in the analysis of any single relevant trial. This may be due to the limited patient numbers in these studies.

Adverse events in the treatment groups were generally higher than in controls for 5 CHMs, but a statistically significant difference was found in only one study on the CHM *Lei gong teng* [43]. Adverse reactions in these studies were reported as mild. Therefore, we feel further investigation is needed to confirm these reports.

In this review we also examined secondary outcomes, measures of effect post-PCI. Recurrent angina was followed up for 6 months in 33 studies involving 22 CHMs. In 21 of these studies with 11 CHMs, patients in the treatment group had significantly lower incidence of recurrent angina than those in the control group. *Dan shen* (capsule or pill) in 5 studies [44, 50, 57, 85, 86], *Xiong shao capsule* in 4 studies [40, 67, 80, 81], *Tong xin luo capsule* in 3 studies [79, 82, 90], and *Tong mai yu xin concentrated pill* in 2 studies [60, 62] were significantly better than the control at reducing recurrent angina after PCI. Minimum lumen diameter in 7 studies with 6 CHMs, late loss of lumen in 6 studies with 5 CHMs, net gain in lumen diameter in 4 studies with 4 CHMs, and lesion area net gain in 5 studies with 4 CHMs were measured, with results of meta-analyses being statistically significant. Studies on the following CHMs revealed angiographic results in the treatment groups were significantly better than in the control group: *Guan tong formula* [54] for minimum lumen diameter, late loss of lumen, net gain in lumen diameter, and lesion area net gain; *Tong xin luo capsule* [82] for late loss of lumen and area net gain in lumen diameter; *Shu xue tong* [56] for late loss of lumen and area net gain in lumen diameter; *Xiong shao capsule* [67] for minimum lumen diameter; *Lei gong teng* [64] for late loss of lumenand lesion area net gain.

Our meta-analysis found possible benefit in Chinese herbal medicine compared to control in the rates of

restenosis, cardiac mortality, recurrent angina, and in MLD, NG, LLL, and LANG. Specifically, the CHMs *Xiong shao capsule* and *Dan shen* appeared to markedly reduce rates of restenosis and recurrent angina, and the CHM *Tong xin luo* was found to significantly reduce restenosis, recurrent angina, LLL, and LANG. The baseline characteristics of most studies, such as age, gender, severity of CAD, degree of coronary stenosis before PCI, and stent type were not significantly different between the treatment and control groups. Nevertheless, concluding that CHMs have definitive preventive effects on restenosis after PCI would be premature because most of the studies were of low quality with shortcomings such as inadequate concealment, nonreporting of dropouts, and their incomplete outcomes data point to the possibility of bias. Additionally, clinical heterogeneity was apparent because different categories of CHMs were used.

The key limitations of our review were quality of the included studies. Ideally, RCTs should adhere to known research design standards. For example, the medication, dosage, and course should be identical in the control groups, and when including patients with different levels of illness, the trial should use stratified randomization. Our examination of these studies did not find enough details of these characteristics though most of the included studies did report comparable baselines between comparison groups. Details about randomization methodology were also lacking. In the 52 studies we reviewed, 19 trials reported randomization using a random number table or computer random number generator such as SAS software, and 10 trials mentioned using sealed, opaque envelope concealment without further explanation. In addition, in some studies post-PCI angiographic assessment for restenosis was not carried out in comparable patient numbers between the study groups. For example, the authors of one study reported 30 patients in the treatment group and 45 patients in the control group. Post-PCI angiography was done on only 19 patients with 24 lesion vessels in the treatment group and on 26 patients with 29 lesion vessels in the control group, with 8 vessels in the treatment group and 13 vessels in control group determined to have restenosis [64]. No explanation was given as to why angiographic assessment was not carried out on comparable patient numbers. Thus this type of incomplete outcome data can lead to selection bias.

Studies that involve therapeutic trials should also report adverse events regardless of whether or not they occurred. Reporting of adverse effects is very important for evaluating the safety of interventional measures even though there is no certainty that the adverse event is related to the interventional measure. Furthermore, adverse events can affect study dropout rates. In our review, only 22 of the 52 trials we investigated reported adverse events, rendering it difficult to systematically evaluate the safety of CHMs for restenosis.

Most of the 52 studies did not mention type of stent deployed, bare metal versus drug eluting. Therefore, the level of effectiveness of CHMs is unknown when different stent types are used. Future research on this topic will help elucidate this.

Another area that did not receive attention in the studies is the effect of diabetes on restenosis. Persons with diabetes who undergo PCI with stent placement have a high rate of restenosis [94, 95]. Though diabetes was included in baseline patient characteristics in the treatment and control groups in most of the 52 studies of this review, none of the reports indicated the impact diabetes may or may not have had on restenosis. Furthermore, in China, CHMs are widely used in the treatment of diabetes [26]. Researchers may want to factor in these issues when designing future studies.

5. Conclusion and Recommendations

From this review, we conclude CHMs may have moderate efficacy in preventing restenosis following percutaneous coronary intervention with stent placement. This is despite the fact that our investigation revealed unclear methodological quality, clinical heterogeneity, and some possible bias in the identified studies. Among the CHMs, *Xiong shao capsule* appears to be somewhat effective in preventing restenosis because studies involving this CHM were of low bias and had sufficient patient numbers. The CHM *Dan shen (capsule or pill)* appears to have latent beneficial efficacy in preventing restenosis because there were relatively more studies and patient numbers for this CHM. Therefore, we recommend that *Dan shen* should be a priority for further research. We did not find evidence of a beneficial effect for administering CHMs to prevent major adverse cardiac effects due to restenosis after PCI.

Future trials on CHMs as therapy to prevent restenosis post-PCI need to adhere to established design standards to overcome the limitations presented in this review. In particular, they should ensure adequate concealment of allocation and blinding of primary outcomes assessors.

Abbreviations and Acronyms

AMI: Acute myocardial infarction
CABG: Coronary artery bypass graft
CAD: Coronary artery disease
CHM: Chinese herbal medicine
DES: Drug-eluting stent
ISR: In-stent restenosis
LANG: Lesion area net gain
LLL: Late loss of lumen
MI: Myocardial infarction
MLD: Minimum lumen diameter
NG: Net gain in lumen diameter
PCI: Percutaneous coronary intervention
RCT: Randomized controlled trials
RR: Risk ratio
RWM: Routine western medicine.

Conflict of Interests

The authors declare no conflict of interests.

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Research Article

In Vitro Assessment of Cytochrome P450 2C19 Potential of Naoxintong

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The effects of Buchang Naoxintong Capsules (BNCs) on S-mephenytoin 4′-hydroxylation activities in human liver microsomes in vitro were assessed. Human liver microsome was prepared by different ultracentrifugation. Human liver microsome incubation experiment was carried out to assay BNC on S-mephenytoin 4′-hydroxylation activities. The 4′-hydroxylation of S-mephenytoin, a representative substrate toward CYP2C19, was increased by phenytoin sodium (positive control). After the incubation, the metabolites of the substrates (4′-OH-mephenytoin) were determined by HPLC. Results showed that both phenytoin sodium and BNC showed obvious increase effect on CYP2C19. The enzymatic reaction of BNC was observed with concentrations ranging from 5 μg/mL to 250 μg/mL. Compared to blank, the increase effect of BNC showed significant difference from the beginning of concentration of 150 μg/mL ($P < 0.001$). The conclusion was that BNC showed obvious increase effect on the catalytic activities of drug-metabolising CYP2C19 enzyme.

1. Introduction

The efficacy of clopidogrel in combination with acetylsalicylic acid (ASA) therapy has been clearly established in well-designed randomized controlled trials that have showed a reduction in recurrent coronary events following acute myocardial infarction, as compared with ASA monotherapy [1]. However, there is substantial individual variability in response to clopidogrel, with inhibition of ADP-induced platelet aggregation ranging from less than 10% to almost complete inhibition of platelet aggregation with a wide distribution across this range, such that there is no dichotomous separation into "responders" and "nonresponders or resistance" [2]. Such variations in response have repeatedly been associated with adverse cardiovascular outcomes in patients undergoing percutaneous coronary intervention (PCI) [3].

The pharmacology of clopidogrel is key to understanding this phenomenon. Clopidogrel is a prodrug that must be converted to an active metabolite (R-130964). The metabolite inhibits platelet aggregation (the rationale for clopidogrel's use in high-risk cardiovascular disease) by irreversibly binding to the platelet $P2Y_{12}$ adenosine diphosphate receptor. In vivo, 85% of the clopidogrel dose is inactivated by plasma esterases. The remaining 15% is bioactivated in a 2-step pathway that depends on the cytochrome P450 isoenzyme system. The specific isoenzymes involved include cytochrome P450 1A2, 2B6, 2C9, 2C19, and 3A4 [4]. The cytochrome P450 2C19 and 3A4 isoenzymes play the major role.

One mechanism for resistance to clopidogrel involves genetic polymorphisms that alter expression of cytochrome P450 isoenzymes that act on the drug. Of these, CYP2C19*2 is the most common genetic variant reproducibly associated with variability in clopidogrel active metabolite bioavailability, antiplatelet effects, and clinical outcomes [5, 6]. Another mechanism for clopidogrel resistance is competitive inhibition of cytochrome P450 isoenzymes needed for the metabolic activation of clopidogrel. To overcome deficits in clopidogrel responsiveness, someone suggested the addition of CYP inducers to enhance clopidogrel conversion [7]. Our prevenient study found that adjunctive Buchang Naoxintong Capsules (BNC) to clopidogrel can enhance the antiplatelet

effect in volunteers with the CYP2C19*2 gene mutation [8]. In the present study, the effect of BNC on the 4′-hydroxylase activity of S-mephenytoin human liver microsome in vitro was assessed.

