Therapeutic Strategies in
PULMONARY ARTERIAL HYPERTENSION

A. J. Peacock · J. A. Barberà
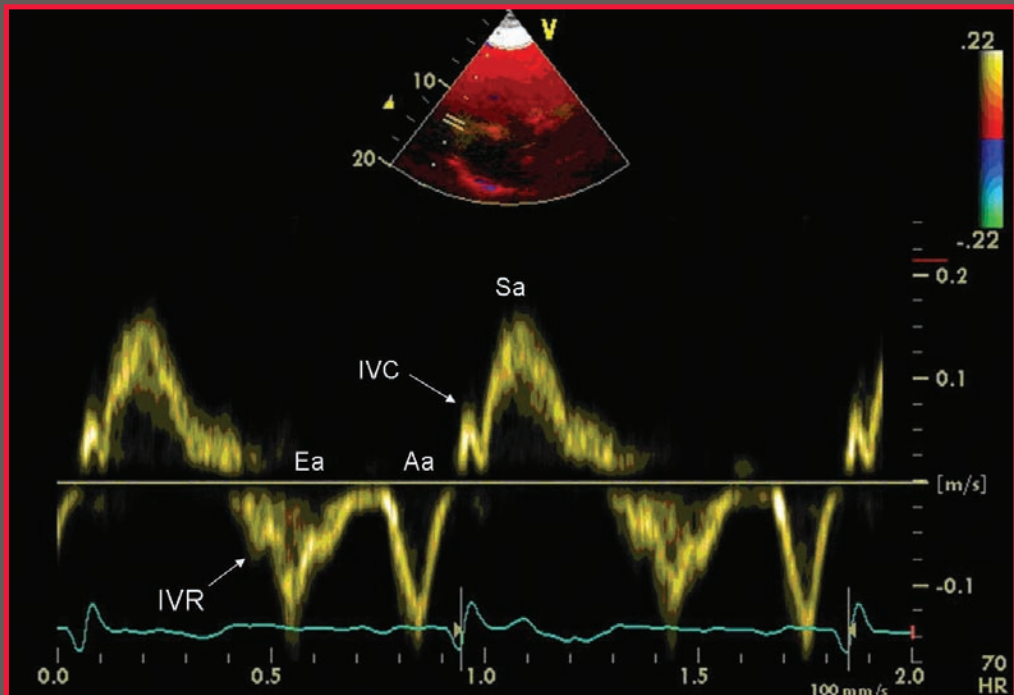
PULMONARY ARTERIAL HYPERTENSION

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Imaging of the right heart and pulmonary circulation

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INTRODUCTION

Imaging techniques play an important role in the diagnosis of pulmonary arterial hypertension (PAH). However, the role of imaging in the follow-up of patients with PAH has been limited until now, despite the fact that right ventricular (RV) failure is the primary cause of death in this disease. Reasons for this might be the technical difficulties encountered in accurately measuring RV structure and function, as well as a lack of studies confirming the clinical relevance of these techniques in assessing the effects of treatment and prognosis. Echocardiography is a well-established and accessible imaging modality for the screening and diagnosis of pulmonary hypertension (PH). One limitation of this technique is that it is highly operator-dependent. In addition, volumetric measurements rely upon geometric assumptions that are difficult to apply to the complex shape of the right ventricle. Emerging techniques for an accurate assessment of RV structure and function are magnetic resonance imaging (MRI) and computed tomography (CT). Both of these techniques enable the imaging of the pulmonary vasculature and perfusion. This chapter provides an overview of the technical possibilities and limitations of these imaging modalities in the imaging of the right ventricle and pulmonary circulation.

ECHOCARDIOGRAPHY

In clinical practice, echocardiography is the mainstay of evaluation of RV structure and function, being easily accessible and versatile in comparison with other imaging modalities. However, echocardiography of the RV is challenging because of the multipartite, multiplanar morphology of the RV, its anterior position and the poor visualisation of the RV anterior free wall. In addition, its multiplanar geometry makes it difficult to use summation-type volume calculations.

Echocardiography can be used as a non-invasive screening tool in the field of PH and plays an important role in the diagnostic algorithm. It provides not only an estimate of PH at rest and during exercise, but can also help to exclude left-sided heart disease as a cause

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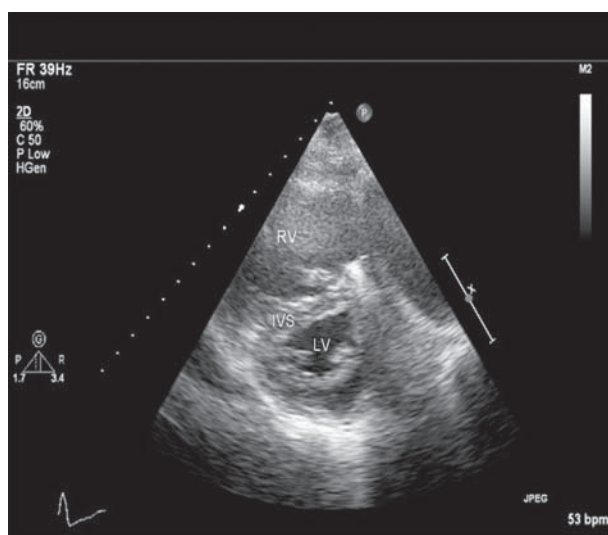
Figure 1.1 Transthoracic echocardiogram: parasternal short-axis view. Due to elevated pressures in the right ventricle (RV), the interventricular septum (IVS) is displaced, leading to a D-shaped left ventricle (LV) with reduced systolic and diastolic volume.

of PH, predict prognosis, monitor treatment effects and detect preclinical stages of the disease [1]. Knowing the characteristic echocardiographic features of the RV is important in both initial and serial evaluation of PH patients. Multiple echocardiographic techniques are available for the assessment of these characteristics.

ONE- AND TWO-DIMENSIONAL IMAGING (M-MODE AND 2D)

Right and left ventricular size and function, the morphology of cardiac valves, abnormal anatomical connections, atrial pathology and the presence of pericardial effusion are well established with 2D echocardiography (Figure 1.1).

Most patients with PH present with enlarged right-sided chambers, pulmonary artery (PA) dilatation, RV hypertrophy and reduced global RV systolic function due to chronic RV pressure overload [2]. This is accompanied by a systolic flattening of the interventricular septum (IVS) and an increased thickness with an abnormal IVS/posterior left ventricular wall ratio >1. Due to the displacement of the IVS to the left, the ventricle appears D-shaped with reduced systolic and diastolic volumes but preserved global systolic function. Interatrial right-to-left septum bowing might be another characteristic of PH. Pericardial effusion can be seen due to impaired venous and lymphatic drainage secondary to elevated right atrial pressure.

Assessing the RV volume, the simplest and most routinely used methods include linear dimensions and areas obtained from single tomographic echocardiographic planes. The best correlations between single-plane measurements and RV volumes have been obtained with the maximal short axis dimension and the planimetered RV area in the 4-chamber view. The area–length method, which uses an ellipsoidal or pyramidal model, correlates better with RV volume than the Simpson's rule, using disk volumes.

In clinical practice, right ventricular ejection fraction (RVEF), the ratio of change in ventricular volume during the cardiac cycle, is the most commonly used index of RV contractility. Two-dimensional assessment of RVEF can be estimated with Simpson's rule and the

area–length method, although the correlation with MRI and radionuclide-derived RVEF is modest [3].

Right ventricular fractional area change (RVFAC) represents the ratio of systolic area change to diastolic RV area, measured in the 4-chamber view. In end-stage pulmonary disease, a good correlation exists between RVFAC and RVEF [4].

A quantitative measurement of RV systolic performance is the tricuspid annual plane systolic excursion (TAPSE). This method reflects the longitudinal systolic excursion of the lateral tricuspid annulus towards the apex. It is measured with M-mode imaging in the apical 4-chamber view. A moderate correlation exists between TAPSE and radionuclide-derived RVEF [5].

(COLOUR-) DOPPLER ECHOCARDIOGRAPHY

Colour-Doppler echocardiography can detect intracardiac shunts and regurgitation of cardiac valves. In 86% of cardiovascular patients, a tricuspid regurgitation (TR) of measurable quality can be detected. The development of TR in patients with PH is likely to be related to the presence of annular dilatation, altered RV geometry and the apical displacement of tricuspid leaflets [6]. Using the systolic regurgitant tricuspid flow (v), an estimation of the systolic pulmonary artery pressure (sPAP) can be made by Doppler echocardiography. In the absence of pulmonary outflow tract obstruction, sPAP is equivalent to the RV systolic pressure, which can be calculated with the simplified Bernouilli equation:

$$RVSP = 4\,v^2 + \text{right atrial pressure (RAP)}$$

v is measured with a continuous wave Doppler signal and the RAP is an estimated value using characteristics of the inferior vena cava.

Peak early diastolic and end-diastolic velocities of pulmonary regurgitation correlate significantly with mean and diastolic PA pressure [7].

RV outflow tract acceleration time, defined as the interval from onset to the maximal velocity of forward flow in a pulsed wave Doppler derived signal, has a negative correlation with mean pulmonary artery pressure (mPAP). A RV outflow tract acceleration time <100 ms reflects an increased mPAP.

RV myocardial performance index (TEI index), which is the ratio of isovolumetric time intervals to ventricular ejection time, can be calculated from the pulsed wave Doppler derived inflow and outflow durations. This parameter has been described as a global non-geometric index of systolic and diastolic ventricular function. The normal value of this index is 0.28 ± 0.04 and this value increases in the presence of RV dysfunction [8].

Left ventricular diastolic filling is frequently abnormal in patients with PH. Doppler echocardiography can analyse this abnormal filling pattern by determining the ratio between the early diastolic peak transmitral flow velocity (E) and the late diastolic peak velocity (A) [9] with a ratio E/A <1 being indicative for abnormal left ventricular diastolic filling. Reasons for this abnormal filling pattern in PH might be a reduced left atrial filling, abnormal left ventricular (LV) relaxation, the presence of abnormal LV geometry because of RV enlargement and leftwards septal displacement at the early diastolic phase, or possible myocardial oedema [10].

TISSUE DOPPLER IMAGING

Tissue Doppler imaging (TDI) has been introduced to estimate RV function by measuring the deformation and velocity of the RV structures during the cardiac cycle. The tissue velocity along a long axis in the 4-chamber view relates to longitudinal shortening and gives a one-dimensional view of unit velocity at predefined anatomical sites. Tissue Doppler imag-

ing may be useful in detecting early RV dysfunction, which could have an important impact on treatment and prognosis.

There are five major deflections visualised on TDI of the RV tricuspid annulus: the isovolumetric contraction wave, systolic velocity (Sa), isovolumetric relaxation wave, early diastolic velocity (Ea) and late diastolic velocity (Aa). A peak systolic tissue Doppler signal Sa <11.5 cm/s identifies the presence of ventricular systolic dysfunction (RVEF <50%) [11]. Moustapha and colleagues [12] have found that Sa and Ea were significantly lower in patients with PH compared to controls, indicating depressed RV function. Caso and co-workers [13] reported a prolonged myocardial relaxation time by TDI in patients with pulmonary disease and PH.

Tissue Doppler imaging may be useful in estimating the mean pulmonary capillary wedge pressure (PCWP). The early diastolic velocity of the mitral annulus corrected for the early diastolic mitral inflow velocity (E/Ea) relates well to the PCWP (E/Ea >15 = PCWP >20 mmHg) and may be used to estimate LV filling pressures [14, 15]. Low filling pressures in PH patients are indicative for a non-cardiac aetiology of PH [9].

The myocardial performance index can also be derived with TDI and more accurately compared to Doppler imaging. It has a higher frame rate and samples a discrete segment of the ventricular myocardium. The systolic and diastolic time intervals are measured in the same cardiac cycle, eliminating beat-to-beat variation.

Using conventional TDI, a non-functional segment being sampled can still possess adequate velocities by being tethered to a normally functional adjacent segment. Strain analysis, measuring deformation, and strain rate measuring velocity of deformation, can overcome this limitation. In various disease entities, tissue Doppler derived strain is a sensitive index of segmental contractile function and correlates to invasive and magnetic resonance measures of RV [16]. Lopez-Candales and colleagues [17] found that PH patients had lower RV longitudinal free wall strain compared with healthy volunteers.

Although strain imaging has overcome the problem of segmental myocardial tethering, strain is still angle- and load-dependent. Speckle imaging and velocity vector imaging, two novel two-dimensional measures of myocardial motion, may overcome this problem and merit further investigation.

THREE-DIMENSIONAL ECHOCARDIOGRAPHY (3D)

Three-dimensional echocardiography is a promising method for more accurate assessment of the RV volume and function. Although less limited by geometric assumptions when compared with 2D echocardiography, 3D echocardiography is still dependent on an adequate acoustic window and problems with delineation of the anterior wall and identification of the infundibular plane need to be overcome. These limitations might explain the inaccuracy of 3D echocardiography to measure RVEF [18].

EXERCISE ECHOCARDIOGRAPHY

Because of non-specific and subtle signs, particularly in its early stages, the detection of PH requires a high clinical index of suspicion. Along with clinical assessment, electrocardiogram (ECG), radiographic investigations and Doppler echocardiography, exercise echocardiography can be an excellent tool to screen patients for exercise-induced PH. However, the full physiological range of pulmonary pressure responses to exercise in relation to gender and age in athletes and non-athletes as a reference for cardiovascular evaluation and counselling still has to be defined. For example, in healthy people, moderate exercise leads to only a mild increase in sPAP in contrast to well-conditioned athletes who are capable of reaching sPAP levels of around 60 mmHg with exercise as a consequence of increased flow and left atrial pressure [1].

Another advantage of exercise echocardiography is the diagnosis of diastolic dysfunction, which does not manifest itself under resting conditions [19].

PROGNOSTIC AND THERAPEUTIC IMPLICATIONS

The prognosis of PH is relatively poor and related to the severity of RV dysfunction which can be evaluated by echocardiography. Together with a number of haemodynamic and non-invasive parameters, various echocardiographic indicators of right heart impairment, including indexed right atrial area, the degree of septal shift in diastole, a high RV myocardial performance index and the severity of pericardial effusion, have been associated with unfavourable outcomes [1].

Besides providing prognostic information, Doppler echocardiography can be a helpful tool in the assessment of the effect of medical interventions. Galie and colleagues [20] evaluated the effects of the oral endothelin receptor antagonist bosentan in comparison to placebo on echocardiographic and Doppler measures in a group of PAH patients. Their results showed that patients treated with bosentan showed less RV dilatation, increased LV dimensions, greater stroke volume and higher cardiac index compared to the placebo group. There was also an improvement in RV ejection, LV early diastolic filling and a beneficial effect on the diameter of the inferior vena cava and pericardial effusion. The treatment effect on 6-minute walking distance (6MWD) was 37 m in favour of bosentan. This study showed that echocardiography can be successful in detecting changes in cardiac structure and function associated with medical treatment and can be used to monitor the efficacy of new therapeutic interventions.

MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging, although expensive and technically demanding to use accurately, is an emerging technique in the study of the right ventricle and pulmonary circulation. Technical MRI requirements for this type of imaging are a cardiac imaging package for acquiring ECG triggered cine images, and a flow package for measuring blood flow. In addition, a cardiac or body surface coil is required to obtain images with a sufficient signal-to-noise ratio. With regard to the magnetic field strength, any field strength above 0.5 Tesla will suffice. In addition, the physician needs access to post-processing software tools for quantification of volumes, flow and perfusion. For the patient, the MRI must be safe and thus any contraindications must be excluded (e.g. the presence of an infusion pump or cardiac pacemaker).

ASSESSMENT OF RIGHT AND LEFT VENTRICULAR FUNCTION AND MASS BY MRI

Right and left ventricular function assessment is reached by localizing the cardiac 4-chamber view in a standardised procedure [21], and by obtaining a contiguous stack of short-axis cine images of the RV and LV from which RV and LV mass, volume and function are derived. The patient is instructed to hold their breath in relaxed expiration during all image acquisitions, and also during 'scout' imaging for localisation of the heart. If breath holding in expiration is too difficult for the patient, then the patient is asked to hold their breath in a relaxed inspiration instead (Figure 1.2).