2. Materials and Methods

2.1. Drugs. BNC 0.4 g (Compilation of The National Standard of Chinese Traditional Medicine no. WS-10001 (ZD-0001)-2002; Med-drug Permit no. Z20025001) were supplied by the Buchang Pharmaceutical Co. Ltd. The ingredients of BNC include Radix Astragali, Radix Angelicae Sinensis, Radix Paeoniae Rubra, Radix Salviae Miltiorrhizae, Rhizoma Chuanxiong, Semen Persicae, Flos Carthami, Resina Olibani, Myrrha, Caulis Spatholobi, Radix Achyranthis Bidentatae, Ramulus Cinnamomi, Ramulus Mori, Pheretima, Scorpio, and Hirudo.

BNC was flayed, triturated, quantified, and then was dissolved in dimethylsulfoxide (Tianjin Fuchen Chemical Reagents Factory, concentration less than 1/1000).

2.2. Human Liver Microsomes. The ten human liver samples were obtained from patients who underwent a partial hepatectomy at the Department of Hepatobiliary Surgery, Fujian Provincial Hospital (Fuzhou, China). Surgery was performed for the removal of liver trauma from the liver. The use of the human liver for the study had been approved by the Institutional Ethics Committee. None of the subjects had a reported history of alcohol or drug abuse. The livers were removed within 2 h, frozen in liquid nitrogen, and stored at −80°C until used for microsomal preparation. Liver microsomes were prepared by different ultracentrifugation as described previously [9]. Liver samples are homogenized and centrifuged at a lower force (9000 r/min) for 15 min. The resulting supernatant is then centrifuged at a higher force (100000 r/min) for 60 min to precipitate the microsomes. The microsomal is resuspended in a final suspension buffer and is then ready for use in incubation studies. Protein and CYP contents were determined using the Bradford Protein Assay Kit (Shanghai Majorbio Bio-Pharm Technology Co. Ltd) and the method of Omura and Sato [10], respectively. The concentration of the protein in liver microsomes was 18 mg/mL, and the total amount of CYP450 enzyme was 589 pmole/mg.

2.3. Incubation Conditions. Microsomes (0.5 mg protein) were incubated at 37°C for 60 min with 20 μL S-mephenytoin (250 μmol/L, sigma company, black group) and an NADPH (Sigma-Aldrich Co. Ltd, Shanghai) generating system in the presence or absence of 20 μL BNC (trial group) or 20 μL phenytoin sodium [an inducer of the CYP2C19 enzyme (http://drugs.medsort.com/), sigma company, 15 μg/mL (0.3 μg/mL as the incubation final concentration), positive control group] in a final volume of 1 mL. BNC was dissolved in dimethylsulfoxide (Tianjin Fu Chen Chemical Reagents Factory) and added to the incubation mixture of microsomes. The incubation final concentrations of BNC used were 5 μg/mL, 50 μg/mL, 100 μg/mL, 150 μg/mL, 200 μg/mL, and 250 μg/mL. The same volume of dimethylsulfoxide was added to the black group and positive control group. One sample was divided into five tubes. Adding 3 mL cold dichloromethane (Hao Fly Chemical Co. Ltd, Zhengzhou), the reaction was terminated by cooling on ice. 100 μL phenacetin (2.795 μg/mL, sigma company) was added as an internal standard. The mixture was shaken for 5 min, then centrifuged at 2000 g for 10 min. The upper organic phase was transferred to another tube and evaporated to dryness under nitrogen. The residue was dissolved in 100 μL of eluate and 20 μL was injected into Agilent 1200 high-performance liquid chromatography (HPLC, Agilent Technologies Co. Ltd, USA) system.

2.4. Determination of 4′-Hydroxymephenytoin. 4′-Hydroxymephenytoin was purchased from Research Biochemicals International (Natick, MA). Determination of 4′-hydroxymephenytoin was carried out by the HPLC method as reported previously [8]. The mobile phase consisted of methyl alcohol, acetonitrile, and water pH value was adjusted to 8.0 by triethylamine (Hou Wang Chemical Co. Ltd, Nanjing) in the proportion of 17/19/64 and was delivered to a Welchrom C18 column (Shiseido Co. Ltd, Tokyo, Japan; 4.6 mm × 250 mm, 5 μm) at a column temperature of 25°C and a flow rate of 1.0 mL min^{-1}. The eluate was monitored at a wavelength of 204 nm. The calibration curve was generated by processing the authentic standard substance through the entire procedures. The coefficient of variation for the intraassay and inter-assay was less than 3.75% and 4.41%, respectively.

2.5. Statistical Analysis. Data were analyzed using SPSS (version 16.0, SPSS Inc., America) and expressed as mean and SD. Analysis of variance (ANOVA) was used as statistical methods to compare group means. A value of $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Chromatogram. Figures 1(a), 1(b), and 1(c) show chromatogram of buffer solutions blank, chromatogram of 4′-OH-mephenytoin, phenacetin, and S-mephenytoin, and chromatogram of incubated microsomes sample, respectively.

3.2. Accuracy. The results from the accuracy studies are give in Table 1.

3.3. Recovery Studies (Table 2). The extraction recovery was given in Table 2. The absolute recovery of 4′-OH-mephenytoin at the three levels of 230, 2300, and 5800 ng/mL ranged from 96.3 to 98.2%.

3.4. Linear Range and Detection and Detection Limit (Figure 2). A linear calibration graph was obtained for 4′-OH-mephenytoin in the range 70.5–5800 ng/mL with a correlation coefficient (r^2) of 0.9969. The regression equation was written as $Y = 2678.1X + 93.9$. This method had a limit of detection of Ca. 39.1 ng/mL.

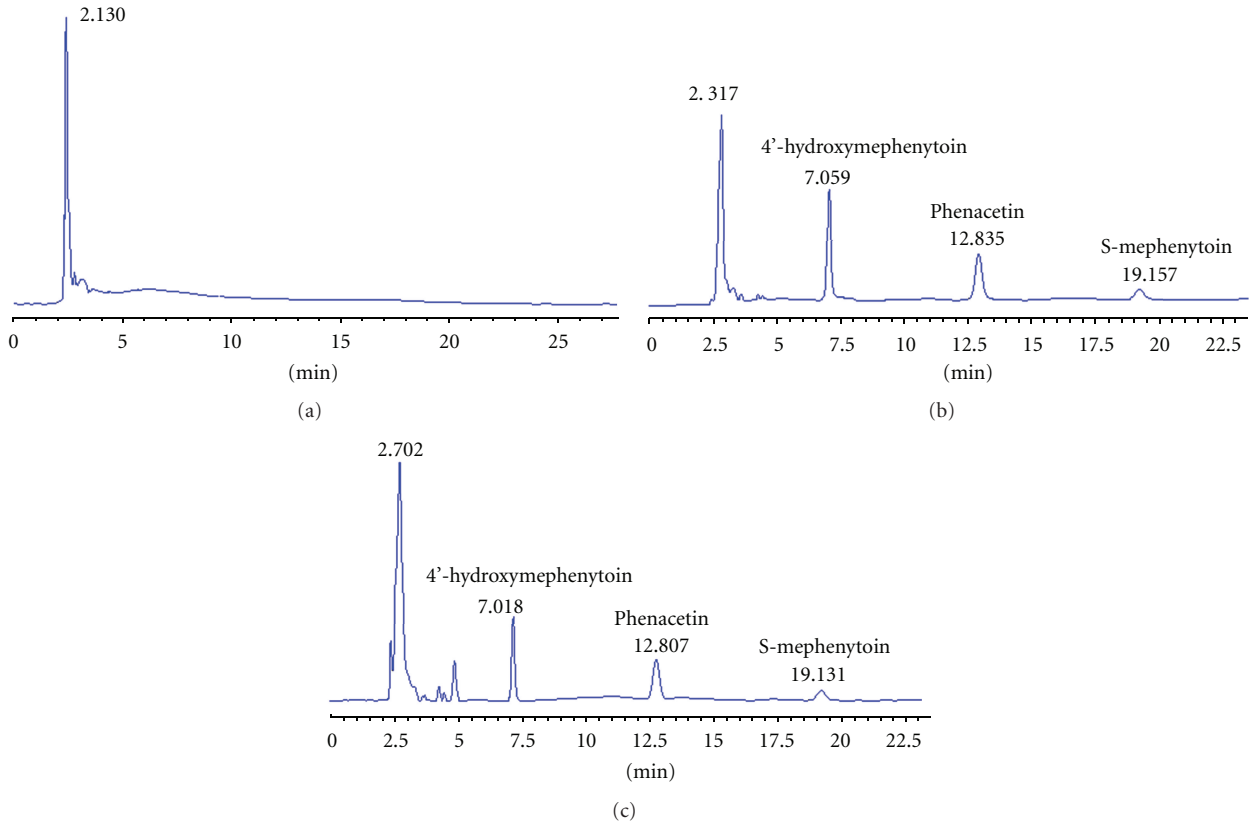

Figure 1: Chromatogram of buffer solutions blank (a), chromatogram of 4′-OH-mephenytoin (7.0 min), phenacetin (12.8 min), and S-mephenytoin (19.1 min (b)), and chromatogram of incubated microsome sample (c), respectively.

Table 1: Precision of the determination of 4′-OH-mephenytoin (mean ± SD, $n = 5$).