Several studies have been performed to explore the clinical significance of the different MRI parameters derived from cine imaging. In a recent study, RV mass was used as a study endpoint in the comparison of the effects of sildenafil and bosentan [22]. The results of this study showed that sildenafil reduced RV mass more than bosentan. However, the interpretation of these study findings is difficult, since a change in RV mass may reflect a change in PA pressure, or may be a sign of a normal adaptation to the disease [23]. Indeed,
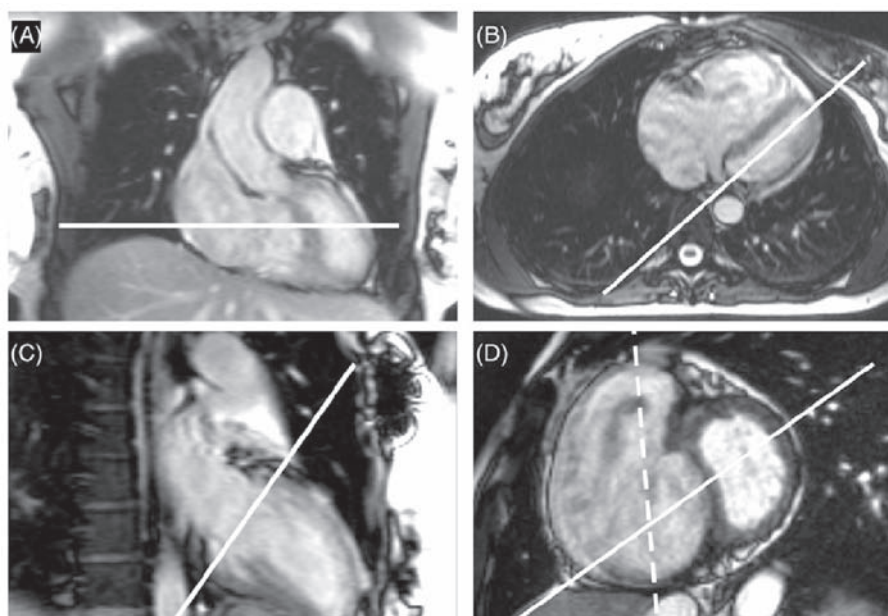
Figure 1.2 Consecutive steps in the planning of the stack of short-axis cines. (A) Coronal view, (B) transverse view, (C) vertical long-axis view, and (D) short-axis view. The white lines indicate the projection lines used for planning the next step in localizing. The line on the coronal view defines the transversal view, and similarly the next views are obtained. Signs of PAH are enlarged right atrium [(A) and (B)] and pulmonary artery (A), leftward ventricular septal bowing [(A), (B) and (D)], and increased pericardial fluid [(B) and (D)] (with permission from [27]).

a recent study investigating the prognostic value of different MR parameters at baseline and their change during therapy in 64 idiopathic PAH patients showed that the prognostic value of RV mass is limited in comparison to RV end-diastolic volume and stroke value [24]. A decrease in right ventricle end-diastolic volume and an increase in stroke volume during therapy reflected an excellent long-term prognosis in this study. The finding that RV end-diastolic volume is an important parameter to look for during therapy is in agreement with an earlier echocardiographic study [20]. The curvature of the IVS can also be calculated from these short-axis cines and this was shown to have a close relationship with the PA pressure [25]. To derive indices of RV diastolic function, additional long-axis cine images are required with sufficient temporal resolution (of the order of 15 ms) to visualise the pulmonary and tricuspid valves in cine mode. Although the investigation of the diastolic function of the RV has until now been limited, one study indicates that RV diastolic dysfunction is a measure of disease severity in PAH, and can be improved by medication [26] (Figure 1.3).

PULMONARY ARTERY FLOW AND DISTENSIBILITY

In order to obtain the flow characteristics of the PA, phase-contrast flow quantification in the main PA, in an image plane perpendicular to the main PA, is required (Figure 1.4).

The flow pattern in the PA contains significant information on the characteristics of the pulmonary vascular bed [27], although this has been poorly studied by MRI. In addition,
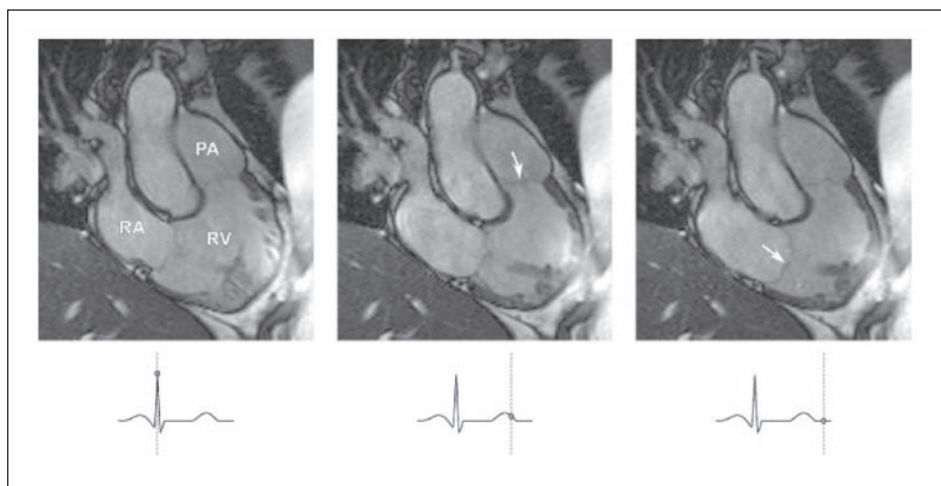
Figure 1.3 MRI RV, two-chambered cine images of a PH patient. The ECG marks the time-points within the cardiac cycle where the MRI images were obtained. The images were acquired at end-diastole (left), the onset of pu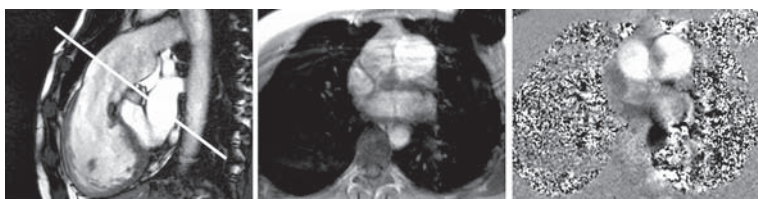lmonary valve closing (middle), and tricuspid valve opening (right). The white arrows depict pulmonary valve closing (middle) and tricuspid valve opening (right). The images were acquired 0 ms (left), 311 ms (middle), and 457 ms (right) from the ECG R wave, with an R-R interval of 622 ms. PA = pulmonary artery; RA = right atrium; RV = right ventricle (with permission from [26]).

Figure 1.4 Magnetic resonance phase-contrast flow quantification in the main pulmonary artery. (A) Planning on the RV outflow tract cine, (B) magnitude image, (C) velocity-encoded image. Signs of PAH are hypertrophic RV (A) and enlarged pulmonary artery (all images) (with permission from [27]).

stroke volume can be calculated from the integrated area under the flow curve. Two MRI-based studies using stroke volume measurements derived from the pulmonary flow curve showed that stroke volume can be used to monitor therapy and contains important prognostic information [24, 28].

Another characteristic of the pulmonary vascular bed is the distensibility of the large pulmonary vessels. A recent study showed that increased stiffness of these vessels assessed by MRI is associated with a poor outcome in PAH [29] (Figure 1.5).

MR PULMONARY ANGIOGRAPHY AND PERFUSION MEASUREMENTS

Although digital subtraction angiography of the PA is still regarded as the reference technique for the diagnosis of chronic thromboembolic PH, recent studies showed that MR angiography is a sensitive non-invasive alternative for the depiction of central thromboembolic material [30]. Typically, one static 3D image acquisition is performed, tailored for spatial resolution and signal-to-noise during a breath hold of about 15 seconds. The advantage of MRI is not only that it provides high quality 3D images of the pulmonary vasculature, but also that these measurements can be combined with the assessment of RV function and
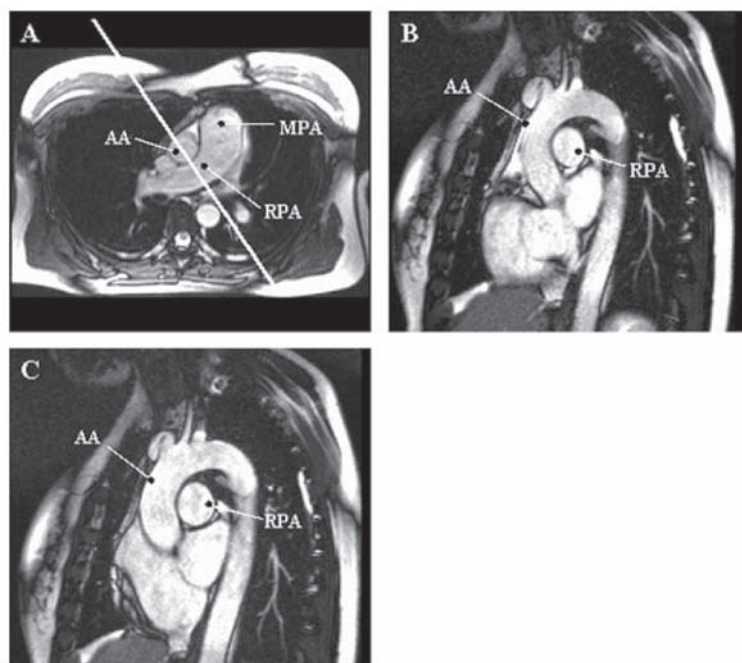
Figure 1.5 Top left, A: Transverse MRI of a patient showing the ascending aorta (AA), main pulmonary artery (MPA), and the right pulmonary artery (RPA). The white line, orthogonal to the right pulmonary artery, displays the position of the image plane applied for cine imaging of the RPA cross-section. Top right, B: Right pulmonary artery cross-section with trigger delay of 0 ms. Bottom, C: Right pulmonary artery cross-section with trigger delay of 298 ms (with permission from [29]).

perfusion measurements [31]. MRI-based dynamic pulmonary perfusion imaging is a technique visualizing the passage of a contrast bolus of MRI contrast agent through the lungs in a 3-dimensional way, enabling the visualisation of subsegmental perfusion defects in chronic thromboembolic PAH [32]. In addition, post-processing of the perfusion images make it possible to quantify regional pulmonary blood flow, blood volume and mean transit time [33] (Figure 1.6).

NEW DEVELOPMENTS

The MRI protocol can be extended with more advanced techniques that have recently been used for the study of the RV in PAH. Examples are delayed contrast enhancement imaging and myocardial tagging. Delayed contrast enhancement imaging is performed about 10 minutes after injection of MRI contrast agent. In healthy myocardium, the contrast agent has washed out, but in non-viable myocardium the contrast agent is still present in the damaged and fibrotic tissue. Thus, 'bright is dead' with this technique. In a study in PAH, delayed contrast enhancement was observed at the insertion regions of the RV to the septum and LV wall [34]. Presumably, this delayed enhancement is a manifestation of regional myocardial injury.

Myocardial tagging is a MRI method to label the myocardial tissue with parallel lines or a grid (typical distance 7 mm) of magnetic presaturation at the beginning of the cardiac cycle. These lines remain visible as 'dark' lines in MRI cine images and thereby display the myocardial strain over the cardiac cycle by changes in the line- or grid-pattern. In PAH, the
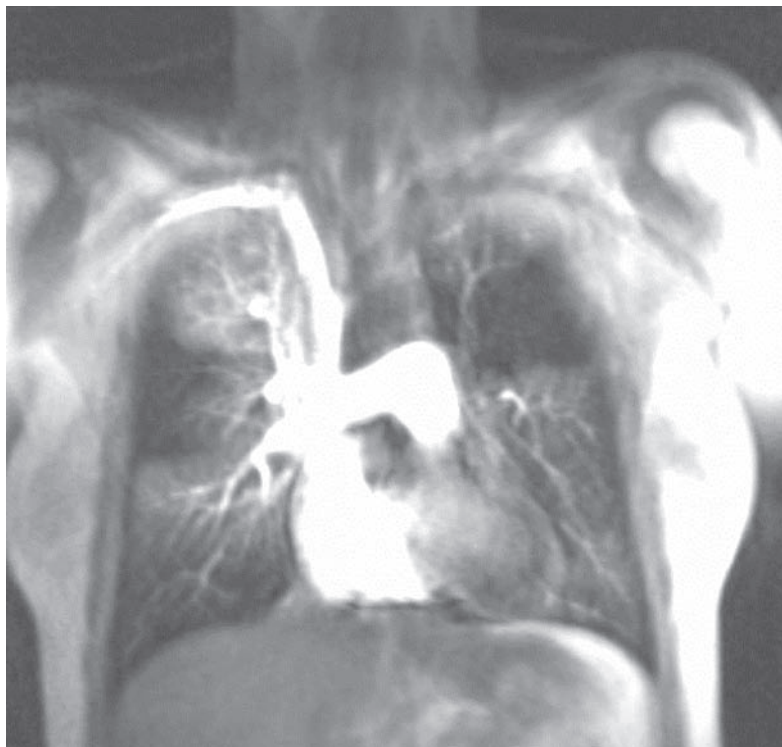
Figure 1.6 Example image of dynamic pulmonary perfusion in a patient with chronic thromboembolic pulmonary hypertension. One slice position is shown, at the ninth temporal phase of the 3D acquisition. Wedge-shaped perfusion deficits are manifest.

RV myocardial wall is thick enough to explore with this tagging technique. In a study in PAH patients, it was shown that there is a left to right asynchrony in the peak of circumferential shortening, which is caused by RV overload and plays a role in the leftward septal bowing and impaired LV filling [35].

COMPUTED TOMOGRAGPHY (CT)

CT scanning is a rapidly evolving technique in cardiovascular imaging. Recent technical advances such as the development of multislice CT (MSCT) and multidetector-row CT make it possible to measure RV volumes and function in an acceptable period of time [36, 37]. Although the main role of CT in phenotyping PH is well established, the role of CT in the longitudinal assessment of PAH is largely unexplored. However, it is reasonable to expect a similar imaging quality of the right ventricle with the now widespread availability of 64-slice scanners, permitting ECG-gated cardiac imaging and cardiac function in comparison to MRI. An advantage of multidose CT over MRI is that imaging of the right ventricle, pulmonary vessels and perfusion can be combined with high resolution imaging of the lung parenchyma in an acceptable timescale. A disadvantage of this technique is the high radiation doses required. At this point in time, the application of MSCT in the study of the right ventricle and PH remains limited.

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2

Exercise testing and haemodynamics

R. J. Oudiz

INTRODUCTION

EXERCISE PHYSIOLOGY IN PULMONARY ARTERIAL HYPERTENSION

Pulmonary arterial hypertension (PAH) is a disease of the pulmonary circulation, often termed a pulmonary vasculopathy. Several mechanisms are important in inducing dyspnoea and fatigue with exercise in patients with PAH (Figure 2.1). A major factor is that O_2 delivery to muscles fails to increase normally in response to exercise, because pulmonary blood flow and therefore left ventricular output does not increase appropriately. In patients with pulmonary vascular disease, the blunting of the increase in cardiac output (or pulmonary blood flow) is reflected in the blunting of the increase in oxygen consumption ($\dot{V}O_2$), which is inadequate for meeting the increased demands of cellular respiration in response to exercise [1].

The reason for the inadequate O_2 transport is that the right ventricle fails to overcome the increased Pulmonary vascular resistance (PVR) to sufficiently increase pulmonary blood flow to meet the muscle O_2 demands of exercise. In the presence of inadequate O_2 delivery, anaerobic glycolysis is required to provide high energy phosphate for the exercising muscles; thus lactic acidosis ensues. This increases CO_2 production relative to O_2 consumption due to release of CO_2 from bicarbonate as it buffers lactic acid. This additional CO_2 production and the metabolic acidosis add to the ventilatory drive. Eventually, the source of ATP regeneration from anaerobic glycolysis is inadequate. Consequently, muscular contraction cannot be maintained, and the muscles fatigue.

The pulmonary vasculopathy in PAH also results in an increase in the resting ventilatory dead space fraction of the tidal volume (VD/VT) and its failure to decrease with exercise, because the regions of ventilated lung that are underperfused at rest remain underperfused during exercise, despite an increased pulmonary artery pressure. This decrease in ventilatory efficiency increases the ventilatory requirement of PAH patients during exercise and further contributes to the symptoms of dyspnoea.