Joined concentration (ng/mL)	Intraday (ng/mL)	RSD (%)	Interday (ng/mL)	RSD (%)
230	227.00 ± 8.52	3.75%	227.83 ± 10.05	4.41%
2300	2278.30 ± 58.60	2.57%	2269.30 ± 77.19	3.40%
5800	5849.30 ± 122.44	2.09%	5779.30 ± 141.53	2.45%

Table 2: The extraction recovery of 4′-OH-mephenytoin (mean ± SD, $n = 5$).

Joined concentration (ng/mL)	Extraction recovery (%)	RSD (%)
230	98.2 ± 4.3	4.4%
2300	96.3 ± 3.9	4.0%
5800	97.7 ± 2.541	2.6%

3.5. The Effects of BNC on S-Mephenytoin 4′-Hydroxylation Activities in Human Liver Microsomes. Compared with blank group, the amount of 4′-OH-mephenytoin was significantly increased in positive control group and trial group after pre-incubated with phenytoin sodium (0.3 μg/mL, $P < 0.000$) and BNC from the beginning of concentration of 150 μg/mL ($P < 0.001$, Table 3) in human liver microsomes. The production rates of 4′-OH-mephenytoin in black group were defined as 100%, these production rates of 150 μg/mL, 200 μg/mL and 250 μg/mL were increased 8.6%, 11.1%, and 12.9%, respectively.

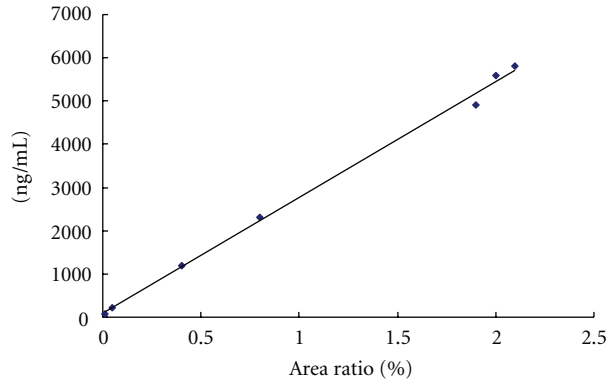

Figure 2: Standard curve of 4′-OH-mephenytoin.

Table 3: Comparison of the amount of 4′-OH-mephenytoin among blank group, positive control group, and trial group.

	Blank group ($n = 5$)	Positive control group (phenytoin sodium, $n = 5$)	Trial group (BNC, $n = 5$)					
Incubation final concentration (μg/mL)		0.3	5	50	100	150	200	250
4′-OH-M (nmol/mg·h)	6.8 ± 0.3	7.7 ± 0.3	6.6±0.4	6.8±0.3	7.1±0.3	7.3 ± 0.4	7.5 ± 0.3	7.6 ± 0.2
		▲▲▲				▲▲, △△, ♀	▲▲, △△△, ♀♀, †	▲▲▲, △△△, ♀♀♀, ††

Note: values are presented as mean ± SD.
▲▲▲, △△△, ♀♀♀: compared with blank, 5 μg/mL, 50 μg/mL, 100 μg/mL, respectively, $P < 0.000$.
▲▲, △△, ♀♀, ††: compared with blank, 5 μg/mL, 50 μg/mL, 100 μg/mL, respectively, $P < 0.001$.
♀, †: compared with 5 μg/mL, 50 μg/mL, respectively, $P < 0.05$.

4. Discussion

The active metabolite (R-130964) of clopidogrel is a secondary metabolite, and multiple cytochrome P450 enzymes (CYPs), including CYP3A, CYP2C19, CYP2C9, CYP2B6, and CYP1A2, contribute to the two sequential metabolic steps resulting in the formation of R-130964. Of these, CYP2C19 is responsible for approximately 45% of the first step (the formation of 2-oxo-clopidogrel) and approximately 20% of the final step—the generation of the pharmacologically active thiol metabolite. CYP2C19 also is a major metabolizing enzyme of several clinically important drugs such as proton-pump inhibitors (PPIs) like omeprazole and lanzoprazole, antiepileptics-like mephenytoin, diazepam, and selective serotonin reuptake inhibitors like citalopram. PPIs are often given concomitantly with clopidogrel to minimize the chances of gastrointestinal bleeding in patients with acute coronary syndrome especially percutaneous coronary interventions. Recent attention has been placed on a potential interaction observed between clopidogrel and the widely used PPIs. Some evidence suggested that omeprazole interacted with clopidogrel, reducing clopidogrel antiplatelet effects as measured by various laboratory tests [11, 12]. Most data indicated that the interaction involves the competitive inhibition of the CYP2C19 isoenzyme. The interaction appears to be clinically significant, as several retrospective analyses have shown an increase in adverse cardiovascular outcomes when PPIs and clopidogrel are used concomitantly [13–15].

Bent evaluated in vitro the dose-dependent induction potential of six commonly used trade herbal products on CYP2C19 and CYP2E1 metabolic activities in cultured human hepatocytes. They found that St John's wort was the most potent CYP-modulating herb, showing a dose-dependent induction/inhibition of CYP2C19, with induction at low dosages and inhibition at higher ones [16]. Previous investigations in man have shown that CYP2C19 activity is susceptible to induction by herbs and natural products; examples include St John's wort, G. biloba and the Chinese herbal mixture Yin Zhi Huang (also called Jaundiclear) [17–19]. Induction of cytochrome P450 isoenzymes, leading to an enhanced platelet inhibitory effect of clopidogrel, has also been described, which suggests a means for overcoming clopidogrel resistance. In a small prospective study, administration of St John's wort enhanced the platelet inhibitory effect of clopidogrel in volunteers known to be unresponsive to the drug and in patients with stable coronary artery disease [20]. Their further studies suggested that St John's wort on the pharmacodynamic response of clopidogrel in hyporesponsive volunteers and patients could increase platelet inhibition by enhancement of CYP3A4 metabolic activity [21]. Our study showed that BNC showed obvious increase effect on the catalytic activities of drug metabolism CYP2C19 enzyme. The increase effect of BNC was from the beginning of concentration of 150 μg/mL. This result might explain why adjunctive BNC to clopidogrel can enhance the antiplatelet effect in volunteers with the CYP2C19*2 gene mutation. It was important clinical meaning. In China, BNC is an approved traditional Chinese medicine (TCM) for stroke [22], which is widely used, and is well tolerated. BNC combined with aspirin could enhance the antiplatelet effect in patients with cardio-cerebrovascular diseases [23], but there is a lack of study of possible drug-herb interactions. Our current study showed that BNC combined dual antiplatelet therapy (DA, clopidogrel plus ASA) enhanced the anti-microembolization (CME) effect of either therapy alone and reduced the risk of the DA therapy-associated bleeding, demonstrating an improved benefit/risk ratio in the rat model of CME by inhibiting platelet aggregation and myocardial apoptosis, balance the pro- and anti-inflammatory cytokines as well as serum ET-1 and eNOS [24, 25]. This result suggested that integrated Chinese and Western medicine might provide a multitarget therapy with potential superior therapeutic efficacy and a better safety profile.

Understanding drug interactions that impair or increase therapeutic efficacy is important, especially with multidrug treatment. Although our findings are provocative, future

studies designed to investigate the BNC on CYP2C19 metabolic activities in vivo experimental methods.

5. Conclusions

BNC showed obvious increase effect on the catalytic activities of drug metabolism CYP2C19 enzyme. The effect of BNC begins from the beginning of concentration of 150 μg/mL.

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Review Article

Tanshinone IIA: A Promising Natural Cardioprotective Agent

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Tanshinone IIA (Tan IIA) is a member of the major lipophilic components extracted from the root of *Salvia miltiorrhiza* Bunge, which is currently used in China and other neighboring countries to treat patients suffering from myocardial infarction (MI), angina pectoris, stroke, diabetes, sepsis, and other conditions. However, Tan IIA is not easy to be absorbed through intestinal pathway. To raise the bioavailability of the herb, sodium tanshinone IIA sulfonate (STS) was developed. This paper discussed the pharmacology of Tan IIA, STS, and their potential cardioprotective effects.

1. Introduction

Salvia miltiorrhiza Bunge (Danshen) belongs to the Labiatae family of the plant kingdom. It is considered to have the function of activating blood circulation and removing blood stasis, entering the "heart", "pericardium", and "liver" channels according to the theory of traditional Chinese medicine (TCM). Danshen has been widely used in oriental countries, especially China, to treat various circulatory disturbance-related diseases for its special pharmacological actions, including vasodilatation, anticoagulation, antiinflammation, and free radical scavenging. In recent years, traditional medicines have been playing more and more important roles in the maintenance of health, the prevention and treatment of diseases, as well as plant-based drug discovery [1, 2]. Although many practitioners are used to prescribing nature products, more and more doctors and researchers are fascinated in chemical compounds of *Salvia miltiorrhiza* Bunge.

There are two main active compounds: the lipophilic (Tanshinone I, IIA, IIB; cryptoTanshinone; other related compounds) and the hydrophilic (polyphenolic acids, danshensu, protocatechuic aldehyde, and protocatechuic acid). Tanshinone IIA (Tan IIA), which is a member of the major lipophilic components extracted from *Salvia miltiorrhiza* Bunge, has indicated significant therapeutic effects on various diseases *in vivo* and *in vitro*. Since Tan IIA is not easy to be absorbed through intestinal pathway, sodium tanshinone IIA sulfonate (STS) was developed to raise the bioavailability. The chemical structure of STS is shown in Figure 1. In this paper, the pharmacology of Tan IIA and STS in the treatment for cardiovascular diseases was reviewed.