In addition to VD/VT, increased lactic acidosis and CO_2 production, a third ventilatory stimulus is often present, arterial hypoxaemia. The latter is especially marked in patients who develop a right-to-left shunt through a patent foramen ovale during exercise [2]. If right-to-left shunting occurs during exercise, the functional dead space is further increased, which further increases the ventilatory requirement. This is because $PaCO_2$ is regulated near resting levels during exercise, despite the shunting of venous CO_2-rich blood into the arterial circulation [3]. The shunting results in a reduced arterial O_2 saturation, along with an increased arterial CO_2 and H^+ stimulus to the carotid bodies. The increases in arterial PCO_2 and H^+ are barely measurable because of the simultaneous hyperventilation of the pulmonary

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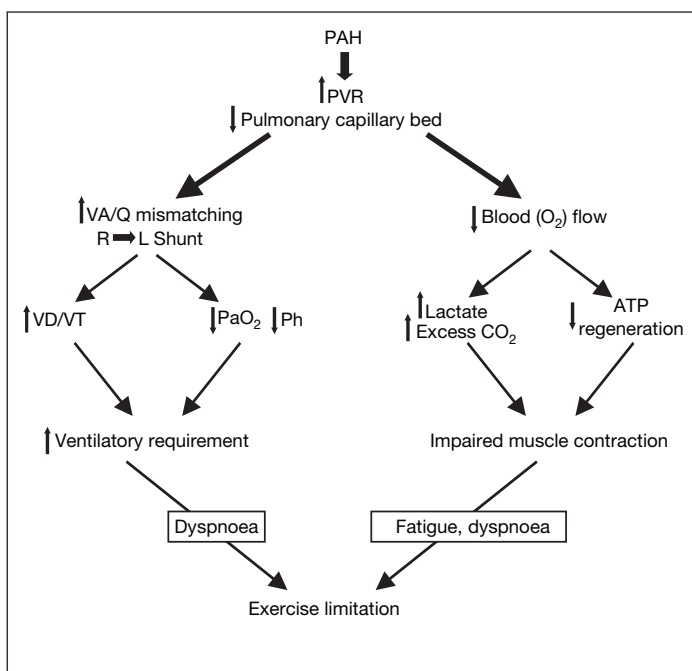
Figure 2.1 Pathophysiology of exercise limitation in PAH patients (adapted with permision from [5]). Longer arrows show pathways leading to dyspnoea and fatigue with exercise. Shorter arrows indicate how each response differs from normal. Once the pulmonary vasculopathy of PAH ensues, a loss of pulmonary capillary bed and an increase in PVR are manifest. These changes produce two important consequences: V/Q mismatching and decreased pulmonary and systemic blood flow. The V/Q mismatching leads to an increased ventilatory requirement and the symptom of dyspnoea, while the decrease in blood flow leads to impaired muscle contraction and the symptom of fatigue. ATP = adenosine triphosphate; L = left; PaO_2 = arterial O_2 pressure; PPH = primary pulmonary hypertension; PVR = pulmonary vascular resistance; R = right; VA/Q = alveolar ventilation/perfusion ratio; VD/VT = dead space volume/tidal volume ratio.

blood flow due to stimulation of chemoreceptors sensitive to the H^+ stimulus. These added stimuli to ventilatory drive presumably promote the sensation of dyspnoea.

In summary, the symptoms of dyspnoea and fatigue in PAH result from:

1. An inadequate increase in O_2 transport to tissues during exercise; this can be measured with cardiopulmonary exercise testing (CPET) as a decrease in peak and anaerobic threshold (low work rate lactic acidosis) compared to normal.
2. Early lactic acidosis, causing increased CO_2 output and ventilatory drive, measured as a decrease in the anaerobic threshold, or AT.
3. Underperfusion of ventilated lung, measured as a high at the AT [4] (reflects 'ventilatory efficiency').
4. Arterial hypoxaemia present without or with right-to-left shunting through a patent foramen ovale. The latter is particularly frequent in PAH because right atrial pressure increases during exercise allowing anyone with a potentially patent foramen ovale (in about 45% of patients with PAH [2]) to develop marked exercise hypoxaemia.

The physiologic abnormalities described above represent the underlying cardiopulmonary impairments in PAH, and can be objectively quantified with CPET, which can be useful

for describing the cardiopulmonary impairments as well as for measuring PAH severity [5, 6], and for prognosis [7].

HAEMODYNAMICS IN PAH

The definition of PAH is a haemodynamic one. Haemodynamics have been assessed in PAH patients using non-invasive and invasive measurements [8]. Despite reports of good correlation between estimated pulmonary arterial pressure (PAP) by echocardiography and by cardiac catheterisation [9, 10], obtaining reliable haemodynamic estimates using echocardiography in PAH patients is not usually feasible [11, 12] and cannot reliably estimate left ventricular diastolic pressure, an essential measurement for diagnosing PAH [8]. Thus, the gold standard for documentation of the presence of PAH remains cardiac catheterisation. Catheterisation is important not only for documenting an abnormally elevated PAP and ensuring that left-sided heart disease is not present as a cause or contributor to PAH [8], but also for prognostication [13].

EXERCISE HAEMODYNAMICS

While not yet definitively proven, it is likely that the initial development of pulmonary vascular disease results in progressively increasing PVR, which ultimately leads to the right ventricle having to hypertrophy to generate more contractile force in order to maintain pulmonary blood flow. Thus, pulmonary hypertension (PH) may not initially be present under resting conditions, and pulmonary vascular disease may only be detectable when the demands of increased pulmonary blood flow, such as during exercise, 'unmask' its presence [14]. The currently accepted haemodynamic definition of PAH thus encompasses either overt resting PAH (mean pulmonary arterial pressure at rest >25 mmHg) or exercise-induced PAH (mean pulmonary arterial pressure during exercise >30 mmHg) [15].

There are few data to validate this 30 mmHg threshold as an accurate predictor of PAH severity. In fact, some studies suggest that, at least at rest, the PAP itself does not predict survival in PAH. Rather, right atrial pressure and cardiac output may be more important predictors of outcome [13, 16]. This is likely because most of these studies only measured resting PAP. Clearly, because PAH is a disease manifested by impaired pulmonary blood flow with exercise (exertional dyspnoea is the most frequent presenting symptom of PAH [17, 18]), there is a need to better define the haemodynamic effects of exercise in PAH and to examine the effects of PAH drugs upon exercise haemodynamics.

Unfortunately, there is neither standardisation nor validation of exercise haemodynamics as an endpoint in PAH therapeutic studies. Chemla and colleagues [19] nicely outlined the complexities of understanding the haemodynamic response to exercise in PAH, noting that consideration must be given to arterial and venous resistance partitioning, variable pulmonary arterial pressure/flow relationships and right ventricular function.

WHY MEASURE EXERCISE HAEMODYNAMICS IN PAH?

Haemodynamics measured during exercise in PAH patients may be useful in three particular instances:

1. To evaluate the physiological response to exercise.
2. To unmask the presence of pulmonary vascular disease that is not evident on resting measurements.
3. To evaluate the effects of treatment upon the exercise response.

1. Physiological response to exercise

Sun and colleagues [5] described the pathophysiological response to exercise using CPET. Markowitz and Systrom [6] took this one step further, performing both CPET and simultaneous invasive haemodynamics in 130 patients with exertional symptoms. They defined a pulmonary vascular limit to exercise haemodynamically as a combination of elevated PVR and an impairment in peak oxygen uptake (peak $\dot{V}O_2$ <80% predicted). Although their threshold of peak $\dot{V}O_2$ may be considered somewhat arbitrary, their study found that abnormal ventilatory efficiency agreed with abnormal exercise haemodynamics when pulmonary circulation abnormalities were present. This study used a symptom-limited ramp exercise protocol on a cycle ergometer, with catheterisation of the pulmonary artery via the internal jugular vein.

Frantz and co-workers [20] studied continuous ambulatory haemodynamics using a surgically-implanted haemodynamic monitor in 24 patients with PAH. They found that changes in mean pulmonary artery pressure correlated with changes in 6-minute walk distance (6MWD) at 12 weeks, however the correlation was modest (r^2 <0.5). Nevertheless, this modality provided some insight into the daily fluctuations in haemodynamics, particularly with exercise, during routine ambulation. Moreover, the lack of better correlation of haemodynamics with 6MWD may in fact be due to the inability of the 6MWD to track physiological PAH severity rather than any shortcoming of the haemodynamics under observation.

2. Unmasking pulmonary vascular disease

Exercise-induced PH may represent an early clinical stage of PAH, and thus an opportunity for earlier treatment. James and colleagues [21] studied 13 patients with exertional dyspnoea using pulmonary artery catheterisation at rest and during upright bicycle exercise. Four patients had normal pulmonary artery (PA) systolic pressure, six had elevated PAP at rest that increased further with exercise, and three of the patients had normal PAP at rest but demonstrated elevated PAP during exercise, to the same degree as the six patients with elevated resting PAP. This study suggests that a certain percentage of patients with exertional dyspnoea may not manifest abnormal PAP at rest and that, unless exercise is used to 'unmask' pulmonary vascular disease, the diagnosis of PAH and the opportunity for treatment may be missed. Indeed, Raeside and colleagues [22] showed that ventilatory inefficiency as measured by CPET correlates with the elevation of PAP with exercise, thus corroborating the concept that impaired pulmonary blood flow during exercise can be detected non-invasively, possibly at an earlier stage.

Steen and colleagues [23] evaluated the use of exercise echocardiography with confirmatory invasive exercise haemodynamics for identifying PAH in 54 patients with scleroderma. They defined an abnormal response to exercise as an increase of >20 mmHg in right ventricular (RV) systolic pressure estimated by echocardiography. Almost half of the patients with suspected PH had an abnormal echo response to exercise. Of these, 19% had resting PH, while 61% had PH with exercise, defined during invasive haemodynamics by a mean PAP >30 mmHg and pulmonary artery occlusion pressure (PAOP) >18 mmHg at peak exercise. The invasive exercise measurements were performed in the supine position by lifting dumbbells with both arms until either exhaustion or 85% of predicted maximum heart rate was reached first.

Finally, Grünig and colleagues [14] hypothesised that asymptomatic gene carriers could be detected by an abnormal haemodynamic response to exercise. They found that an abnormal increase in PA pressure correlated with the presence of the family's risk haplotype in family members of patients with familial PAH. Their protocol used supine bicycle exercise echocardiography.

3. Treatment effects and exercise haemodynamics

To date, none of the approved PAH drugs have been extensively studied for their effects upon exercise haemodynamics. Provencher and colleagues [24] demonstrated only fair cor-

Table 2.1 Exercise haemodynamic protocols used in PAH patients

Exercise protocol	Haemodynamic modality	Reference
Dumbbell weight lifting	Echocardiography, confirmation with catheterisation	[23]
Supine bicycle	Echocardiography	[14]
Ramp CPET	Catheterisation	[6]

CPET = cardiopulmonary exercise testing.

Table 2.2 Selected physiological parameters and exercise variables contributing to inconsistencies of the exercise response

Parameter	Comments
Subject fitness/activity level	Deconditioning may affect haemodynamic response
Subject body habitus	Lower extremity work against gravity may affect
WR delivery	Constant vs incremental (ramp) WR may produce a more steady-state response; haemodynamic response may also depend on slope of ramp
Exercise modality	Cycle vs treadmill affects physiological response
Body position	Upright vs recumbent vs supine

WR = work rate.

relation between change in exercise haemodynamics and change in exercise tolerance after treatment of 42 patients with idiopathic PAH. This may have been due in part to a lack of standardisation of the exercise protocol, as well as a lack of validation documenting the clinical utility of these measures.

Castelain and co-workers [25] studied rest and exercise haemodynamics in seven patients with idiopathic PAH before and 6 weeks after treatment with epoprostenol. Interestingly, although exercise capacity improved, resting haemodynamics did not. However, the pressure–flow relationship expressed as the slope of mean PAP versus cardiac index during exercise decreased after treatment, indicating an improvement in PVR (pressure divided by flow) during exercise in response to epoprostenol.

An important application of exercise haemodynamics would be to prove the hypothesis that early PAH manifested by an elevated exercise PAP in the face of a normal resting PAP is a significant clinical entity and that (early) treatment can improve clinical outcome. Such a study has not been done, but the above studies provide strong rationale for such a study.

STANDARDISATION OF AN EXERCISE PROTOCOL FOR HAEMODYNAMIC ASSESSMENTS

Table 2.1 lists selected studies involving PAH patients and measurements of exercise haemodynamics. Unfortunately, there is non-uniformity among these studies with respect to the exercise protocol used, making direct comparison of the findings impossible.

Table 2.2 lists selected parameters and exercise protocols that may modulate the physiological response to exercise. The considerable variability in these parameters and protocols makes generically comparing haemodynamic responses to exercise among individuals an unsound strategy. Thus, a standardised protocol based on what is already known about the physiology of exercise and haemodynamics might normalise the exercise response for most

individuals and allow proper interpretation of the haemodynamics. Such a protocol has not yet been developed for the PAH population and is long overdue.

It is logical then to turn to CPET for obtaining an exercise 'prescription' for haemodynamic assessments based on the subject's aerobic and ventilatory response, and also for modelling the exercise protocol itself. A constant work rate (WR) test during the haemodynamic measurements would be preferable to a ramp test, as this would ensure that a steady-state is achieved prior to measuring haemodynamics. The WR should be chosen to correspond with the subject's AT (based on a prior CPET performed using a ramp WR) to allow adequate cardiovascular stress, avoiding early exhaustion or prolonged testing periods.

Several considerations must be given to the design of a proper exercise haemodynamic protocol, some of which may not be alterable. These are shown in Table 2.2. Going forward, both for clinical trials and clinical practice, consensus-driven standardisation of an exercise haemodynamic protocol should be sought.

SUMMARY

The physiology of exercise in PAH patients is fairly well understood, however the haemodynamic response to exercise in these patients has yet to be well characterised. Dynamic measurements of pressure–flow relationships may be the ideal measures of the haemodynamic response to exercise and may provide both insight into the mechanisms of improvement seen with PAH drugs as well as a useful endpoint for clinical trials of new PAH drugs. A standardised, consensus-driven protocol with appropriate definitions of normal and abnormal responses to exercise needs to be developed in order to properly quantify haemodynamic severity in PAH and provide a means for documenting patient responses to new therapies.

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3

Epidemiology of pulmonary arterial hypertension

M. Humbert

DEFINITION AND CLASSIFICATION OF PULMONARY ARTERIAL HYPERTENSION

Pulmonary arterial hypertension (PAH) is a rare disease (i.e. one that affects less than 1 in 2000 individuals in the general population) [1]. By nature, rare diseases impact on only a small number of patients, families and healthcare providers and this often has negative consequences for the patients who may suffer from less than optimal care [1]. Rare respiratory diseases are no exception and it is time to improve awareness as well as understanding of the epidemiology, pathophysiology and management of these conditions [1]. PAH describes a group of rare pulmonary diseases causing breathlessness, loss of exercise capacity and death due to elevated pulmonary artery pressure and subsequent right-heart failure [2–4]. PAH is characterised by remodelling of the small pulmonary arteries leading progressively to their obstruction [2–4]. Although cardiac echo-Doppler is an excellent tool for screening of the condition, a definite diagnosis of PAH requires strict right-heart catheter criteria [2, 3]. Indeed, PAH is defined by an elevation of the mean pulmonary artery pressure (mPAP) above 25 mmHg at rest and/or 30 mmHg during exercise without elevation of the pulmonary capillary wedge pressure (<15 mmHg), and elimination of frequent causes such as hypoxia, respiratory diseases and thromboembolic disease [2, 3]. Half of PAH cases referred to pulmonary vascular centres have no identifiable risk factor, corresponding to idiopathic (sporadic) and familial PAH. The other PAH subcategories include a number of associated conditions (connective tissue diseases, congenital heart diseases, portal hypertension and human immunodeficiency virus [HIV] infection) or exposure to appetite suppressants and other drugs and toxic agents [3, 5] (Table 3.1).

Right-heart catheterisation and pulmonary vasoreactivity testing are established and safe diagnostic tools that should be performed in all patients with PAH [2, 5]. Less invasive tools such as cardiac echo-Doppler are of interest for PAH screening, but one has to bear in mind that a significant proportion of patients with echocardiography parameters compatible with PAH may have a strictly normal pulmonary circulation and/or another condition mimicking PAH such as left-heart diastolic disease [6]. This was well emphasised in a recent multicentre cross-sectional analysis of 599 patients with systemic sclerosis where only 18 of 33 patients with an echo-Doppler compatible with PAH had a definite diagnosis of PAH after right-heart catheterisation, while 3 of 33 had diastolic left-heart dysfunction and 12 normal or near-normal values [6]. These data explain why pulmonary vascular specialists do not rely on inexpert PAH diagnosis but always require strict measurements to confirm the diagnosis.