2. Cardiovascular Pharmacology

2.1. Vasodilative Effect. Cheng et al. [3] demonstrated that Tan IIA produced a concentration-dependent relaxation in isolated spontaneously hypertensive rat (SHR) aortic rings precontracted with phenylephrine or potassium chloride (KCl) through ATP-sensitive K(+) channel to lower $[Ca(2+)]_i$. Wu et al. [4] investigated the effects of Tan IIA on isolated rat coronary arteriole and the underlying mechanisms. The results showed that endothelium denudation, inhibition of nitric oxide synthase (NOS), inhibition of the cytochrome P450 epoxygenase, and blockade of the large conductance Ca(2+)-activated potassium channels (BKca) significantly decreased the vasodilation elicited by Tan IIA, which indicated that Tan IIA induces an endothelium-dependent vasodilation in coronary arterioles; nitric oxide (NO) and cytochrome P450 metabolites contribute to

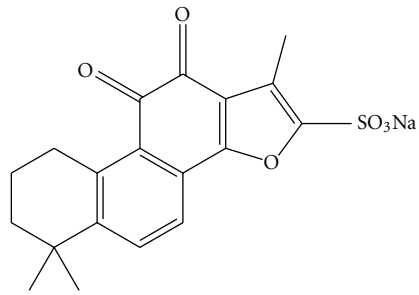

Figure 1: The chemical structure of STS.

the vasodilation; activation of BKca channels plays an important role in the vasodilation. Kim et al. [5] concluded that topical Tan IIA increased both normalized arteriolar diameter and periarteriolar NO concentration in the two–kidney, one-clip renovascular hypertension model. N(G)–monomethyl-L-arginine inhibited Tan IIA-induced vasodilation. Tan IIA prevented the hypertension-induced reduction of endothelial NOS (eNOS) and increased eNOS expression to levels higher than sham-operated control. Topical Tan IIA increased normalized arteriolar diameter more in the cremaster muscle of control mice than that in cremasters of eNOS knockout mice. In ECV-304 cells transfected with eNOS-green fluorescent protein, Tan IIA significantly increased eNOS protein expression and eNOS phosphorylation. The results also indicated that eNOS stimulation was one mechanism by which Tan IIA induced vasodilation and reduces blood pressure.

2.2. Inhibition of Left Ventricular Hypertrophy. Accumulative studies had demonstrated that Tan IIA could inhibit left ventricular hypertrophy (LVH) with different mechanisms. Overwhelming postloading is a main factor promoting LVH, therefore controlling hypertension is very important in LVH inhibition. Tan IIA has shown vasodilatation effect through adenosine triphosphate (ATP)-sensitive K(+) channel to lower the concentration of Ca^{2+} in myocytes, regulate the condition of hypertension, and inhibit the formation of hypertrophy [3]. Tan IIA could also prevent LVH through inhibiting angiotensin receptor (ATR) expression or blocking free Ca^{2+} influx in rats with hypertrophic myocardium caused by abdominal aorta constriction, and its effect on lowering hypertension had no significant difference compared with Valsartan [6, 7]. Additionally, Tan IIA has been reported to block the transforming growth factor (TGF) beta1/Smads signal pathway and inhibit the formation of myocardial hypertrophy [7], attenuate enhanced collagen type I expression and collagen synthesis as well as depressed matrix metalloproteinase-1 (MMP-1) expression and activity by angiotensin II (Ang II) [8]. Furthermore, Tan IIA depressed the intracellular generation of reactive oxygen species (ROS), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, and subunit p47 (phox) expression, which are the factors inducing LVH. Fang et al. [9] concluded that Tan IIA conferred its beneficial effects on the collagen metabolism probably through its regulation of transcript levels of the MMPs/tissue inhibitor of metalloproteinases (TIMPs) balance. Although the balance regulation had no difference as compared with Valsartan, Tan IIA showed slight improvement in attenuating cardiac dysfunction. At least three studies assessed proto-oncogene c-fos mRNA expression of cardiocytes when investigated the mechanism of Tan IIA, indicating that Tan IIA could prevent LVH induced by Ang II, which might be related to its inhibition of proto-oncogene expression [10–12]. Similar conclusion was drawn by Tu et al. [13] that Tan IIA had the definite function in preventing LVH by its action on the protein kinase B (PKB/Akt) signaling pathway, which could regulate the expression of proto-oncogene c-fos. Two *in vitro* studies [14, 15] had demonstrated that Tan IIA dose-dependently inhibited the increment of the total protein level induced by Ang II and the p-extracellular signal regulatory kinase (ERK)1/2 expression stimulated by Ang II, which indicated the mechanism that Tan IIA inhibited the myocardial cell hypertrophy induced by Ang II may be associated with the inhibition of p-ERK1/2.

2.3. Restraining Smooth Muscle Cell Proliferation and Intimal Hyperplasia. Tan IIA could significantly decrease intimal thickening, suppress cell proliferation and migration, inhibit the expression of various growth factors, induce the differentiation, maturity, and apoptosis of the vascular smooth muscle cell (VSMC), and ameliorate the function and condition of vascular smooth muscle [16]. Since there are so many beneficial effects on VSMC, Tan II is playing an important role in the treatment of arteriosclerosis, restenosis after angioplasty or stenting, brain arteriovenous malformations, and pulmonary hypertension. However, the mechanism of Tan II has not been very clear. Various studies [17–21] had found that Tan IIA could suppress cell proliferation and BrdU incorporation into DNA, block cell cycle in G0/G1 phase, and inhibit ERK1/2 phosphorylation and c-fos expression. Initial proliferation might be inhibited by blocking mitogen-activated protein kinase (MAPK) signaling pathway and downregulating c-fos expression. Pan et al. [22] demonstrated that Tan IIA could significantly inhibit the proliferation of VSMCs in a dose-dependent manner, and the mechanism might be related to the downregulation of calponin (CaN) activities and the inhibition on calcineurin mRNA and proliferating cell nuclear antigen (PCNA) expressions. Jin et al. [23] illuminated that Tan IIA exhibited multiple effects on inhibiting human aortic SMCs migration, the mechanisms of which might inhibit IkappaBalpha phosphorylation and p65 nuclear translocation through inhibition of Akt phosphorylation, suppress tumor necrosis factor-alpha (TNF-α)-induced ERK and c-jun phosphorylation, and block nuclear factor-kappaB (NF-κB) and activator protein-1 (AP-1) DNA-binding; all these factors played important roles in human aortic SMCs migration.

2.4. Attenuation of Atherosclerosis. In addition to VSMC proliferation and intimal hyperplasia, injury of vascular endothelium, lipid deposition, oxidative stress, and inflammatory reaction also play important roles in the formation and

progression of atherosclerosis. Endothelial cells can secrete two kinds of substances with opposite functions, one can induce VSMC apoptosis (such as NO), and the other can inhibit VSMC apoptosis (such as endothelin-1 (ET-1), Ang II, and growth factors). Unbalance between them decides whether endothelium is injured. NO is the key factor in signal transduction, it can relax blood vessels and activate genes relative to VSMC apoptosis. Li et al. [24] and Huang et al. [25] concluded that Tan IIA could inhibit the negative effect of Ang II on NO production and eNOS expression in porcine aortic endothelial cells. Another *in vitro* study showed that Tan II might inhibit ET-1 production and cell apoptosis, inducing protective effect on vessel endothelium [26]. Tan IIA could also reduce plaque area in endothelium, decrease lipid deposition, and significantly inhibit the formation of atherosclerosis, although the level of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) in serum had not been changed by Tan IIA [27].

To verify the antioxidant effect on atherosclerosis formation, at least five experiments [28–32] had been established. Tang et al. [28] demonstrated that Tan IIA could attenuate atherosclerotic lesion in apolipoprotein E (apoE)(−/−) mice, which might be attributed to its properties of both antioxidation and downregulation of scavenger receptors. Furthermore, antagonism of peroxisome proliferators-activated receptor gamma (PPARγ) might be involved in the downregulation of CD36 by Tan IIA. Fang et al. [29] found that the superoxide dismutase (SOD) activity was significantly increased while the level of malondialdehyde (MDA) was decreased in Tan IIA group, which showed that antioxidant effect of Tan IIA might be a potential mechanism involved in antiatherosclerosis. Tang et al. [30] suggested that Tan II A significantly attenuated the atherosclerosis in rat model, which might be attributed to its inhibition of oxidized low density lipoprotein (oxLDL) production, independent of the serum levels of lipids, calcium, and 25-OH Vitamin D. Increasing of Cu/Zn SOD activity as well as mRNA and protein expression by Tan IIA might protect LDL against oxidation induced by superoxide anion in vessel. Active oxygen free radical is a major factor inducing endothelial injury; hydrogen peroxide can promote the formation of free radicals, which can penetrate cell membrane, combine with Fe^{2+} or Cu^{2+}, induce lipid peroxidation, and lead to endothelium injury and formation of atherosclerosis finally. Lin et al. [31, 32] indicated that Tan IIA could protect ECV-304 cell damage induced by hydrogen peroxide through its anti-oxidant effect and CD40 anti-inflammatory approach.

Inflammatory effect can induce endothelium injury, foam-cell appearance, and leukocytes adhesion, all of which play important roles in the formation of atherosclerosis. At least two studies [29, 33] had verified that Tan IIA could decrease inflammatory effect and attenuate atherosclerosis of vessels. Fang et al. [29] indicated that expression reduction of CD40 and MMP-2 activity might be the potential mechanisms of antiatherosclerosis effect of Tan IIA. Fang et al. [33] demonstrated that Tan IIA could dose-dependently inhibit atherosclerotic lesion through downregulation of protein expression and activities of MMP-2 and MMP-9 as well as serum VCAM-1 and interleukin (IL)-1β in rabbits fed high-fat diet.

Platelet activation and aggregation can accelerate the formation of atherosclerosis. Jiang et al. [34] indicated that Tan IIA could inhibit the increasing P-selectin expression of thrombin-activated platelets in a concentration-dependent manner, which may also be a mechanism of Tan IIA to inhibit atherosclerosis.

2.5. Lipid-Lowering Effect. Kang et al. [35] demonstrated that human HepG2 cells treated with Tan IIA for 24 h exerted a dose-dependent inhibitory effect on apolipoprotein B (apoB) secretion together with TG. However, another secretory protein, albumin, was unaffected by Tan-IIA treatment, indicating that the effect of Tan IIA is specific for apoB secretion. Tan IIA decreased the transcription level of microsomal TG transfer protein gene, suggesting that lipoprotein assembly is likely to be involved in the inhibited ApoB secretion. Gong et al. [36] reported that Tan IIA inhibited 3T3-L1 preadipocyte differentiation and transcriptional activities of full-length PPARγ and PPARγ ligand-binding domains. The effects of Tan IIA are mediated through its property as a natural antagonist of PPARγ. Tan IIA treatment reduced adipose mass and body weight, improved glucose tolerance, and lowered the LDL/HDL ratio without changing the food intake in a high-fat-diet-induced obese animal model.

2.6. Inhibitory Effect on the Inflammatory Responses. Various studies demonstrated that inflammatory response was involved in the process of myocardial infarction (MI), endothelium injury, atherosclerosis, and cardiovascular hypertrophy [23, 29, 33, 37, 38], which have been mostly introduced in the former paragraphs. However, mechanisms underlying this effect have not been fully understood. NF-κB activation by NF-κB-inducing kinase (NIK)-lkappaB alpha kinase (IKK) pathway and MAPKs pathway is known to be involved in the inflammatory response. Jang et al. [39] determined the inhibitory effect of Tan IIA on the activation of NF-κB and IkappaB alpha phosphorylation and also examined phosphorylation of NIK and IKK as well as the activation of MAPKs such as p38 MAPK (p38), ERK1/2, and c-Jun N-terminal kinase (JNK) in RAW 264.7 cells stimulated with Lipopolysaccharides (LPS). The result suggested that Tan IIA might inhibit LPS-induced lkappaB alpha degradation and NF-κB activation via suppression of the NIK-IKK pathway as well as the MAPKs (p38, ERK1/2 and JNK) pathway in RAW264.7 cells, and these properties might provide a potential mechanism which could explain the anti-inflammatory activity of Tan IIA. Another *in vitro* study [40] suggested that Tan IIA had a similar structure with 17 beta-estradiol (E2) and the result indicated Tan IIA exerted anti-inflammatory effects by inhibition of inducible NOS (iNOS) gene expression and NO production, as well as inhibition of inflammatory cytokine (IL-1β, IL-6, and TNF-α) expression via estrogen receptor-dependent pathway. Therefore, it could serve as a potential selective estrogen receptor modulator (SERM) to treat inflammation-associated neurodegenerative and cardiovascular diseases without increasing the risk of

breast cancer. Similar results were demonstrated by other studies [41, 42].

2.7. Antioxidant Effect. Oxidation reaction was involved in various pathological mechanisms, inducing different diseases including MI, angina pectoris, and restenosis after PCI, LVH, and so on. Tan IIA can inhibit these reactions, which have been mentioned in the above experiments [8, 28–32]. To test the hypothesis that Tan IIA can alter the expression and/or activity of specific antioxidant enzymes to prevent cells from oxidant damage, at least three experiments [38, 43, 44] were conducted and demonstrated that the cell protective effect of Tan IIA was mediated primarily by induction of glutathione peroxidase (GPx) gene expression and activity, as well as other antioxidant enzyme activities in the heart. At least four experiments [44–47] indicated that Tan IIA could scavenge the free radicals produced in the superoxide approach, which might be one of the important mechanisms in myocardiocyte damage. Other studies [30, 48] suggested that Tan IIA significantly attenuates myocardiocyte or vasculocyte damage, which might be attributed to its inhibition of ox-LDL production.

2.8. Antiplatelet, Anticoagulant, and Antithrombotic Effect. Tan IIA can decrease the blood viscosity obviously, inhibit the activation of thrombin, and promote fibrin degradation; it can inhibit the function of platelets and the formation of thrombus. Li et al. [49] showed that Tan IIA could significantly decrease the platelet number, with efficacy similar to aspirin. Jiang et al. [34] also found that Tan IIA could reduce the number of blood platelets by inhibiting P-selectin expression in a concentration-dependent manner. Li et al. [50] demonstrated that Tan IIA could inhibit the thrombus formation and platelet aggression in *in vivo* study, and it exerted more significant effect on antiplatelet than anticoagulation.

To investigate the effects of Tan IIA on procoagulant activity (PCA) of human ECV304 cells induced by acute promyelocytic leukemia cell line NB4 cells, Zhang et al. [51] showed that the conditional media of NB4 cells treated with Tan IIA (Tan IIA-NB4-CM) can increase the levels of PCA and tissue factor (TF) activity of ECV304 cells through some unidentified factor; however, Tan IIA can obviously decrease the PCA and TF activity of ECV304 cells induced by Tan IIA-NB4-CM.

CD41 and CD62p are two of the most important inflammatory factors, which can induce platelets aggregation and promote blood coagulation. Jia et al. [52] found that Tan IIA could decrease the expression of CD41 and CD62p, which might inhibit platelet aggregation and blood coagulation.

2.9. Antiarrhythmia Effect. Jia et al. [52] indicated that Tan IIA could decrease the expression of adhesion molecule in blood platelet to prevent arrhythmia. In addition, high-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) in vascular smooth muscle also play important roles in controlling the vascular tone by determining the level of membrane potential and Ca^{2+} influx through voltage-gated Ca^{2+} channels. Agents that can alter the activity of Ca^{2+} channels or BK_{Ca} thus affect the vascular tone in both physiological and pathological conditions. Experiments [53, 54] showed that Tan IIA could block L-type Ca^{2+} channel, decrease concentration of intracellular Ca^{2+}, ameliorate calcium overload in myocardiocytes, and prevent or even treat arrhythmia finally. Except for Ca^{2+} and K^+, microRNA-1 (miR-1) level is also one of the important factors in ischemic arrhythmia. Shan et al. [55] indicated downregulation of miR-1 and consequent recovery of Kir2.1 might account partially for the efficacy of Tan IIA in suppressing ischemic arrhythmia and cardiac mortality. These findings support the proposal that miR-1 could be a potential therapeutic target for the prevention of ischemic arrhythmias. On gene level, Sun et al. [56] indicated that Tan IIA could activate human cardiac KCNQ1/KCNE1 potassium channels (I_{Ks}) in HEK 293 cell directly and specifically through affecting the channels' kinetics, which would be a promising therapeutic medicine in arrhythmia.

2.10. Antimyocardial Hypoxia. Reducing oxygen consumption and increasing the tolerance in hypoxygen of myocardiocytes are beneficial to coronary heart disease. Huang et al. [57] detected the left ventricle end diastole pressure (LVEDP) after ligating coronary artery of dogs, the result indicated Tan IIA could decrease LVEDP and heart volume and reduce myocardial oxygen consumption. Shao et al. [58] demonstrated that the activation of ATP enzyme in myocardium was decreased in patient with hyperthyroidism; however, Tan IIA could protect it and increase the tolerance in hypoxygen of the myocardiocytes. Various studies [4, 59, 60] have suggested that Tan IIA might dilate coronary artery, inhibit vascular contraction, increase coronary blood flow and reduce oxygen consumption of myocardiocytes with different mechanisms. Sun et al. [61] suggested that Tan IIA could decrease intracellular calcium overload and K+ outflow, inhibit Na+ inflow, keep the balance of membrane potential, and therefore protect myocardiocyte in hypoxia.

2.11. Reduction of Myocardial Infarct Size. Tan IIA can dilate coronary artery and increase coronary blood flow, which is beneficial for reducing MI size. Various experiments [62–65] have demonstrated that Tan IIA might recover cardiac function and reduce MI size significantly with different mechanisms. Zhang et al. [62] indicated that the possible mechanism responsible for the effect of Tan IIA was associated with the phosphatidylinositol 3-kinase (PI3K/Akt)-dependent pathway, which was accompanied with decreased cardiac apoptosis and inflammation. In addition, Tan IIA was found to reduce MI size by $53.14 \pm 22.79\%$ as compared to that in the saline control, simultaneously, and significantly prolonged the survival of cultured human saphenous vein endothelial cells rather than human ventricular myocytes *in vitro* (these cells were separately exposed to xanthine oxidase (XO)-generated oxyradicals), which may suggest that Tan IIA could reduce MI size through prolonging survival of endothelial cells [63]. Xu et al. [64] have assessed the effect of Tan IIA on endothelial cells of MI in rats, and they suggested

that Tan IIA could reduce MI size and myocardial ischemia injury through promoting angiogenesis and upregulating vascular endothelial growth factor (VEGF) expression. Jiang et al. [65] found that Tan IIA might establish extensive collateral circulation and increase blood flow in ischemic area.