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Table 3.1 Clinical classification of pulmonary hypertension (with permission from [3])

1. Pulmonary arterial hypertension
  • Idiopathic PAH
  • Familial PAH
  • Associated with:
    – connective tissue diseases
    – congenital systemic to pulmonary shunts
    – portal hypertension
    – HIV infection
    – drugs and toxins
    – other: thyroid, glycogen storage, or
      Gaucher disease; HHT; MPD;
      haemoglobinopathies; splenectomy
  • PAH with venous or capillary involvement
    (PVOD, PCH)
  • PPHN
2. PH with left heart disease
  • Atrial or ventricular
  • Valvular
	3. PH with lung diseases / hypoxaemia
	  • COPD
	  • Interstitial lung diseases
	  • Sleep-disordered breathing
	  • Chronic exposure to high altitude
	  • Developmental abnormalities
	**4. PH due to chronic thrombotic
	   and/or embolic disease**
	  • TE obstruction of proximal PA
	  • TE obstruction of distal PA
	  • Non-thrombotic pulmonary embolism
	    (tumour, parasitis, foreign material)
	5. Miscellaneous
	  Sarcoidosis, histiocytosis X, LAM,
	  compression of PV (tumour adenopathy,
	  fibrosing mediastinitis)

COPD = chronic obstructive pulmonary disease; HHT = hereditary haemorrhagic telangiectasia; LAM = lymphangioleiomyomatosis; MPD = myeloproliferative disorders; PA = pulmonary arteries; PAH = pulmonary arterial hypertension; PCH = pulmonary capillary haemangiomatosis; PPHN = persistent pulmonary hypertension of the newborn; PV = pulmonary vessels; PVOD = pulmonary veno-occlusive disease; TE = thromboembolic.

DATA FROM THE FRENCH REGISTRY OF PULMONARY ARTERIAL HYPERTENSION

Information on the natural history of PAH is derived from a national registry produced in the United States of America in the early 1980s, where 187 patients with primary pulmonary hypertension (corresponding to idiopathic PAH in the recent classification) were described and followed for up to 5 years [7, 8]. This study confirmed that 'primary' pulmonary hypertension has a very poor prognosis, with a median survival of 2.8 years post-diagnosis [8]. Significant medical advances have occurred in the last 15 years, including a more systematic assessment of patients with objective parameters (e.g. the 6-minute walk test and acute vasodilator challenge) and availability of new treatments (e.g. prostacyclin, endothelin receptor antagonists and type 5 phosphodiesterase inhibitors) [9, 10]. Prompted by the rapid evolution of knowledge in the field of PAH and the absence of a multicentre registry since the 1980s, the French Reference Centre for Pulmonary Arterial Hypertension (Hôpital Antoine Béclère, Clamart, France) set up a French Network on Pulmonary Arterial Hypertension in the year 2000 [5]. This network initiated a national prospective registry with the goals of collecting data on PAH patients in the modern era and describing the evolution of PAH during a 3-year follow-up. In this recent registry, established in 2002–2003, we studied 674 patients with a strict catheter diagnosis of PAH in a network of 17 university pulmonary vascular centres situated throughout the country [5].

The female/male sex ratio was 1.9. Our registry confirmed the female predominance in most subtypes of PAH including idiopathic, familial, anorexigen-, connective tissue disease- and congenital heart disease-associated cases [5]. PAH associated with portal hypertension or HIV infection was characterised by a moderate male predominance, presumably reflecting the epidemiology of portal hypertension and HIV infection in France. This registry also underlined that PAH may develop at all ages, with one-quarter of cases occurring after the age of 60 years, and that the condition may be first detected in patients in their eighties [5].
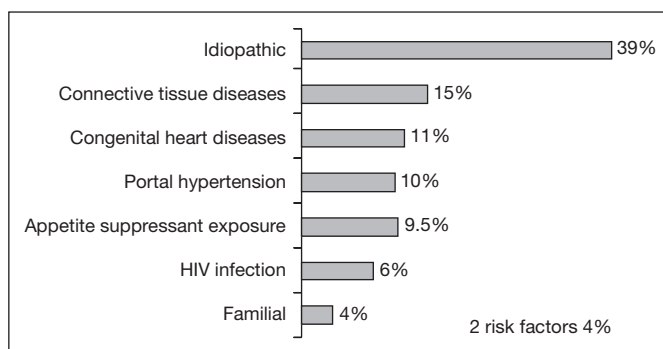
Figure 3.1 Distribution of patients with pulmonary arterial hypertension in the 2002–2003 French Registry (with permission from [5]). HIV = human immunodeficiency virus.

Indeed, the mean (±SD) age of patients enrolled was 50±15 years and was similar for both females and males. A significant proportion of the population was older than 70 years at the time of diagnosis (9.1%). Body mass index (BMI) was normal (24.4±5.5 kg/m^2). A BMI >30 was observed in 14.8% of cases, a proportion similar to that of the adult French population as a whole [5].

More than half of enrolled patients (52.6%) presented with idiopathic (39.2%), familial (3.9%) or anorexigen-associated PAH (9.5%), connective tissue diseases, congenital heart diseases, portal hypertension and HIV infection corresponded to 15.3%, 11.3%, 10.4% and 6.2% of the population, respectively (Figure 3.1). Among connective tissue diseases, systemic sclerosis and systemic lupus erythematosus were the two leading causes, representing 76% and 15% of the cases, respectively. Two-thirds of the systemic sclerosis cases were limited forms and one-third were diffuse forms. Twenty-nine patients (4.3%) displayed two coexisting conditions known to be associated with PAH. HIV infection and portal hypertension were the most common coexisting conditions (n = 13).

Germline mutations in the bone morphogenetic protein receptor 2 (*BMPR2*) gene are detected in at least 70% of familial PAH cases [11] and *BMPR2* mutations can also be detected in 11–40% of apparently sporadic cases [11]. The distinction between idiopathic and familial *BMPR2* mutation carriers may thus be artificial, as these subjects have an inherited condition and may all correspond to a potential familial disease. Recent expert discussion favours the use of the term 'heritable' PAH to describe these genetic forms of the disease. In any case, *BMPR2* mutation represents the major genetic predisposing factor for PAH. However, the penetrance of *BMPR2* mutations is low (around 20%), and neither the factors involved in the initiation of the disease in affected subjects, nor the precise molecular mechanisms underlying the responsibility of *BMPR2* haploinsufficiency in the disease are identified [12]. Previous reports have suggested that the clinical and haemodynamic presentation of familial PAH was not different from idiopathic PAH, with similar pathological lesions as well as molecular and cellular abnormalities described in both subtypes [12]. However, a significant proportion of so-called idiopathic cases were in fact associated with *BMPR2* mutations and this might have given a biased comparison [11]. Recent findings have indicated that *BMPR2* mutation carriers with familial or idiopathic PAH were less likely to display vasoreactivity than non-carriers, raising the possibility that monoallelic *BMPR2* mutation identifies patients who may respond poorly to long-term vasodilator therapy [13]. In a recent analysis of patients from the French Registry, we hypothesised that a mutated *BMPR2* status might be associated with distinct disease phenotypes. To test this hypothesis, the French Network of Pulmonary Hypertension obtained data on consecutive patients displaying idiopathic or familial PAH in whom point mutation and large size rearrangements of *BMPR2* were screened for [11].
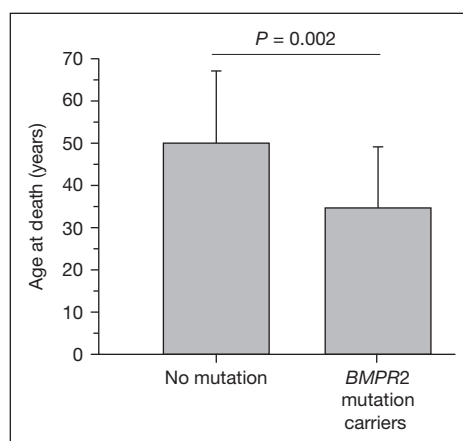
Figure 3.2 Age at death in patients with pulmonary arterial hypertension according to *BMPR2* mutation status (with permission from [11]). *BMPR2* = bone morphogenetic protein receptor 2.

Clinical, functional and haemodynamic characteristics, as well as outcomes, were compared in *BMPR2* mutation carriers and non-carriers. Sixty-eight *BMPR2* mutation carriers (28 familial and 40 idiopathic PAH) were compared to 155 non-carriers (all displaying idiopathic PAH). When compared with non-carriers, *BMPR2* mutation carriers were younger at diagnosis of PAH (36.5±14.5 versus 46.0±16.1 years; P <0.0001), had higher mean pulmonary artery pressure (64±13 versus 56±13 mmHg; P <0.0001), lower cardiac index (2.13±0.68 versus 2.50±0.73 l/min/m²; P = 0.0005), higher pulmonary vascular resistance (17.4±6.1 versus 12.7±6.6 mmHg/l/min/m²; P <0.0001), lower mixed venous oxygen saturation (59±9% versus 63±9%; P = 0.02), shorter time to death or lung transplantation (P = 0.044), younger age at death (P = 0.002), but similar overall survival (P = 0.51) [11]. *BMPR2* mutation carriers with PAH present approximately 10 years earlier than non-carriers with a more severe haemodynamic compromise at diagnosis [11]. Age at death was more than 10 years younger in patients with a *BMPR2* mutation as compared to non-carriers [11] (Figure 3.2).

The use of anorexigens (mainly aminorex and fenfluramine derivatives) has been associated with an increased risk of PAH in studies performed in Europe and North America [14–16]. This proportion was still 3% in incident cases in 2002–2003, more than 5 years after the withdrawal of fenfluramine derivatives from the French market. As previously indicated by Abenhaim and colleagues [16], obesity was not a confounding factor explaining appetite suppressant exposure in our population, as the distribution of BMI was similar in idiopathic and anorexigen-associated PAH patients, and in a proportion similar to that of the adult French population as a whole. The duration of exposure and the delay between the last anorexigen intake and the first symptoms of PAH varied markedly between cases, indicating that anorexigen-associated PAH could be described even after short exposures of less than 3 months and a long time after last anorexigen intake [5]. A history of anorexigen exposure was found in 9.5% of patients with PAH, 77% of cases corresponding to fenfluramine derivatives. The duration of exposure to fenfluramine derivatives ranged from 1 to 300 months, 15.3% being exposed for less than 3 months, 19.4% from 3 to 6 months, 36.1% from 6 to 12 months and 29.2% more than 12 months. The delay between the last intake of appetite suppressant and the first symptoms of pulmonary hypertension was within 2 years of exposure in 24.2%, from 2 to 5 years in 32.3% and more than 5 years in 43.5% of cases [5].

More recently, the author and colleagues retrospectively studied the records of all patients with a diagnosis of fenfluramine-associated pulmonary arterial hypertension (fen-
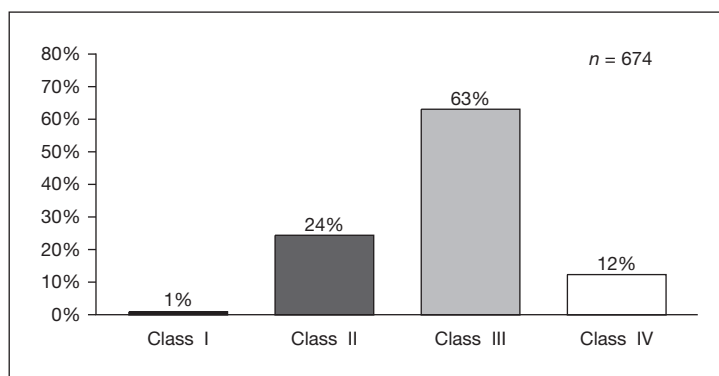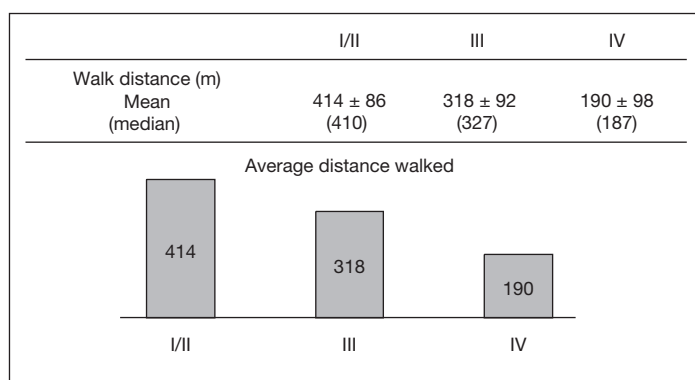
Figure 3.3 New York Heart Association functional class at diagnosis in patients with PAH from the French Registry (with permission from [5]).

PAH) evaluated at our centre between 1986 and 2004 [17]. Baseline clinical and haemodynamic data were collected, as well as the survival time. The median duration of exposure was 6 months, with 4.5 years between exposure and the onset of symptoms. Nine of 40 patients (22.5%) evaluated for the presence of germline *BMPR2* mutations were positive. In these patients, duration of exposure to fenfluramine was significantly lower than in patients without mutation ($P = 0.007$) [17]. Median survival was 6.4 years with no significant difference between fen-PAH and a control group of idiopathic and familial PAH patients referred to our centre over the same timeframe and treated identically. The duration of fenfluramine exposure was not related to survival, whilst the cardiac index was the only independent predictor at multivariate analysis [17]. Thus fen-PAH shares clinical, functional, haemodynamic and genetic features with idiopathic PAH, as well as the same overall survival. It has therefore been concluded that fenfluramine exposure characterises a potent trigger for PAH without influencing its clinical course [17].

Systemic sclerosis was the leading cause of PAH among connective tissue diseases [5]. In systemic sclerosis, the occurrence of PAH is known to have a major impact on outcome and survival [18]. Since more than 10% of patients with systemic sclerosis will develop PAH, an early identification of this complication by means of a systematic echocardiography-based screening programme is recommended [6]. When such strategies are widely applied, it is likely that larger numbers of PAH cases will be reported, thus increasing prevalence of this condition. Similarly, congenital heart diseases were certainly underrepresented in the present registry performed in pulmonary vascular centres. This presumably reflects the fact that these patients have a relatively stable disease course and that they are rarely referred to pulmonary vascular centres, unless they need to be listed on a heart–lung transplantation programme or treated with complex specific PAH therapies such as continuous intravenous epoprostenol. Thus, we assume that only a subset of patients with systemic sclerosis or congenital heart diseases with PAH were followed-up in pulmonary vascular centres in France in 2002–2003.

In the whole cohort of patients, delay between the onset of symptoms and diagnosis was 27 months and a majority of patients had severe symptoms at presentation, with 75% in New York Heart Association (NYHA) functional class III or IV (1% in class I, 24% in class II, 63% in class III and 12% in class IV) (Figure 3.3). Exercise capacity had been evaluated at the time of diagnosis through a 6-minute walk test which was abnormal in most patients, and as low as 60% and 55% of reference values for men and women, respectively [19]. Six-minute walk distance correlated with NYHA functional class (Figure 3.4) in all forms of PAH, except for HIV-associated PAH, possibly due to the small size of the subgroup [5].

	I/II	III	IV
Walk distance (m) Mean (median)	414 ± 86 (410)	318 ± 92 (327)	190 ± 98 (187)

Average distance walked

Figure 3.4 Six-minute walk distance correlates with New York Heart Association functional class at diagnosis in patients with PAH from the French Registry (with permission from [5]). I/II, III, IV = New York Heart Association functional class I/II, III and IV.

Right-heart catheterisation demonstrated a severe haemodynamic compromise, which correlated with clinical severity assessed by the NYHA functional class (Table 3.2). Acute vasodilator challenge was performed either with inhaled nitric oxide (95.8%), intravenous prostacyclin (2.4%) or both (1.8%). As previously described [20, 21], the rate of acute vasodilator response was low (5.8%), and slightly higher in NYHA functional class I/II patients (9.8%), as compared to patients in functional class III (4.8%) or IV (3.0%) [5].

Based on the 674 PAH cases in our registry, the low estimate of prevalence in France is 15 cases/million adult inhabitants [5]. The low estimate of prevalence for idiopathic PAH is 5.9 cases/million inhabitants. Regional prevalence was evaluated according to the region where the patients lived. A wide variation in PAH regional prevalence was observed, from 5 to 25 cases/million adult inhabitants. In 2002–2003, the low estimate of PAH incidence was 2.4 cases/million adult inhabitants/year. Recent studies have allowed estimation of the prevalence and incidence of PAH. When analysing data from the Scottish Morbidity Record Scheme, a prevalence of 52 PAH cases/million was obtained [22]. Despite the many limitations of this aspect of the study, the first comment is that this number, which may overestimate the true prevalence of the disease, still meets the criteria for a rare disease. Conversely, it is likely that the expert data based on gold-standard procedures from the reference centre (Scottish Pulmonary Vascular Unit) underestimate the true frequency of the disease. Based on the experience of this expert centre, the corresponding prevalence was 26 cases/million in Scotland [22]. Therefore, the prevalence of PAH in Western European currently lies somewhere between 25 and 50/million inhabitants [1, 4, 22].

One-year survival was 88.4% in the whole incident group (*n* = 121) and 89.3% in the group of 56 incident patients with idiopathic, familial and anorexigen-associated pulmonary arterial hypertension [5]. Although preliminary unpublished analysis of the 3-year survival of idiopathic PAH demonstrates that PAH remains a severe life-threatening condition, this 1-year survival data compared favourably with the estimated 1-year survival calculated with the National Institutes of Health (NIH) equation (71.8%) [8].

CONTEMPORARY REGISTRIES IN DEVELOPED AND DEVELOPING COUNTRIES

DEVELOPED COUNTRIES

Prompted by the rapid evolution of knowledge in the field of PAH and the absence of a multicentre registry since the 1980s, prospective registries were initiated in France [5],

Table 3.2 Pulmonary haemodynamics at diagnosis in patients with PAH from the French Registry (with permission from [5]).

	I/II	III	IV	Total
Catheter data available	94%	96%	93%	95%
RAP (mmHg)	6 ± 4	9 ± 5	11 ± 7	8 ± 5
mPAP (mmHg)	51 ± 17	56 ± 15	57 ± 13	55 ± 15
Cardiac output (l/min)	5.0 ± 1.6	4.2 ± 1.4	3.5 ± 1.5	4.3 ± 1.5
SvO$_2$ (%)	67 ± 8	62 ± 8	54 ± 9	63 ± 9
TPR (Wood units)	9.5 ± 6.8	13.0 ± 6.9	16.2 ± 8.2	12.5 ± 7.3

I/II, III, IV = New York Heart Association functional class I/II, III and IV; mPAP = mean pulmonary artery pressure; RAP = right atrial pressure; SvO$_2$ = mixed venous oxygen saturation; TPR = total pulmonary resistance.

Scotland [22] and the United States [23]. A US-based registry from a single large referral centre in Chicago established that PAH patients were referred late to specialised centres in the USA, with 80% in NYHA class III or IV [7]. This registry also emphasised that medical management was often inappropriate, with an excessive use of oral calcium channel blockers in PAH patients showing no acute vasodilator response [7, 9]. Finally, it was apparent from this registry that referral of PAH patients with connective tissue diseases (mainly systemic sclerosis) was increasing, while referral of HIV-infected patients remained low [7]. This latter feature is markedly different from the French registry [5] and presumably indicates the under-appreciation of PAH in HIV-infected patients in the USA. This single tertiary centre registry may not reflect US national trends, which might be better evaluated by the REVEAL (Registry to Evaluate Early and Long Term PAH Disease Management) Registry, a multicentre, observational, industry-sponsored USA-based registry currently enrolling patients in the USA.

Screening programmes
PAH is a condition that is notoriously difficult to diagnose [2, 6]. In the early stages of disease, patients are generally asymptomatic. Initial symptoms including dyspnoea, exercise intolerance and fatigue are often rather unspectacular and may lead patients, relatives and physicians to assume that they are simply 'out of shape'. Later, the described symptoms are often attributed to a more common cardiorespiratory disease. As a result, there is commonly a substantial delay of two or more years in the diagnosis of PAH and initiation of treatment [5]. Thus, early detection of PAH is still inadequate. The implementation of screening programmes targeting high-risk patient groups should help in identifying patients earlier. Recent screening programmes (based on cardiac echo-Doppler evaluation followed by right-heart catheterisation if PAH is suspected) have demonstrated that early diagnosis of PAH is possible in patients displaying HIV infection [23], systemic sclerosis [6], and sickle cell disease [24]. These screening programmes have allowed the diagnosis of patients with markedly lower mean pulmonary artery pressure and pulmonary vascular resistance compared with patients diagnosed with symptomatic PAH [5–8, 23, 24]. These screening programmes have also demonstrated that left-heart disease is common in these patients, emphasizing the importance of a complete evaluation, including right-heart catheterisation, in order to properly distinguish patients with pre-capillary from those with post-capillary pulmonary hypertension [6, 25]. For instance, in a prospective multicentre study of 599 patients with

systemic sclerosis, PAH was confirmed in 8%, while left ventricle diastolic dysfunction was found in 18% [26]. In this study, more than 10% of systemic sclerosis patients with a peak velocity of tricuspid regurgitation >2.5 m/s had evidence of diastolic left-heart dysfunction and post-capillary pulmonary hypertension [6].

Haemodynamics and cardiopulmonary function were evaluated in 43 patients with sickle cell disease, including 26 patients with a mean pulmonary artery pressure of ≥25 mmHg (pulmonary hypertension group) [27]. Upon catheterisation, 54% of the patients with pulmonary hypertension had PAH, while 46% had post-capillary pulmonary hypertension [27]. Thus, evaluating the mechanisms of pulmonary hypertension in patients with sickle cell disease requires a complete evaluation including right-heart catheterisation. In sickle cell patients with PAH, mean pulmonary artery pressure was moderately elevated and the cardiac output was high, in contrast to what is normally seen in idiopathic PAH [5, 27]. Further investigation is warranted to assess the potential benefits and risks of using PAH-specific therapies in sickle cell disease-related pulmonary hypertension [27, 28].

DEVELOPING COUNTRIES

Improving awareness, diagnosis, prevention and treatment

Pulmonary hypertension is certainly much more prevalent than reported in developing countries where relatively common diseases such as schistosomiasis, sickle cell disease, HIV infection, liver cirrhosis, and others including auto-immune and congenital heart disease may promote pulmonary vascular disease [1, 3, 29]. In addition, hypoxia is a major risk factor for pulmonary hypertension with more than 140 million individuals living above 2500 metres worldwide, including 80 million in Asia and 35 million in South America [30]. Improving awareness, diagnosis, prevention and treatment of pulmonary hypertension in developing countries is currently supported by a World Health Organization (WHO) programme of the Global Alliance Against Chronic Respiratory Diseases (GARD) [29, 31]. Pulmonary hypertension is now being formally studied in developing countries such as China and Brazil [29, 32, 33].

THE COMPLEX NATURE OF INTERACTIONS BETWEEN THE PULMONARY AND CARDIOVASCULAR SYSTEMS

Pulmonary hypertension is frequently detected in patients with chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis, sarcoidosis, histiocytosis X, neuromuscular or chest wall disorders, and disorders of ventilatory control including sleep apnoea syndromes and obesity hypoventilation syndrome [34]. In a majority of cases, pulmonary hypertension is mild-to-moderate (20–35 mmHg) but sometimes severe, with major consequences on the right-heart [34–38]. In COPD patients, a mean pulmonary artery pressure exceeding 35–40 mmHg may not just be the consequence of chronic lung disease and is likely to produce symptoms and affect the clinical course [35–37]. Similarly, patients with idiopathic pulmonary fibrosis may develop pulmonary hypertension, which will in turn impact prognosis [38]. There is thus a subset of patients with respiratory diseases with 'out of proportion' pulmonary hypertension who share some clinical features with idiopathic PAH [34–38]. A better description of the mechanisms linking respiratory diseases and 'out of proportion' pulmonary hypertension may help in the understanding of some of the clinical manifestations and the devastating disease course that afflicts some of these patients [38]. Pathological examination of lung explant specimens from patients with end-stage idiopathic pulmonary fibrosis showed thickening of the arterial and venous walls with severe luminal narrowing in dense fibrotic zones in all patients [39]. In architecturally preserved lung zones, occlusion of venules and small pulmonary veins was

observed in 65% of the patients, although there were only mild changes in the muscular pulmonary arteries [39]. This study indicated that in many patients with idiopathic pulmonary fibrosis, non-fibrotic lung areas demonstrated an occlusive vasculopathy, the significance of which remains undetermined. Similarly, pulmonary hypertension may be a severe life-threatening complication of sarcoidosis [40–42]. Understanding the mechanism of pulmonary hypertension is of major importance, since it may be due to cardiac disease leading to post-capillary pulmonary hypertension [41]. In the absence of left-heart disease, different phenotypes can be identified according to the presence or absence of pulmonary fibrosis [42]. In non-fibrotic cases, a specific and sometimes steroid-sensitive vasculopathy may contribute to pulmonary hypertension [42]. In cases with fibrosis, pulmonary destruction, hypoxaemia and extrinsic pulmonary artery compression may be implicated [42]. Similarly, pulmonary hypertension might be related to an intrinsic pulmonary vascular disease in pulmonary histiocytosis X, in which the pulmonary circulation is involved independent of small airway and lung parenchyma injury [43].

THE GLOBAL BURDEN OF PULMONARY HYPERTENSION

It is widely understood that pulmonary hypertension is a rare condition [1]. Although this is true for PAH [1, 5, 7, 22, 23], the global burden of pulmonary hypertension as a whole is currently unknown and largely underestimated. Hypoxia is a major worldwide risk factor for pulmonary hypertension [29]. The predominant causes of hypoxia are inadequate oxygenation of arterial blood as a result of either lung disease such as chronic obstructive pulmonary disease, impaired control of breathing, or residence at high altitude. Indeed, chronic mountain sickness is a public health problem in mountainous regions around the world [29, 30]. In addition, up to 4% of all patients with acute pulmonary embolism may develop chronic thromboembolic disease and pulmonary hypertension [44–47]. Altogether, pulmonary hypertension is certainly underestimated both in developing and developed countries and further well-designed studies are needed to better understand the burden of the disease in populations exposed to a variety of different risk factors [1].

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4

Current treatment of PAH: prostanoids, phosphodiesterase-5 inhibitors and stimulators of soluble guanylate cyclase

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INTRODUCTION

Currently, the mainstays of pulmonary arterial hypertension (PAH) therapy are prostanoids, phosphodiesterase-5 (PDE-5) inhibitors and endothelin receptor antagonists. This chapter will cover prostanoids, PDE-5 inhibitors and a novel class of drugs that act by stimulation or activation of soluble guanylate cyclase (sGC). Endothelin receptor antagonists are dealt with elsewhere in this book.

PROSTANOIDS

Endogenous prostacyclin, an arachidonic acid metabolite of vascular cells, is a potent vasodilator in the pulmonary and the systemic circulation with additional antiplatelet as well as antiproliferative properties [1]. Most of the effects of prostacyclin are mediated via the IP receptor which is coupled to Gs proteins and activates the intracellular second messenger cyclic AMP. However, prostacyclin also activates the EP receptor and the peroxisome proliferator activated receptor-δ (PPARδ). It is unknown which mechanisms are the most important in terms of treating pulmonary hypertension. Prostacyclin is an important regulator of pulmonary vascular tone and vascular homeostasis, and diminished production of prostacyclin is an important component of endothelial dysfunction. In patients with PAH, prostacyclin synthesis by pulmonary vascular endothelial cells is markedly diminished. For several years, prostanoid treatment has been playing a fundamental role in the modern approach to pulmonary hypertension.

INTRAVENOUS EPOPROSTENOL

The introduction of intravenous epoprostenol treatment was a major advance in the management of patients with severe PAH. Originally used in the early 1980s as a bridge to lung

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transplantation, it was soon discovered that many patients had substantial and long-term improvement with epoprostenol treatment. Some patients could even be removed from the transplantation waiting list. After the first randomised study ever performed in PAH showed improvements in haemodynamics, exercise capacity and survival in epoprostenol-treated patients, the drug was approved in the USA and several other countries for patients with idiopathic PAH (IPAH) [2]. Of 81 patients enrolled in the study, 41 received epoprostenol and 40 conventional therapy: 8 patients died, all of whom had been assigned to conventional therapy (P = 0.003). To date, this study remains the only randomised, controlled trial in the field of pulmonary hypertension to have shown a survival benefit in the treatment group. Approval of epoprostenol was later extended to PAH associated with connective tissue disease after another randomised trial in patients with the scleroderma-spectrum of disease confirmed improvements in haemodynamics and exercise capacity in this patient population [3].

The mechanisms of action of intravenous epoprostenol are not fully understood. Clinical observations suggest that the effects of epoprostenol are not limited to pulmonary vasodilation but affect pulmonary vascular remodelling by inhibition of pulmonary artery smooth muscle cell proliferation, modulation of endothelial cell proliferation and angiogenesis, as well as other mechanisms. This hypothesis is supported by the observation that the acute haemodynamic response to epoprostenol in the catheter laboratory is not predictive of the long-term response, or in other words, that patients can have a substantial benefit from epoprostenol therapy even if the drug exerts no acute vasodilatory effects at all.

Recently, two large single-centre studies reported on the long-term outcome with epoprostenol treatment in IPAH. Sitbon and colleagues studied 178 patients with IPAH in functional class III or IV at baseline. Survival rates at 1, 2, 3 and 5 years were 85%, 70%, 63% and 55%, respectively, which was significantly better than the survival rates of a historical control group receiving conventional supportive care prior to the prostacylin era with respective survival rates of 58%, 43%, 33% and 28% [4]. Similar results were published by McLaughlin and colleagues [5] who reported survival rates at 1, 2 and 3 years of 88%, 76% and 63%, respectively with epoprostenol treatment.

Epoprostenol is usually started at a dose of 2 ng/kg/min and this dose is then gradually increased depending on symptoms and side-effects. The average dose at 1 year ranges between 20 and 35 ng/kg/min. Most expert centres perform regular right-heart catheterisations to prevent underdosing as well as overdosing.

Epoprostenol has several side-effects including headache, jaw pain, nausea, diarrhoea, hypotension and leg pain. Most of these symptoms are preventable or manageable with careful dose adjustments. The major problems with epoprostenol treatment are related to the delivery system. Epoprostenol has a half-life of 2–3 minutes and must be administered by continuous intravenous infusion. The delivery system consists of a portable pump and a permanent means of central venous access, usually a Hickman catheter or a port catheter. Pump failure or a dislocation of the catheter may cause rapid and life-threatening haemodynamic deterioration. The most relevant complication, however, is catheter-related sepsis with a reported incidence between 0.1 and 0.6 per patient-year, with some of these cases being fatal. These numbers have been reported by large volume centres and it seems likely that the incidence of septic complications may be higher where epoprostenol treatment is initiated in centres with less experience.

It is mainly for reasons of the risks, costs and inconveniences associated with epoprostenol that this treatment is no longer considered first-line therapy for class III PAH patients by most experts. However, intravenous epoprostenol remains the treatment of first choice for patients in functional class IV, especially when they show signs or symptoms of haemodynamic instability.

INTRAVENOUS ILOPROST AND INTRAVENOUS TREPROSTINIL

Iloprost and treprostinil are prostacyclin derivates with higher stability and longer plasma half-lives than epoprostenol (see below), which makes these compounds attractive alternatives for continuous intravenous therapy as they are easier to handle and less prone to complications. Based on a similar mechanism of action, it is widely believed that the efficacy of iloprost and treprostinil should be similar to epoprostenol, although there are limited data to verify this assumption.

The use of intravenous iloprost in patients with PAH has never been studied in a randomised controlled trial, but the drug is widely used in Germany and the United Kingdom as well as some other countries, although regulatory approval has been granted only in New Zealand. The daily dose of intravenous iloprost ranges from 1–10 ng/kg/min with most patients receiving dosages of 2–5 ng/kg/min. Side-effects are the same as with epoprostenol and long-term survival data with intravenous iloprost have not been published.

Intravenous treprostinil has been approved in the United States for the treatment of PAH. There has been no formal randomised, placebo-controlled trial with intravenous treprostinil and approval was mainly based on the demonstration of bioequivalence to subcutaneous therapy. A small study in 27 PAH patients has provided preliminary evidence that patients can be safely transitioned from intravenous epoprostenol to intravenous treprostinil, although haemodynamic parameters tended to be slightly worse with treprostinil. Side-effects of both drugs were comparable. However, published data on the long-term safety and efficacy of intravenous treprostinil are still limited. There have been reports of a higher incidence of gram-negative infections of the vascular access (usually a Hickman catheter or a port catheter) but it is still unknown whether these were chance findings or whether the risk of infection is really higher with treprostinil. In addition, for unknown reasons, the average dose of intravenous treprostinil is close to 100 ng/kg/min, which is substantially higher than the dosage used for subcutaneous administration (average 40 ng/kg/min).