2.12. Inhibiting Ischemia Reperfusion Injury. Ischemia reperfusion (IR) exerts disturbance of microcirculation and leads to many diseases, including myocardial stunning and reperfusion arrhythmia. Production of oxygen free radicals, calcium overload in myocytes, endothelial cell injury, adhesion of leukocyte, energy supply reduction, mitochondrial damage, and myocardiocytes apoptosis are considered to be involved in this process. Tan IIA can inhibit the activation of proteases and ameliorate calcium overload in myocytes, which have been introduced in the previous paragraph [53, 54, 61]. In addition, Tan IIA can increase the SOD content in the injured myocytes, decrease the MDA concentration, and influence electron transfer reaction in mitochondria, thus scavenge the free acids, reduce the lipid peroxidation, and protect myocytes and vascular endothelial cells in the IR process [45–47]. ET, which can induce constriction of the vessel, increases significantly in IR, and Tan IIA can inhibit the production and release of ET, promote secretion of NO, and decrease IR injury of the heart [24–26]. Jiang et al. [34] indicated that Tan IIA could inhibit HL-60 cell adhesion to human umbilical vein endothelial cells through concentration dependently inhibiting TNF-alpha and ameliorate microcirculation disturbance. Fu et al. [46] suggested that Tan IIA might markedly inhibit H_2O_2-induced oxidation *in vitro*, significantly inhibit IR-induced cardiomyocyte apoptosis by attenuating morphological changes and reducing the percentage of terminal transferase dUTP nick end-labeling (TUNEL)-positive myocytes and caspase-3 cleavage, as well as ameliorate IR injury by upregulating Bcl-2/Bax ratio.

3. Final Comments

In the beginning of 21st century, we are facing serious challenges of cardiovascular diseases (CVDs). Although it is becoming less lethal, CVD prevalence is incessantly increasing and it is still the most common cause of death. As a representative of complementary and alternative medicines, TCM has a history of thousands of years and has made great contributions to the health and wellbeing of the people and to the maintenance and growth of the population. It provides us with great treasure of herbal medicines or natural products, which can be served as lead compound or new drug candidates in the battle against CVDs.

Herbal medicines with the function of activating blood circulation (ABC) have been investigated extensively and made remarkable achievements in recent years [66, 67]. *Salvia miltiorrhiza* Bunge is the most common used ABC herb in China for treating CVDs and other circulatory disturbance-related diseases. Tan IIA, which is a member of the major lipophilic components extracted from *Salvia miltiorrhiza* Bunge, has indicated significant therapeutic effects and multiple pharmacological actions including vasodilative, antithrombotic, anti-inflammation, antioxidant, anti-ischemia, antiarrhythmia, antihyperplasia, antiatherosclerosis, and lipid-lowering effect. Clearly, Tan IIA appears to be a promising natural cardioprotective agent. Further research is warranted to translate these beneficial effects into clinical practice and definitely address the mechanisms of its multitarget actions.

Conflict of Interests

The authors declare no conflict of interests.

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Review Article

Control Strategy on Hypertension in Chinese Medicine

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Hypertension is a clinical common disease, with high mortality and disability. Although there have also been significant advances in therapeutic concepts and measures, it has shown a certain value and significance in the treatment of Chinese medicine. The control strategy on hypertension is described from the following aspects such as differentiation of symptoms, pathogenesis, formula syndrome, and herb syndrome. As the common clinical manifestations of hypertension are dizziness, headache, fatigue, lassitude in the loins and knees, and so on, the pathogeneses of them are analysed. The author found that the main pathogenesis of the disease is heat, excessive fluid, and deficiency, which occurred incorporatively and interacted with each other in patients. Although the pathogenesis of the disease is complicated, the distribution of formula syndromes and herb syndromes is regular. The common formula syndromes include *Banxia Baishu Tianma Tang* (Decoction of *Pinellia ternata, Atractylodes* and *Gastrodia elata*), *Da Chaihu Tang* (Major Bupleurum Decoction), and *Liu Wei Dihuang Wan* (Pill of *Rehmannia*). And the common herb syndromes include Tian Ma (*Gastrodia elata*) syndrome, Sheng Di Huang (Radix Rehmanniae) syndrome, Niu Xi (*Achyranthes Root*) syndrome, and Chuan Xiong (*Ligusticum wallichii*) syndrome.

1. Introduction

Hypertension is a clinical common disease, with high mortality and disability. Additionally, it is also an independent risk factor for stroke, coronary heart disease, heart failure, renal insufficiency, peripheral vascular diseases, early death, and many other major diseases [1]. In numerous studies, it is shown that around 30% of the population died from cardiovascular and cerebrovascular events, in which 62% of acute stroke events and 49% of cardiovascular events were directly caused by hypertension. There are about 1 billion hypertensive patients in the world. It was demonstrated that there were about 200 million hypertensive patients in China with more than 10 million patients increased annually [2, 3] in Cardiovascular Report 2006 in China. The antihypertensive treatment has made great progress in modern medicine. The therapeutic drugs include six classes of antihypertensive agents and fixed compound preparation. There have also been significant advances in therapeutic concepts and measures, including paying more attention to the control of earlier-stage hypertension and elevated systolic blood pressure, optimization of combination therapeutic scheme, and new strategy of antihypertensive treatment combined with lipid-lowering therapy [4]. Despite the availability of six classes of antihypertensive agents, control of blood pressure and improving patients' quality of life remain unsatisfactory, and "three lows" status quo, low awareness, low treatment, and low control, still exist. As the complexity of hypertension, there are still some issues to be resolved. For instance, most patients are prescribed single or combined antihypertensive drugs for long-term even lifetime. It also has been confirmed that the emergence of adverse effect and irrational drug use increase the difficulty and dissatisfaction in treatment [5]. Currently, it has shown a certain value and significance in the treatment of Chinese medicine. Now the understandings of hypertension in Chinese medicine and control strategies with classical prescriptions are described as follow.

2. Differentiation of Symptoms

Symptoms are not only the basis for syndrome differential diagnosis but also the therapeutic targets. As Chinese medicine had no idea of blood pressure originally, it treated

patients mainly by differentiating the syndromes according to the symptoms and signs caused by hypertension and paid more attention to relieve symptoms and improve the quality of life. The most common clinical manifestations of hypertension are dizziness, headache, fatigue, shortness of breath, lassitude in the loins and knees, memory loss, dry eye, palpitation, and so on [6].

The disease belongs to "vertigo" in Chinese medicine. The main pathogenesis of vertigo is stagnation of phlegm, excess of liver yang, deficiency of qi and blood, liver-kidney yin deficiency, and stagnation of blood. In addition, there are important pathogenesis such as excessive fluid and pathogenic factor in Shaoyang meridians according to the clinical experience in *Shang Han Lun* (Treatise on Febrile Diseases). Dizziness caused by excessive fluid syndrome is always accompanied by chest distress, palpitation, nausea, and vomiting. Paroxysmal dizziness is one of the typical symptoms in Shaoyang syndrome. Persistent dizziness, headache, nausea, and vomiting can be found in Shaoyang syndrome accompanied by heat and excessive fluid syndrome.

The syndrome differentiation of headache is similar to vertigo. The main pathogenesis of headache is excess of liver yang, deficiency of qi and blood, kidney deficiency, stagnation of phlegm and blood. We found that headache is caused by the flaring-up of fire in patients with hypertension. Flaring-up of stomach fire and intestinal fire followed by meridians are also very common, besides excess of liver yang. Severe headache generally suggests blood stasis; therefore, blood-activating and stasis-dissolving drugs should be used to relieve pain quickly. Moreover, cold accumulation in the liver and stomach should not be neglected, which can result in severe headache in the disease.

Weakness and fatigue are extremely common symptoms of the disease. Generally speaking, it is caused by deficiency of qi, blood, yin, and yang. However, they are not the characteristic symptoms of deficiency syndrome. Some are caused by dampness and water accumulated inside. As the characteristic of pathogenic dampness is weight and sticky, which leads to stagnation of the functional qi and consumption of yang qi, weakness, fatigue, heaviness in head and body, and lassitude in loins and limbs often appear.

In addition, although lassitude in loins and limbs is the characteristic symptom of kidney deficiency, excessive fluid is also an important factor of the symptom. *Fu Ling* (*Poria cocos*) and *Bai Shu* (*Atractylodes*) contained in *Gancao Ganjiang Fuling Baishu Tang* (Decoction of *Glycyrrhiza*, Dried Ginger, *Poria cocos*, and *Atractylodes*) treating lassitude in loins and limbs can invigorate spleen to resolve dampness. *Fu Ling* (*Poria cocos*) and *Ze Xie* (*Alisma*) contained in *Liu Wei Dihuang Wan* (Pill of *Rehmannia*) and *Shen Qi Wan* (Pill of Kidney Qi) have the identical effection.

3. Differentiation of Pathogenesis

The identification of the etiology and pathogenesis of hypertension in TCM viewpoint is directly related to the syndrome differentiation and therapeutic methods. According to the traditional view, the pathogenesis of hypertension is considered as founding on yin deficiency, with yang hyperactivity in the superficiality and phlegm-dampness and blood stasis penetrating all along; therefore, the basic therapeutic methods should be to supplement qi and nourish yin [4]. However, due to the widely used antihypertensive agents, the progression of the disease was blocked in time, which leads to great changing of the natural history and pathogenesis of hypertension. Therefore, we should not only pay attention to yin deficiency and yang hyperactivity syndrome but also follow the pathogenesis and syndromes of the illness with interest in the recognition of the pathogenesis of hypertension and treat the disease according to clinical symptoms. The author found that the main pathogenesis of the disease is heat, excessive fluid, and deficiency, which occurred incorporatively and interacted with each other in patients.