INHALED ILOPROST

Iloprost is a prostacyclin derivative with a very similar pharmacodynamic profile but a longer serum half-life of 20–30 minutes. Administration by inhalation has been suggested to cause selective pulmonary vasodilation, although systemic effects do occur, especially with higher dosages. However, inhaled iloprost is a potent pulmonary vasodilator that has been shown to be more potent than nitric oxide in patients with PAH [6]. Unfortunately, the haemodynamic effects of a single iloprost inhalation are no longer detectable 45–60 minutes after the inhalation. Thus, patients have to inhale the drug 6–9 (or even 12) times per day and even with this inhalation frequency, it is not possible to cover 24 hours. In order to obtain maximum efficacy, it is mandatory to use appropriate nebulisers providing proper alveolar deposition of the drug. The typical dose of a single inhalation is 5 µg, although some patients require only 2.5 µg per inhalation.

Several case series have reported beneficial results of treatment with inhaled iloprost in patients with PAH. Some groups, however, were unable to reproduce these findings. The ensuing debate about the efficacy of inhaled iloprost was partly solved after a 12-week randomised, placebo-controlled trial involving 207 patients, the Aerosolised Iloprost Randomised (AIR) study, found a significant improvement in functional class and 6-minute walking distance (6MWD) in patients who received iloprost compared to placebo [7]. However, haemodynamics were improved only when measured directly after inhalation, while trough values after 12 weeks remained more or less unchanged from baseline. In addition, time to clinical worsening was not significantly better in the iloprost group.

Based on the results of the AIR trial, inhaled iloprost has been approved in Europe for the treatment of idiopathic PAH in functional class III but not for other forms of pulmonary arterial hypertension. The debate continues on the long-term efficacy of inhaled iloprost, which still needs to be addressed by well-designed clinical studies. The only long-term study with inhaled iloprost published to date showed treatment failure within 2 years in more than two-thirds of the patients who received inhaled iloprost as first-line therapy.

Inhaled iloprost has also been studied as adjunctive therapy in PAH patients treated with the endothelin receptor antagonist bosentan. The STEP (Iloprost Inhalation Solution Safety and Pilot Efficacy Trial in Combination with Bosentan for Evaluation in Pulmonary Arterial Hypertension) study included 67 PAH patients in functional classes III and IV who received inhaled iloprost or placebo in addition to bosentan for 12 weeks [8]. At the end of the study period, there was marginal improvement in the 6MWD (+26 m) that was of borderline statistical significance ($P = 0.051$). Changes in functional class and time to clinical worsening, however, were in favour of the iloprost group. In contrast, the COMBI (Combination Therapy of Bosentan and Aerosolised Iloprost in Idiopathic Pulmonary Arterial Hypertension) trial, which had a similar design except that this was an open-label study, found no improvement in any of these endpoints [9]. Thus, the two trials yielded contradictory results and further studies are required to determine the efficacy of inhaled iloprost in combination regimens with bosentan.

INHALED TREPROSTINIL

Treprostinil (formerly, UT-15) is another stable prostacyclin derivative with a serum half-life of 3–4 hours after intravenous administration, which has acute haemodynamic effects comparable with epoprostenol in patients with PAH. Inhaled treprostinil is currently being evaluated in clinical trials. The substance holds promise because of longer lasting acute haemodynamic effects compared to iloprost (60–120 min versus 30–45 min) but this advantage may be offset by the strategy to inhale treprostinil only 4 times per day. Small case series have suggested beneficial effects on haemodynamics and exercise capacity in patients with PAH treated with inhaled treprostinil. The results of a phase III trial, TRIUMPH (Treprostinil Sodium Inhalation Used in the Management of Pulmonary Arterial Hypertension) have not been published at the time of this writing. According to press releases from the manufacturer, the study met the primary endpoint, i.e. improvement in 6MWD, although the net benefit was marginal. In addition to that, the study failed to show significant improvements in clinically important secondary endpoints such as functional class or time to clinical worsening. So far, it has not been convincingly shown that the clinical effects of inhaled treprostinil are sustained over longer periods.

SUBCUTANEOUS TREPROSTINIL

In order to circumvent the problems associated with continuous intravenous delivery of epoprostenol, continuous subcutaneous infusion of treprostinil has been proposed as an alternative way to administer a prostanoid to patients with PAH. Drug delivery is accomplished by micro-infusion pumps similar to those used to administer insulin in diabetic patients. The largest randomised, placebo-controlled trial performed to date in patients with PAH included 470 patients in functional classes II, III and IV and found a statistically significant increase in 6MWD compared to placebo after 12 weeks of treatment [10]. Although statistically significant, the mean improvement was only 16 m. There was a clear dose-dependent relationship with the greatest improvements seen in those patients who could tolerate the highest doses. Several haemodynamic variables including right atrial pressure, pulmonary arterial pressure, cardiac output, pulmonary vascular resistance and mixed

venous oxygen saturation were also significantly improved in the treprostinil group. However, there were no differences in survival and the number of patients with clinical deterioration was not reduced. A major problem with subcutaneous treprostinil is the occurrence of infusion site pain, which was reported in 85% of patients exposed to the drug. This pain can be quite intense and satisfactory methods of dealing with it have yet to be developed. Subcutaneous treprostinil is among the drugs that have been recommended as first-line therapy for PAH patients in functional class III, although its clinical use is limited, mainly because of the side-effects.

Treprostinil has been approved in the United States for treatment of PAH in functional classes II–IV. Approval in Europe is still pending. Because of the side-effects, current guidelines do not recommend the use of subcutaneous treprostinil in functional class II. As with inhaled iloprost, the long-term efficacy of subcutaneous treprostinil is still a matter of debate and requires further study. The largest series published to date utilising this treatment included 860 patients and reported survival rates similar to those that have been demonstrated with intravenous prostacyclin (71% at 3 years). However, 59% of the patients discontinued this therapy prematurely, almost half of them because of clinical deterioration (including death) and more than one-third because of side-effects, mostly infusion site pain, which was described by 92% of the patients [11].

Some patients have been safely transitioned from intravenous epoprostenol to subcutaneous treprostinil, but attempts like this should be performed only in experienced centres with the patient under careful clinical and haemodynamic surveillance.

ORAL PROSTANOIDS: BERAPROST SODIUM AND ORAL TREPROSTINIL

Beraprost is an orally active prostacyclin analogue. Under fasting conditions, beraprost is rapidly absorbed; peak plasma concentrations are reached after 30 min, and the elimination half-life is 30–45 min. Half-life of elimination is increased 4–5-fold in patients with severe renal failure. Beraprost was first introduced by Japanese groups who reported data from several uncontrolled trials suggesting beneficial effects on exercise capacity, haemodynamics and survival in patients with PAH. The first randomised, placebo-controlled study was performed in Europe – the Arterial Pulmonary Hypertension and European Beraprost Trial (ALPHABET) which included 130 patients with PAH in functional class II and III [12]. There was a small but significant increase in 6MWD in the beraprost group but haemodynamics did not improve. A second randomised, placebo-controlled trial was performed in the USA and included 116 PAH patients in functional classes II and III [13]. This study demonstrated some beneficial effects of beraprost after 3 and 6 months of treatment, respectively, but not at either 9 months or 12 months. Drug-related side-effects, especially headache, jaw pain, flushing, diarrhoea and palpitations, were common in both studies. Based on these data, many experts no longer recommend the use of beraprost in PAH. The drug has not been approved in the USA or Europe.

Beraprost is approved only in Japan and Korea, where an extended-release formulation of the drug became available recently. As with the inhaled prostanoids, there are also no robust long-term data with beraprost, and the new extended-release formulation has not been sufficiently evaluated.

Oral treprostinil is currently being studied in an ongoing phase III trial – Following Rehabilitation, Economics and Everyday Dialysis Outcome Measurements (FREEDOM). Data are not available at the time of preparation of this chapter.

THE ROLE OF PROSTACYCLINS IN CURRENT MANAGEMENT STRATEGIES FOR PAH

For several years, intravenous epoprostenol was the only efficacious therapy for the majority of patients with PAH and thus it was used in the majority of patients, indepen-

dently of the functional class at presentation. The situation has changed with the introduction of endothelin receptor antagonists and PDE-5 inhibitors which have now become the preferred treatments for patients in functional classes II and III, sometimes even in functional class IV. Intravenous prostanoids are now reserved for patients with most advanced disease, i.e. those presenting in late class III or class IV despite oral therapy. The role of inhaled and oral prostanoids has not been well defined. In randomised, controlled trials, the efficacy of these compounds has been modest (Table 4.1) and none of these drugs seem to have a profound and lasting effect on haemodynamics. Long-term efficacy has not been convincingly demonstrated for any oral or inhaled prostanoid to date. Thus, there is a growing debate on the usefulness of these compounds and further trials are needed to define the role of oral and inhaled prostanoids in modern treatment strategies for PAH.

PROSTACYCLIN RECEPTOR AGONISTS

The main problem with oral prostanoids to date has been tolerability, as these drugs frequently cause unpleasant side-effects, especially headache, jaw pain, gastrointestinal discomfort and diarrhoea, often limiting the maximum achievable (or tolerable) dose. A new substance currently under investigation is NS-304, an orally available non-prostanoid prostacyclin receptor agonist. NS-304 is a prodrug of the active compound MRE-269 which has a half-life of approximately 10 hours. Since NS-304 itself has no activity on the prostanoids receptor, it is expected to have fewer systemic side-effects than orally administered prostanoids. So far, there are no data on the safety and efficacy of NS-304 in PAH as the first clinical trial with this compound is still in progress.

PHOSPHODIESTERASE TYPE 5 INHIBITORS (PDE-5 INHIBITORS)

Phosphodiesterases are a superfamily of enzymes that inactivate cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), the second messengers of several hormones and mediators including prostacyclin and nitric oxide. At least eleven isoforms have been identified, which have different substrate affinities and tissue locations. The isoform PDE-5 is abundantly expressed in the lung vessels of patients with pulmonary hypertension, where enzyme activity has been reported to be much higher than in normal lung vessels. PDE-5 inactivates cGMP, thereby inhibiting the vasodilatory effects of nitric oxide and atrial natriuretic peptides. Of note, cGMP is a potent endogenous inhibitor of PDE-3, a major decaying enzyme of cAMP, the second messenger of prostacyclin and other endogenous vasodilators. Thus, PDE-5 inhibition increases the intracellular concentrations of both cAMP and cGMP. The effects of PDE-5 inhibitors are most prominent in tissues with an abundant expression of the PDE-5 isoenzymes, which include the lungs, the corpus cavernosum and the lower oesophageal sphincter muscle. In contrast, PDE-5 isoenzymes show a much lower expression in the systemic circulation. These features make PDE-5 inhibitors promising new substances for treatment of PAH [14].

SILDENAFIL

Among several PDE-5 inhibitors, sildenafil has been most extensively studied in patients with pulmonary hypertension. The drug is approved in several countries for the treatment of erectile dysfunction. Sildenafil is rapidly absorbed after oral administration; the mean oral bioavailability is 41% and peak plasma concentrations are reached 60 min post-dose. The terminal plasma half-life of sildenafil is 3–5 hours and the drug is cleared primarily by the CYP3A4 (major) and CYP2C9 (minor) pathways. Several case reports and case series have found a beneficial effect of sildenafil in patients with various forms of pulmonary

Table 4.1 Changes from baseline in haemodynamic variables after 3 months of therapy with various drugs as shown in randomised, placebo-controlled trials

	Inhaled iloprost* (6 × 5 μg)	Beraprost (4 × 80 μg)	SC treprostinil (9.3 ng/ kg/min)	Bosentan (125 mg bid)	Sitaxentan (100 mg qd)	Sildenafil (20 mg tid)
Study	AIR	ALPHABET	Treprostinil	AC 351	STRIDE-1	SUPER
mPAP (mmHg)	−0.1	−1	−2.3[†]	−1.6[†]	−3	−2.1[†]
CI (l/min/m²)	+0.03[‡]	+0.2	+0.12[†]	+0.5[†]	+0.3[†]	+0.2
PVR (dynes)	−9[†]	−104	−160[†#]	−223[†]	−221[†]	−122[†]

[†] = significantly better compared to the placebo group; * = values measured at trough, i.e. before inhalation; [‡] = in the original paper, numbers were given for cardiac output, which improved by 0.05 l/min; for the purpose of comparability, this value was divided by an assumed value of 1.75 to calculate cardiac index; [#] = in the original paper, numbers were given for pulmonary vascular resistance index, which improved by 280 dynes; for the purpose of comparability, this value was divided by an assumed value of 1.75 to calculate cardiac index; CI = cardiac index; mPAP = mean pulmonary artery pressure; PVR = pulmonary vascular resistance; SC = subcutaneous.

hypertension either alone or in combination with prostanoids. SUPER-1 (Sildenafil Use in Pulmonary Arterial Hypertension), the pivotal trial studying the effects of sildenafil in PAH compared three dosages of sildenafil: 20 mg, 40 mg and 80 mg respectively, three times a day with placebo [15]. This study enrolled 279 patients with PAH in functional classes II or III, who were 1:1:1:1 randomised to either placebo or sildenafil at doses of 20 mg tid, 40 mg tid or 80 mg tid, respectively. After 12 weeks, the placebo-corrected improvements in 6MWD were similar with all three dosages, ranging from +45 m to +50 m. Based on these results, sildenafil has been approved in many countries at a dose of 20 mg tid. However, SUPER-1 indicated that the haemodynamic effects of sildenafil were dose-dependent and the highest dose of 80 mg tid had the strongest effect on pulmonary vascular resistance (PVR) (-310 dyn versus -171 dyn and -192 dyn with 20 mg tid and 40 mg tid, respectively). All dosages were equally well tolerated and the open-label extension of the study had a target dose of 80 mg tid [unpublished data]. For the time being, there are virtually no long-term data on the 20 mg tid dosage and many experts believe that higher dosages might be more efficacious in the longer term.

A randomised, controlled clinical trial, PACES (Pulmonary Arterial Hypertension Combination Study of Epoprostenol and Sildenafil), that studied the effects of sildenafil when used in patients treated with intravenous epoprostenol has just been concluded. The study was performed in a double-blind fashion and patients received either placebo or sildenafil at a target dose of 80 mg tid in addition to intravenous epoprostenol. The results of this trial have not been fully published but preliminary presentations have shown that the change in 6MWD was significantly better in the epoprostenol plus sildenafil group. Perhaps more importantly, there was a significant improvement in time to clinical worsening in sildenafil-treated patients, although the study lasted only 12 weeks. There were seven deaths, and all of them occurred in the group of patients treated with epoprostenol only. This study provides the strongest evidence available to date that PDE-5 inhibitors can improve the outcome of patients with PAH. The problem remains, however, that the dose of sildenafil used in this study (80 mg tid) is higher than the currently approved dose of 20 mg tid.
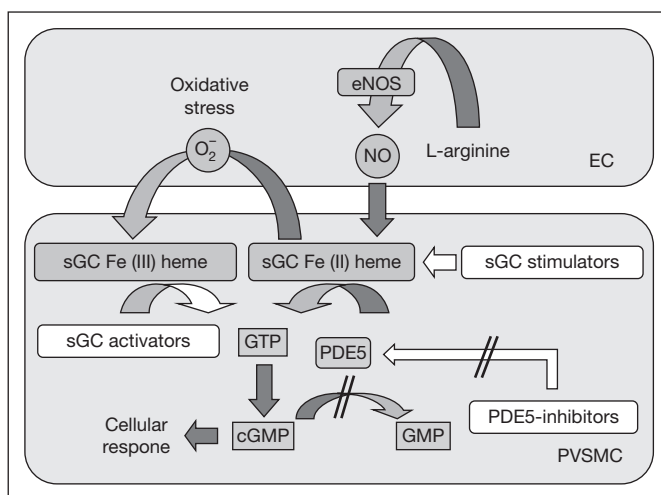
Figure 4.1 Pharmacological targets of the nitric oxide (NO) – soluble guanylate cyclase (sGC) – cyclic GMP (cGMP) system. In the physiological state, NO activates sGC upon binding to Fe(II) heme. Under conditions of oxidative stress oxygen radicals (O_2^{-}) inactivate sGC by reducing the heme-iron (Fe(III) heme). sGC stimulators and activators increase cGMP production, sGC stimulators by acting preferentially on the non-oxidised Fe(II) heme, sGC activators by acting preferentially on the Fe(III) heme. Based on their actions, sGC stimulators require the presence of NO whereas sGC activators act independently of NO. Phosphodiesterase-5 inhibitors augment intracellular cGMP by blocking its degradation. EC = endothelial cell; eNOS = endothelial nitric oxide synthase; GTP = guanosine triphosphate; PVSMC = pulmonary vascular smooth muscle cell.