Heat syndrome can be found in various stages of hypertension, especially when target organ damage is not found. Heat syndrome includes liver fire, heart fire, stomach fire, and intestinal fire. Liver fire syndrome often shows clinical symptoms such as vertigo, headache, facial flush with perspires, conjunctival congestion, bitter taste in the mouth, irritability, wiry-rapid-powerful pulse, or powerful cunkou pulse alone, or wiry and long pulse even well beyond cunkou pulse. *Longdan Xie Gan Tang* (Decoction of Radix Gentianae for Purging Liver Fire) noted in *Yi Fang Ji Jie* (Collection of Prescriptions with Notes), *Tianma Gouteng Yin* (Decoction of *Gastrodia* and *Uncaria*) noted in *Za Bing Zheng Zhi Xin Yi* (New Meanings in Syndrome and Therapy of Miscellaneous Diseases), and *San Cao Jiang Ya Tang* (Decoction of Antihypertensive Effect) which is Professor Liu Duzhou's experienced prescription containing Long Dan Cao (Gentian), Xia Ku Cao (*Prunella vulgaris*), Yi Mu Cao (*Leonurus japonicus*), Shao Yao (Chinese Peony), and Gan Cao (Glycyrrhiza) all can be used for clearing liver fire and lowering blood pressure. Patients with heart fire syndrome often present with distraction, distress in chest, vexation, palpitation, nervousness, insomnia and dreaminess, hard to sleep, and easy to wake. *Zhizi Chi Tang* (Decoction of *Gardenia* and Lobster Sauce) and *Huanglian Jie Du Tang* (Detoxicant Decoction of *Coptis*) all can be used for clearing away heart fire and lowering blood pressure. Patients with stomach fire syndrome often present with dry mouth, thirst with desire for cold drinks, easy to starve, foul breath, smelly stool, and right guan pulse powerful alone. *Bai Hu Tang* (White Tiger Decoction) can be used to clear away stomach fire and lower blood pressure. Intestinal fire syndrome often shows foul breath, constipation, abdominal distension and pain, strength, and deep-hidden-powerful pulse. *Da Chaihu Tang* (Major *Bupleurum* Decoction) can be used for clearing away intestinal fire, dredging intestines, and descending turbid substance. Liver fire and heart fire often appear simultaneously, which result in hyperactivity of heart fire and liver fire syndrome in the disease. In addition, the liver restricting the spleen and stomach may also result in hyperactivity of liver fire, stomach fire, and intestinal fire.

Fluid retention syndrome is a special type of the disease, containing morbid fluid retained in Shangjiao, Zhongjiao, and Xiajiao syndromes, cold fluid retention syndrome, fluid retention turning into heat syndrome, and downward

flow of damp-heat syndrome. Morbid fluid retained in Shangjiao syndrome often shows dizziness aggravated by body position change, chest distress, palpitation, and cough. Morbid fluid retained in Zhongjiao syndrome often shows gastric distension, abdominal distension, nausea, vomiting, poor appetite, thirst without desire to drink, or not thirsty, daytime sleepiness, and greasy fur. Morbid fluid retained in Xiajiao syndrome often shows soreness, lumbar heaviness, low back pain, weakness and heaviness in lower extremity, edema, abnormal leucorrhea, dysuria and thick-greasy fur of tongue root. Cold fluid retention syndrome can appear retching, headache, cold hands and feet in syncope and dysphoria. Fluid retention turning into heat syndrome and downward flow of damp-heat syndrome can shows weakness and heaviness of lower extremity, thirst, yellow and ropiness leukorrhea, beriberi, and thick-greasy fur of tongue root. *Zexie Tang* (Decoction of American water Plantain), *Fuling Xingren Gancao Tang* (Decoction of *Poria cocos*, Almond, and *Glycyrrhiza*), *Wu Ling San* (Wuling Powder), *Ling Gui Zhu Gan Tang* (Decoction of Poria cocos, Cassia Twig, *Atractylodes macrocephala* and *Glycyrrhiza*), *Banxia Baishu Tianma Tang* (Decoction of *Pinellia ternata*, Atractylodes Macrocephala and *Gastrodia elata*), *Wu Zhu Yu Tang* (Decoction of *Evodia rutaecarpa*), *Er Miao Wan* (Ermiao Pill), *San Miao Wan* (Sanmiao Pill), and *Si Miao Wan* (Simiao Pill) all can be used for dissipating excessive fluid.

Deficiency syndrome includes spleen deficiency syndrome and kidney deficiency syndrome. As spleen promotes transportation and transformation of dampness, excessive fluid retention is related to dysfunction of spleen. Cang Zhu (Rhizoma Atractylodes), Bai Zhu (*Atractylodes macrocephala*), Fu Ling (*Poria cocos*), Ze Xie (American water Plantain), and Gan Cao (*Glycyrrhiza*) all can be used for removing dampness by strengthening spleen and replenishing qi. Kidney deficiency syndrome includes kidney yin deficiency syndrome and kidney yang deficiency syndrome. As prolonged disease involves kidney, we found that kidney deficiency syndrome is always related to hypertension. The longer the course of illness, the higher the incidence rate of kidney deficiency syndrome. Furthermore, it is not to be neglected that the antihypertensive drugs can also lead to kidney deficiency. Diuretic and β-blocker have greater impact on sexual function than other antihypertensive drugs. Sexual function will decrease when the above two antihypertensive drugs are sustained using more than ten years. *Liu Wei Dihuang Wan* (Pill of *Rehmannia*), *Shen Qi Wan* (Pill of Kidney Qi), *Qi Ju Dihuang Wan* (Pill of Chinese Wolfberry, *Chrysanthemum*, and *Rehmannia*) and *Ji Sheng Shen Qi Wan* (Pill for Reinforcing Kidney Qi) all can be used for reinforcing kidney.

4. Differentiation of Formula Syndrome

Although the pathogenesis of the disease is complicated, the distribution of formula syndromes is regular. The common formula syndromes include *Banxia Baishu Tianma Tang* (Decoction of *Pinellia ternata*, *Atractylodes* and *Gastrodia elata*), *Da Chaihu Tang* (Major *Bupleurum* Decoction), and *Liu Wei Dihuang Wan* (Pill of *Rehmannia*). *Banxia Baishu Tianma Tang* (Decoction of *Pinellia ternata*, *Atractylodes macrocephala*, and *Gastrodia elata*) is the classical representative famous prescription for calming liver, suppressing liver yang hyperactivity, dissipating excessive fluid, and expelling phlegm in Chinese medicine. It has been used widely in clinical practice for treatment of hypertension, which is also the author's experienced prescription. Prestigious Chinese physicians such as Yue Meizhong and Jiang Erxun are skilled in using the decoction in both China and the world. Japan's Chinese physicians often use it to treat hypertensive patients with weakness of gastrointestinal function. It can treat liver fire and morbid fluid retained in Zhongjiao, the indications of which are dizziness, headache, palpitation, abundant sputum, or without phlegm, poor appetite, nausea, and vomiting aggravated by tooth brushing, abdominal distension, soreness, lumbar heaviness, heaviness in lower extremity, edema, dysuria, constipation, or loose stool, big and light tongue with greasy fur and slippery pulse. The decoction is suitable for patients lacking physical activity for a long time, especially for old women, the physical characteristics of which are obesity of abdominal type with yellow and white skin, soft muscle, ease of dizziness, and headache. It is often combined with *Zexie Tang* (Decoction of American water Plantain), *Wu Ling San* (Wuling Powder), and *Er Miao Wan* (Ermiao Pill) in treating hypertension.

Da Chaihu Tang (Major *Bupleurum* Decoction) syndrome is a special type of the disease, which can be used for reconciling Shaoyang meridian and descending turbid substance. It has been used for treating liver fire, stomach fire, and intestinal fire. The composing herbs such as Chai Hu (*Bupleurum*) and Huang Qin (*Scutellaria*) can sooth liver and clear liver fire. Da Huang (Rhubarb), Zhi Shi (*Citrus aurantium*), and Shao Yao (Peony) can clear stomach fire and relieve constipation. Professor Huang Huang, coming from Nanjing University of Chinese Medicine, is skilled in using the decoction, the indications of which are dizziness, headache, facial flushing, conjunctival congestion, dry mouth, bitter taste in the mouth, halitosis, abdominal distension, constipation, yellow urine, red tongue, and wiry-rapid-powerful pulse. Professor Huang Huang pointed out that the physical characteristics are obesity type, strength, hard fullness, and distending pain of upper abdomin mostly accompanied by biliary-pancreatic diseases, poor appetite, nausea, vomiting, constipation, suppressive emotions, stress, sleep disorders, and so on. We found that the decoction is suitable for the young and middle-aged men with hypertension. Professor Huang Huang often combined *San Huang Xie Xin Tang* (Decoction of Sanhuang for Purging Heart Fire) to clear heart and liver fire [7].