TADALAFIL

Tadalafil, a long-acting PDE-5 inhibitor, is also being studied in PAH. A phase III study, PHIRST (Phosphodiesterase Type-5 Inhibitor Tadalafil in the Treatment of Patients with PAH) has recently been concluded but results have not yet been published.

Direct comparisons between sildenafil and tadalafil are not yet available except for acute haemodynamic data [16]. The longer duration of action is a potential advantage of tadalafil but it is unclear whether both drugs are going to have the same long-term effects on disease progression. Of note, sildenafil is not a truly selective PDE-5 inhibitor as it also blocks the PDE-1 isoenzyme. This effect may be of particular interest as PDE-1 has been shown to be involved in vascular smooth muscle cell proliferation. Tadalafil, in contrast, has no effect on PDE-1. It is not clear whether these considerations are of clinical relevance, but head-to-head comparisons between sildenafil and tadalafil addressing long-term safety and efficacy in PAH are desirable.

THE ROLE OF PDE-5 INHIBITORS IN CURRENT MANAGEMENT STRATEGIES FOR PAH

On a worldwide scale, sildenafil is probably the most prescribed drug for PAH. The drug has several advantages as it is usually well tolerated, widely available, does not need specific monitoring and is less costly than most other PAH medications. The problem is that the ideal dose of sildenafil for long-term therapy is unknown and the long-term effects of this drug have not been sufficiently studied. Thus, long-term studies with different dosages are urgently needed. The same is true for combination trials as combination therapy is becoming the standard of care for patients with PAH and it is to be expected that PDE-5 inhibitors are going to become an integral part of many combination regimens.

STIMULATORS AND ACTIVATORS OF SOLUBLE GUANYLATE CYCLASE

Soluble guanylate cyclase (sGC) is an emerging target for treating PAH. Under physiological conditions, NO-induced cGMP-activation is mediated via sGC, a heme-iron (II)-containing enzyme. In the lung vessels of patients with PAH, sGC is upregulated but at the same time the enzyme may become inactivated by oxidation of the heme-iron. In that case, NO and other sGC-dependent vasodilators such as the natriuretic peptides lose their vasodilatory potential. Two different compounds are now under study in pulmonary vascular disease, sGC stimulators and sGC activators. Stimulators augment the NO effects on the enzyme, whereas activators act preferably on the oxidised (iron-III) heme, thus having the potential to induce vasodilation in the absence of NO (Figure 4.1). Both sGC activators and stimulators partly reverse pulmonary vascular remodelling in experimental models of pulmonary hypertension. Acute haemodynamic studies in 10 PAH patients showed profound haemodynamic effects of BAY 63-2521, an orally available sGC stimulator, with a reduction in PVR by more than 30% from baseline when given as a single dose of 2.5 mg [unpublished data]. BAY 63-2521 caused significant reductions not only in pulmonary artery pressures but also in systemic artery pressures accompanied by a marked improvement in cardiac index [unpublished data]. The systemic hypotensive effect, however, seems to abate with long-term administration. None of the sGC stimulators/activators is currently available for PAH therapy. A phase II trial with BAY 63-2521 in patients with PAH and chronic thrombo-embolic pulmonary hypertension (CTEPH) was recently concluded but the results are not yet available. This compound will now be studied in phase III trials in PAH and CTEPH, as well as in phase II studies in patients with pulmonary hypertension associated with interstitial lung disease (ILD).

SUMMARY

Prostanoids and PDE-5 inhibitors, together with endothelin receptor antagonists, have become the mainstays of PAH treatment. On a global perspective, the PDE-5 inhibitor sildenafil is now the most widely prescribed drug for PAH. The optimal dose of sildenafil in PAH is, however, unknown. Intravenous prostacyclin remains a life-saving therapy for patients with advanced pulmonary hypertension whereas the role of inhaled, subcutaneous and oral prostanoids has been less well defined since robust long-term data are lacking. Stimulators and activators of soluble guanylate cyclase are promising new substances that are currently being investigated in various forms of pulmonary hypertension.

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5

The future treatment of pulmonary hypertension

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INTRODUCTION

The pathogenesis of pulmonary hypertension (PH) is complex and multifactorial. Increased pulmonary arterial pressure (PAP) in patients with PH probably results from a combination of pulmonary vasoconstriction, inward vascular wall remodelling and *in situ* thrombosis. As can be seen in Figure 5.1, the inward pulmonary vascular wall remodelling may typically involve intimal lesion (including neointimal and plexiform lesions), medial thickening due to smooth muscle cell (SMC) hyperplasia and hypertrophy, adventitial thickening due to increased fibroblast proliferation and accumulation of extracellular matrix (ECM) proteins, all of which contribute to a thickening of the pulmonary vascular wall. The central role of endothelial dysfunction in the initiation and progression of PH resulting in the altered production of endothelial mediators has been increasingly recognised in PH. Of those local mediators, nitric oxide (NO), prostacyclin (PGI2), serotonin (5-HT) and endothelin (ET) are among the best studied and most commonly implicated in the pathogenesis of pulmonary arterial hypertension (PAH). As a result, three of the currently approved therapies for the treatment of PAH, PGI2 analogues, phosphodiesterase (PDE) inhibitors and ET receptor antagonists, emerged, in part, from restoring the balance between these mediators. Although postulated for all treatments, evidence for the direct antiproliferative effects of most approaches is missing. Recent developments in antiproliferative approaches for the treatment of pulmonary hypertension are summarised in this chapter.

THERAPIES TARGETING THE VASOCONSTRICTIVE COMPONENT OF PH

RHO KINASE INHIBITORS

Rho-kinase (ROCK) belongs to a family of serine/threonine protein kinases that are activated via an interaction with the small guanosine triphosphate (GTP)-binding protein RhoA [1]. Both RhoA and ROCK are widely expressed in many cell types of the vasculature and participate in a variety of important physiological functions including smooth muscle contraction, cell proliferation, cell adhesion, migration, motility and many aspects of inflammatory responses [2]. As these processes mediate the onset and progression of PH, modulation of the Rho/ROCK signalling pathway became an appealing strategy for the treatment of

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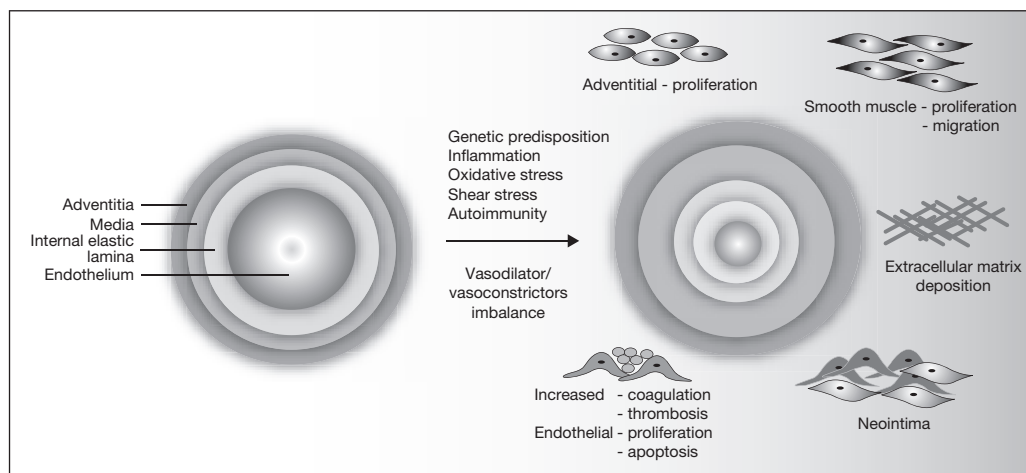
Figure 5.1 Pathogenesis of pulmonary hypertension (PH). Schematic outline of abnormalities observed in PH that may contribute to its cause or progression. An injury and subsequent dysfunction of the endothelium by a variety of stimuli (genetic predisposition, inflammation, oxidative stress, shear stress, auto immunity etc.) causes altered production of endothelial mediators and growth factors that thereby facilitates vasoconstriction and proliferation and migration of pulmonary arterial cell types, endothelial cells, smooth muscle cells and fibroblasts, leading to pulmonary vascular remodelling and *in situ* thrombosis. The reduction of cross-sectional area of the pulmonary microvasculature contributes to the increased pulmonary vascular resistance observed in this disease.

PH. Indeed, the application of two widely employed ROCK inhibitors, fasudil and Y-27632, demonstrated beneficial effects in several experimental models of PH. Oral application of Y-27632 attenuated acute hypoxia-induced vasoconstriction and reduced the development of chronic hypoxia-induced PH and vascular remodelling [3]. Furthermore, the long-term inhibition of ROCK by orally delivered or inhaled fasudil caused a marked improvement in hypoxia-induced, monocrotaline-induced, high flow-induced PH induced by combining the vascular endothelial growth factor receptor (VEGFR) antagonist SU5416 with hypoxia and also in spontaneously hypertensive rats [4–6]. Most interestingly, acute beneficial vasodilatory effects of ROCK inhibitors are also demonstrated in patients with PAH [7]. However, data from large clinical trials are now required to demonstrate the safety and efficacy of long-term treatment with ROCK inhibitors and to develop novel ROCK inhibitors with high selectivity and reduced toxicity.

VASOINTESTINAL PEPTIDE

Vasointestinal peptide (VIP) is a 28-amino acid peptide and one of the most abundant neuropeptides found in the lung [8]. Mediated via specific VPAC1 and VPAC2 receptors, VIP is a potent vasodilator and inhibitor of SMC proliferation, in addition to its potent anti-inflammatory, immunomodulatory functions [9–11], suggesting a possible physiological role in PH. In support of this, a deficiency in VIP levels in the serum and lung tissue was observed in patients with idiopathic PAH [12]. Further VIP administration was shown to reduce proliferation of PA SMCs *in vitro* and attenuated pulmonary vasoconstriction in newborn lambs and in rabbits with monocrotaline-induced PH [13, 14]. In addition, VIP knockout mice developed moderate PAP and right ventricular (RV) hypertrophy [15]. In a preliminary open-label case series, VIP inhalation demonstrated a marked clinical and haemodynamic improvement in eight PAH (formerly primary pulmonary hypertension [PPH]) patients [16].

SEROTONIN RECEPTOR-/SEROTONIN REUPTAKE INHIBITORS

Serotonin (5-hydroxytryptamine; 5-HT) is a well-known potent vasoconstrictor and mitogen [17]. Synthesised by tryptophan hydroxylase 1 (Tph1) in endothelial cells, serotonin is released and passes into the underlying pulmonary smooth muscle cells through the serotonin transporter (SERT; 5-HTT) activating serotonin receptors (7 families, 14 receptors) to stimulate proliferation, migration and contraction. Similar mechanisms are also seen in serotonin-mediated pulmonary fibroblast proliferation [18, 19]. Expression analysis of lung tissues from PAH patients undergoing lung transplantation revealed an increased expression of 5-HTT and, interestingly, isolated PA SMCs from these patients demonstrated an enhanced proliferative growth response to 5-HT. Moreover, patients with PAH had elevated plasma levels of 5-HT [20, 21]. It has been shown experimentally that administration of selective serotonin reuptake inhibitors (SSRI) that antagonise the serotonin transporter 5-HTT (e.g. fluoxetin) reversed monocrotaline-induced PH in rats [22] and 5-HTT overexpressing mice developed spontaneous PH [23], providing direct evidence of 5-HTT in pulmonary vascular remodelling. Furthermore, 5-HT1B/2B receptor- as well as Tph1-deficiency caused resistance to hypoxia-induced PH in mice. In addition, the specific 5-HT1B/1D or 5-HT2B receptor antagonist potentially prevented the increase in PAP in mice that were given a hypoxic challenge [24–26]. Although much work has already been done at the preclinical level in demonstrating the serotonin pathway as a therapeutic opportunity, clinical trials are needed to support this.

SOLUBLE GUANYLATE CYCLASE ACTIVATORS/STIMULATORS

Soluble guanylate cyclase (sGC) is a key enzyme in a NO–cyclic guanosine monophosphate (cGMP) signalling cascade and plays a central role in a large number of physiological processes [27]. sGC consists of an α-subunit and haem-binding β-subunit that is activated by endogenously formed NO and in its active form catalyses the formation of cGMP. Furthermore, cGMP activates cGMP-dependent protein kinases (PKGs) leading to a reduction in cytosolic Ca^{2+} concentration and desensitisation of the actin–myosin contractile system. An increase of sGC protein expression, limited bioavailability of NO and reduced sensitivity to endogenous NO was described in both human and experimental hypoxia-induced PH [28–30]. However, an increasing body of evidence suggests that, under pathophysiological conditions, sGC may become oxidised or rendered haem-deficient and thereby insensitive to NO followed by reduced local cGMP production [31, 32]. This may in part explain the limitations of current therapies addressing the NO pathway in PH.

The pharmacological activation of sGC may therefore be beneficial in restoring NO signalling and thereby to treat PH. Two novel drug classes, namely 'sGC stimulators' and 'sGC activators' have been designed to offer such a possibility. sGC stimulators are compounds that stimulate sGC directly and enhance the sensitivity of the reduced enzyme to low levels of bioavailable NO. Conversely, sGC activators do not modulate NO signalling at all but activate the NO-unresponsive, haem-oxidised or haem-free enzyme [33]. Interestingly, the sGC stimulator BAY 41-2272 was shown to be a systemic and pulmonary vasodilator and to augment the vasodilative response to inhaled NO in acute pulmonary hypertension in lambs [34]. Moreover, both sGC stimulators (BAY 41-2272, BAY 63-2521) and sGC activators (BAY 58-2667) significantly reversed the degree of pulmonary hypertension evolving in response to hypoxia and monocrotaline. This was true for PAP and RV hypertrophy but also for structural changes including the *de novo* muscularisation of small precapillary vessels [28, 35]. These studies provide strong evidence that direct pharmacological stimulation of sGC may be an effective therapeutic intervention in PH. Clinical trials are underway that will address the therapeutic efficiency of BAY 63-2521 in life-threatening advanced PH.
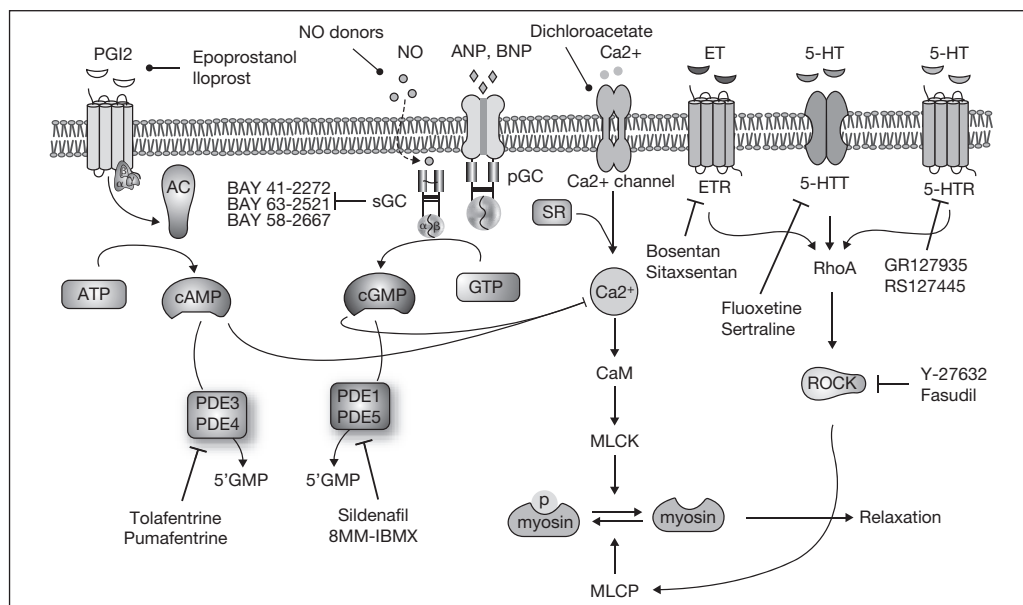
Figure 5.2 Molecules and agents that interfere with the pulmonary vasoconstriction, providing possible therapies for PH. Vasoconstriction is majorly determined by the activities of Ca^{2+}/calmodulin (CaM)-dependent myosin light chain kinase (MLCK, contraction) and RhoA activated Ca^{2+}-independent MLC phosphatase (MLCP, relaxation). Several mediators (PGI2; prostacyclin; NO, nitric oxide; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; ET, endothelin; 5-HT, serotonin) via several downstream signalling molecules (cAMP and cGMP, secondary messengers; PDE, phosphodiesterase; sGC, soluble guanylate cyclase; pGC, particulate guanylate cyclase; ROCK, Rho-kinase) positively or negatively regulate vasoconstriction. 5-HTR = serotonin receptor; 5-HTT = serotonin transporter; AC = adenylate cyclase; ETR = endothelin receptors; SR = sarcoplasmic reticulum.