Liu Wei Dihuang Wan (Pill of *Rehmannia*) is the classical representative famous prescription for nourishing kidney yin, which has been widely used for treating kidney deficiency and excessive fluid syndrome. The indications of the decoction include dizziness, headache, tinnitus, low back pain, lassitude in loins and legs, edema, big and light tongue with less fur, and deep thready pulse. Due to the speciality of the disease, two therapeutic principles should be followed. The first one is that herbs should be prescribed according to the rule of syndrome differentiation and treatment, and

the second one is that the pharmacological action of herbs should act on the pathological mechanism of the disease. *Liu Wei Dihuang Wan* (Pill of *Rehmannia*) not only accords with the pathogenesis but also aims at the pathological mechanism. This compound prescription has broad pharmacological activities, especially the action of protecting vascular endothelial cells, dilating peripheral blood vessels, decreasing peripheral vascular resistance, lowing blood pressure synergistically, and delaying the development and progression of arteriosclerosis. If accompanied by blurred vision, Gou Qi (Chinese Wolfberry) and Ju Hua (*Chrysanthemum*) should be added into the prescription, which composed *Qi Ju Dihuang Wan* (Pill of Chinese Wolfberry, *Chrysanthemum,* and *Rehmannia*). If accompanied by facial flushing and conjunctival congestion, Tian Ma (*Gastrodia elata*) and Gou Teng (*Uncaria*) should be added into the prescription for calming liver and suppressing liver-yang hyperactivity.

5. Differentiation of Herb Syndrome

As the distribution of herb syndromes of hypertension is regular, we could find rules of formula syndromes from herb syndromes if we grasp the treatment rules of herb syndromes neatly and masterly. We found that the common herb syndromes include Tian Ma (*Gastrodia elata*) syndrome, Sheng Di Huang (Radix Rehmanniae) syndrome, Niu Xi (*Achyranthes root*) syndrome, and Chuan Xiong (*Ligusticum wallichii*) syndrome.

Tian Ma (*Gastrodia elata*) can calm liver and suppress liver yang hyperactivity. The indications of Tian Ma (*Gastrodia elata*) are dizziness, tinnitus, distending feeling in head, headache, facial flushing and conjunctival congestion, the pathogenesis of which belongs to flaming up of liver fire and hyperactivity of liver yang. It is noteworthy that if dizziness and headache are more severe, the dose of Tian Ma (*Gastrodia elata*) must be larger to control blood pressure, and we often use 30 g, while routine dose such as 10 to 20 g has unsatisfactory results. The dose of Tian Ma (*Gastrodia elata*) in academician Chen Keji's experienced prescription "*Qing Xuan Jiang Ya Tang* (Decoction for Eliminating Vertigo and Lowering Blood Pressure)" is 30 g, while the dose of another key herb Gou Teng (*Uncaria*) is 30 to 60 g [8].

Sheng Di Huang (Radix Rehmanniae) could remove pathogenic heat from blood, nourish yin, and generate body fluid. In accordance with record of *Shengnong Ben Cao Jing* (Shennong's Classic of Meteria Medica), it can activate blood circulation. Yoshimasu T's "*Yao Zheng* (Indications of Herbs)" recorded that it can treat blood syndrome and fluid retention. Therefore, the indications of Sheng Di Huang (Radix Rehmanniae) are low back pain, lassitude in loins and legs, edema, hypodynamia, dry mouth, constipation, thin and less fur. We often use it to treat liver fire, intestinal fire, kidney fire, kidney deficiency, and excessive fluid. The dose of Sheng Di Huang (Radix Rehmanniae) needs our attention as well as Tian Ma (*Gastrodia elata*). Low dosees enrich yin and nourishes kidney, while large dose could activate blood to dredge vessels. According to the author's experience, the clinical dose is 60 to 120 g. It is not necessary to be too tense if patients present with gastrointestinal symptoms such as diarrhea and smelly stool after taking medicine, which means pathogenic fire is downgoing and eliminating.

According to recordation in *Shengnong Ben Cao Jing* (Shennong's Classic of Meteria Medica), Niu Xi (*Achyranthes root*) can nourish liver and kidney, promote blood circulation to remove blood stasis, guide fire, and fluid downgoing. Therefore, dizziness, headache, facial flushing, conjunctival congestion, lassitude in loins and legs, and edema due to upward attack by qi and fire are the typical indications of Niu Xi (*Achyranthes root*). Professor Xu Wenhua, a prestigious Chinese physician of Jiangsu, is skilled in using Niu Xi (Achyranthes Root) treating pheochromocytoma, malignant abdominal tumors, and so on in clinical practice. The dose of Niu Xi (Achyranthes Root) is very large even up to 250 g sometimes. He had taken herbal decoction with Niu Xi (Achyranthes Root) of 200 g, and no serious side effect was found during the course of the treatment. Motivated by this, Professor Huang Huang often use large dose of Niu Xi (Achyranthes Root) to treat lower limb dropsy due to hemocirculatory disorder, cirrhosis ascites, hypertension patients with obesity, and so on. We often use it to treat liver fire, kidney deficiency, and fluid retention disease. Significant curative effect can occur when the dose is above 60 g, and simultaneous use of both Chuan Niu Xi (*Achyranthes bidentata*) and Huai Niu Xi (*Achyranthes bidentata Blume*) could improve the curative effect.

Chuan Xiong (*Ligusticum wallichii*) could promote qi and activate blood circulation to relieve pain. According to recordation in *Shengnong Ben Cao Jing* (Shennong's Classic of Meteria Medica), it can treat headache. Famous Chinese physician Li Dongyuan also said that Chuan Xiong (*Ligusticum wallichii*) must be used in treating headache. Therefore, the classical indication of Chuan Xiong (*Ligusticum wallichii*) is headache. Severe headache always suggests qi stagnation, blood stasis, and meridians and collaterals impassable. The dose of Chuan Xiong (*Ligusticum wallichii*) also deserves attention greatly. We had treated one case of intractable migraine patients with Xue Fu Zhu Yu Tang (Decoction for Removing Blood Stasis), in which the dose of Chuan Xiong (*Ligusticum wallichii*) is 10 g. However, after taking 10 doses, the pain was not alleviated satisfactorily. It is found that routine dose such as 10 to 20 g cannot get satisfactory effect when headache is severe through many experiments afterwards. However, headache were relieved rapidly when the dosages was increased up to 30 g.

6. Summary

Chinese medicine has been used for over 2500 years and has historically established itself as a system of a holistic medical care in China [9]. What is more, Chinese medicine and integrative medicine health provision in conventional medical clinic and hospital settings has emerged worldwide [10–13]. Seeing the body as a whole, not as separated systems, regarding human beings as more than just their physical bodies, practicing with emphasis on prevention, holoregulation, and comprehensive intervention, are all very strong principles of Chinese medicine, which works

better and costs less than conventional medicine for the management of common diseases [14].

Blood pressure regulation involves multiple system interaction, such as kidneys, central nervous system (CNS), peripheral nervous system (PNS), and endothelial system. Hypertension is caused by a variety of factors such as the interaction of environment and heredity factors leading to disturbances of blood pressure regulation, the pathological mechanism of which includes sympathetic nervous system (SNS), renin-angiotensin-aldosterone system (RAAS), vasopressin (VP), nitric oxide (NO), endothelin (ET), adrenal medulla, and a variety of vasoactive peptide secreted by other endothelial cells and smooth muscle cells [15–17]. Although hypertension is a common cardiovascular disease, it is always combined with hyperlipidemia, coronary heart disease, diabetes, metabolic syndrome, and other diseases, which involve the cardiovascular, endocrine, neurologic, renal medicine, and other departments. Therefore, hypertension is a chronic and complex disease relating to systemic multiple systems. Besides lowering blood pressure steadily, the final purpose of Chinese medicine in treating hypertension is to reduce blood pressure variability and risk factors, protect hypertension target organs from the impairment, modulate factors difficult to control blood pressure, reduce the dosage of western medicine and abate the adverse reactions, relieve symptoms to improve patients' quality of life, improve long-term survival, and reduce the morbidity and mortality maximally [18]. It may be just what the scholars of Chinese medicine need to focus on, reflecting the advantages of Chinese medicine treatment.

Although antihypertensive drugs have great advantage in reducing blood pressure rapidly with reliable effect, Chinese medicine can work at different levels and targets quite different from western drug which works relatively at few targets [19]. Researches had shown that Chinese herbs can regulate the function of RAAS, sympathetic-vagus nerve, and immune system, inhibit the level of inflammatory factor, prevent and reverse left ventricular hypertrophy caused by high blood pressure, influence vasoactive substances significantly, and so on [20–23]. Professor Li had observed the effect of Chinese medicine, Jiangya capsules, which was made up of Radix Cyathulae, Radix Achyranthis Bidentatae, *Pheretima, Laminaria japonica,* Rhizoma Gastrodiae, Rhizoma Chuanxiong, and so on, with multicenter, randomized, double-blind, positive controlled clinical design in elderly patients with isolated systolic hypertension (EISH). It showed that Chinese medical regimen had affirmative effect in treating EISH patients and could lower the systolic blood pressurea and improve quality of life and early renal impairment of the patients [24]. Professor Zhou found out that combined use of Xuezhikang or pravastatin with the antihypertensive therapy could increase the circulating endothelial progenitor cells number and improve their function in essential hypertensive patients with the blood pressure controlled by antihypertensive drugs, leading to benefits independent of pressure-lowering effects [25].

Furthermore, we found that there are laws for treatment according to many of years experience of large number of patients with hypertension. It has important significance in carefully analyzing common symptoms of hypertension, the etiology and pathogenesis, formula syndrome rules, herb syndrome rules, dose rules, combined diseases, and uncontrollable factors of blood pressure under the guidance of theory of Chinese medicine [26, 27].

Conflict of Interests

All authors manifest that there is no conflict of interests.

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