KV CHANNEL ACTIVATORS

In addition to mediators possessing both vasoconstrictor and pro-proliferative capacities, abnormalities in both K^+- and Ca^{2+}-channels have been intimately linked with pathological vasomotor control in the pulmonary circulation, and also with dysregulation of cellular homeostasis and induction of fibroproliferative sequelae, in particular in SMCs [36]. A selective downregulation of voltage-gated K^+ channels (Kv), like Kv1.2 and Kv1.5 in PA SMCs, has been described in both human idiopathic PAH (IPAH) tissue and animal models of PH, with subsequent membrane depolarisation, opening of the voltage-gated Ca^{2+} channels and induction of muscle contraction via Ca-calmodulin and myosin light chain kinase [37], suggesting that activation of K^+ channels is of therapeutic benefit. Accordingly, *in vivo* gene transfer of Kv1.5 reduced PH and restored hypoxic pulmonary vasoconstriction in chronically hypoxic rats [38].

In addition, experimental oral therapies such as sildenafil, a PDE-5 inhibitor, and dichloroacetate (DCA), a metabolic modulator, increased the expression/function of the Kv2.1 channel and subsequently reduced the pulmonary vascular remodelling in rats with chronic–hypoxic PH [39, 40]. Recently, Ca^{2+} signalling via transient receptor potential (TRP) ion channels has been shown to play an important role in IPAH [41]. Similarly, in hypoxia-induced PH, the significance of the TRP ion channel family in regulating SMC Ca^{2+} flux was underlined by the recent finding that the acute hypoxic vasoconstrictor response is exclusively dependent on TRPC6 in mice [42].

The above mentioned molecules and agents, which interfere with the pulmonary vaso-constriction, provide possible therapies for PH, in addition to existing therapies targeting the pulmonary vasoconstriction of PH that are described in Figure 5.2.

THERAPIES TARGETING THE PRO-PROLIFERATIVE COMPONENT OF PH

PLATELET-DERIVED GROWTH FACTOR RECEPTOR ANTAGONISTS

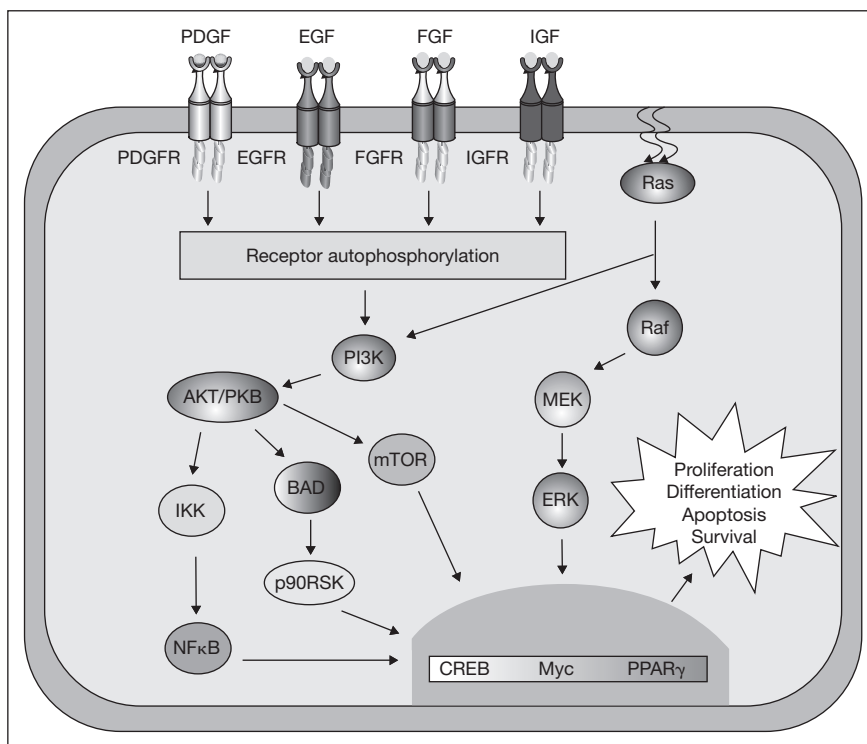Platelet-derived growth factors (PDGF) and their receptors (PDGFR) have served as proto-types for growth factor and receptor tyrosine kinase function for more than 25 years [43]. However, PDGF has recently been identified as a key molecule in the pathogenesis of PAH by Schermuly and colleagues. The PDGF family consists of a family of disulphide-bonded homodimers or heterodimers of four possible subunits (PDGF-A, PDGF-B, PDGF-C, and PDGF-D) that act on cells by binding to homodimers or heterodimers of the two PDGF receptor proteins (PDGFR-α and PDGFR-β) and activating their receptor tyrosine kinase activity. As displayed in Figure 5.3, both PDGFR-α and PDGFR-β engage several well-characterised signalling pathways, such as Ras/mitogen-activated protein kinase (MAPK), phospatidylinositol 3-kinase (PI3K), and phospholipase Cγ (PLC-γ), which are known to be involved in multiple cellular and developmental responses [44].

A complete analysis of the pathogenic role of PDGF in human PAH demonstrated an increased expression of PDGF and PDGFRs by reverse transcription polymerase chain reac-tion (RT-PCR) performed on laser capture microdissected pulmonary arteries from lung-trans-planted patients with IPAH. PDGF-A, PDGF-B, PDGFR-α and PDGFR-β mRNA expression is increased in small pulmonary arteries from patients displaying severe IPAH, as compared to controls [45–47]. This overexpression is more pronounced for PDGF-B and PDGFR-β, as com-pared to PDGF-A and PDGFR-α. In addition, upregulation of PDGFR-β has been demon-strated in several animal models of PH [47–49]. Taken together, these data support the concept that PDGF is overproduced and promotes pulmonary arterial remodelling in PH.

Specifically, strategies to block PDGF signalling with imatinib, an inhibitor of the tyro-sine kinases, PDGF receptor, BCR-ABL and c-kit, [50] in monocrotaline-induced PH in rats and hypoxia-induced PH in mice (treated upon full establishment of PH) demonstrated 'reverse remodelling' potency on a functional level (e.g. improved survival) by improved haemodynamics and by demonstrating a reduction in medial wall thickness and decrease in fully muscularised small pulmonary arteries in histology. In addition, matrix metallo-proteinase (MMP)2 and MMP9, which have previously been shown to be involved in PH, were downregulated by imatinib in an extracellular signal-related kinase (ERK)-dependent manner. Additionally, *in vitro* studies demonstrated that proliferation of rat PA SMCs is potentially inhibited by imatinib, suggesting inhibition of proliferation as the primary mechanism of imatinib's therapeutic effects [47]. In a translational approach, patients with severe PH, who failed to be stabilised even by extensive combined application of approved PAH-specific therapies, were beneficially influenced by compassionate use of imatinib as rescue therapy [51, 52]. A multinational, multicentre, randomised, placebo-controlled, long-term treatment trial with imatinib added to ongoing PH treatment was initiated.

EPIDERMAL GROWTH FACTOR RECEPTOR ANTAGONISTS

The epidermal growth factor (EGF) family consists of four receptor tyrosine kinases: EGFR (or EGFR1, ERBB2, HER1), EGFR2 (or ERBB2, HER2), EGFR3 (or ERBB3, HER3) and EGFR4 (or ERBB4, HER4) and six ligands including the EGF transforming growth factor-α (TGF-α), amphiregulin (AR), heparin-binding EGF-like growth factor (HB-EGF), betacellulin and epi-regulin [53]. Receptor–ligand interaction results in activation of the intrinsic kinase domain and phosphorylation of specific tyrosine residues in the cytoplasmic tail of the receptor. The

Figure 5.3 Growth factor signalling and its role in the promotion of pulmonary vascular remodelling of PH. A number of growth factor receptors (PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor; EGFR, epidermal growth factor receptor; IGFR, insulin growth factor receptor) are autophosphorylated by respective growth factor ligands (PDGF, VEGF, EGF, IGF and others). This leads to activation of two important downstream signalling cascades; the Ras–Raf–MEK–ERK pathway and the phosphatidylinositol 3-kinase (PI3K)-AKT pathway. Subsequently resulting in the activation of numerous transcription factors (CREB, Myc, PPARγ and others) and key cellular processes of pulmonary vascular remodelling (proliferation, survival, apoptosis, differentiation). BAD = Bcl-XL/Bcl-2-associated death promoter; CREB = cyclic-AMP-responsive element-binding protein transcription factor; ERK = extracellular signal-regulated kinase; IKK = Ikappa B kinase; MEK, mitogen-activated protein kinase (MAPK) kinase; mTOR = mammalian target of rapamycin; NF-κB = nuclear factor-kappa B; p90RSK = p90 ribosomal S6 kinase; PKB = AKT/protein kinase B; PPARγ = peroxisome proliferator-activated receptor-γ.

phosphorylated residues become docking sites for multiple proteins, which in turn activate downstream signalling pathways including the PI3K/AKT, STAT and Ras/Raf/MEK pathways [53, 54].

Interestingly, EGF was demonstrated to co-localise with Tenascin C, an ECM component that was deregulated in the obstructive lesions of patients with PAH [55]. Furthermore, an EGF-dependent proliferation and migration of PA SMCs was shown to be dependent on Tenascin C and its interaction with the alpha v beta 3 integrin [56]. Similarly, the EGF receptor inhibitor PKI166 reversed established experimental monocrotaline-induced PH in rats [57]. Conversely, an extensive muscularisation of small pulmonary arteries, vascular remodelling and severe PH was observed in transgenic mice expressing the EGF ligand, TGF-α [58]. However, research into the reverse-remodelling efficacy of other established and clinically approved EGF receptor inhibitors is also warranted.

VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR ANTAGONISTS

The vascular endothelial growth factor (VEGF) family consists of numerous members including VEGF-A, -B, -C, -D and placental growth factor (PlGF). The best characterised member, VEGF-A, exists as a homodimeric glycoprotein comprised of several isoforms, namely VEGF16, VEGF121, VEGF189 and VEGF206 which differ in their affinity to the ECM. VEGF-A and its different isoforms activate the tyrosine kinase receptors VEGFR-1/Flt-1 and VEGFR-2/KDR/Flk-1, VEGFR-3 (Flt-4) as well as Neuropilin (NRP)-1 and NRP-2. In contrast, VEGF-B and PlGF bind to VEGFR1, and VEGF-C and -D bind specifically to VEGFR-2 and -3 and play a crucial role in cell proliferation, permeability and migration [59].

The VEGFRs flt-1 and flk-1 are expressed in the plexiform lesions and are thought to play a role in the pathogenesis of PH by stimulating dysregulated angiogenesis [60, 61]. Experimental data yielded conflicting results, however, showing both up- (hypoxia-, flow-induced pulmonary hypertension) and downregulation (monocrotaline (MCT)-induced pulmonary hypertension) of VEGF and its receptors compared with controls [62, 63]. Interestingly, the combination of hypoxia with the inhibition of VEGFR-1 and -2 by SU5416 resulted in aggravation of pulmonary hypertension, mainly by the presence of proliferating endothelial cells which occlude small pulmonary arteries [64]. Similarly, cell-based gene transfer of VEGF potentially attenuated MCT-induced pulmonary hypertension [65]. On the other hand, inhibition of VEGF may also alter lung structure as shown by emphysema formation after SU5416 application in rodents [66]. The role of chronic VEGF inhibition in pulmonary hypertension has therefore to be interpreted cautiously before considering VEGF inhibitors as therapeutic targets.

FIBROBLAST-/INSULIN-GROWTH FACTOR RECEPTOR ANTAGONISTS

Fibroblast growth factors (FGFs) belong to a family of pleiotropic heparin-binding growth factors. The family of FGFs consists of 23 ligands (FGF-1–FGF-23) and four receptors (FGFR1–4) and is known to stimulate several intracellular signalling pathways in concert with heparin or heparan sulphate proteoglycan [67]. Among all of them, FGF-2 (b-FGF) regulation has been highly studied in its role as a mitogen of PA SMC proliferation [68]. Further studies have suggested upregulation of FGF-2 in response to hypoxia and shear stress in PA SMCs [69] and *in vivo* by high pulmonary blood flow-induced PH in lambs [70].

Equally complex is the insulin growth factor (IGF) family, consisting of ligands (IGF-I, IGF-II), receptors (IGFR-I, IGFR-II), IGF-binding proteins (IGFBP1-6) and insulin receptor substrates (IRS-1–6) [71]. Although claimed to mainly regulate metabolic and developmental processes, IGF is a pleotropic hormone and may have influences on pulmonary vasculature. IGF-I is shown to actively stimulate elastin synthesis and proliferation of PA SMCs [72, 73]. However, exogenous administration of IGF-I promoted anabolism under chronic hypoxia, with no adverse effects on the development of PH [74]. Since the studies on FGF and IGF signalling in PH are at an early stage, intensive investigation is needed to assess their therapeutic efficacy.

MULTIKINASE INHIBITORS

Considering the involvement of several growth factors in progressive vascular remodelling, small-molecule kinase inhibitors that have more than one target (multikinase inhibitors) are generating considerable excitement in the treatment of pulmonary hypertension. One such multikinase inhibitor that has received special attention is sorafenib.

Sorafenib is an inhibitor of the serine-/threonine kinases Raf-1(c-Raf) and b-Raf as well as the tyrosine kinases PDGFR-β, VEGFR-2, VEGFR-3, Flt-3 ligand and c-kit, with IC_{50} values between 6 and 70 nM [75]. Enthusiasm for the compound was initially the result, in part,

of the high regulation of PDGF and VEGF signalling in PH. Furthermore, as shown in Figure 5.3, signalling through many growth factor receptors involves activation of Ras, recruitment of Raf to the membrane and subsequent activation of the mitogen-activated protein kinase (MAPK) –extracellular signal-related kinase (ERK) pathway, which affects a large number of downstream cellular responses [76]. *In vivo* studies by Moreno-Vinasco and colleagues demonstrated that sorafenib treatment of hypoxia-induced PH rats and rats with severe angio-proliferative PH (PH induced by combining the VEGFR antagonist SU5416 with hypoxia) effectively prevented haemodynamic changes and attenuated pulmonary vascular remodelling [77]. In another elegant study, sorafenib treatment reversed established PH and myocardial hypertrophy in monocrotaline-treated rats (Schermuly RT, Klein M; *Circulation* [in press]). Based on its inhibitory effect on tyrosine and serine-/threonine kinases, the beneficial effects of sorafenib appear to be due to a dual pulmonary and myocardial mode of action.

PHOSPHODIESTERASE INHIBITORS

Phosphodiesterases are enzymes that hydrolyse the secondary messengers, cAMP and/or cGMP. They encompass eleven distinct families and play an important role in the regulation of vascular tone and vascular remodelling [78]. In structurally remodelled pulmonary arteries and isolated PA SMCs from patients with PAH and in animal models of PAH, the expression and activities of PDE-1, PDE-3 and PDE-5 are increased compared to healthy controls [79, 80]. Furthermore, sildenafil, a selective PDE-5 inhibitor, promotes selective pulmonary vasodilatation and has been approved for the treatment of patients with PAH [81, 82]. Similarly, treatment with a combined PDE-3 and PDE-4 inhibitor partially reversed established monocrotaline-induced PH in rats [83]. Furthermore, long-term infusion of the PDE-1 inhibitor 8-methoxymethyl 3-isobutyl-1-methylxanthine in hypoxic mice and monocrotaline-injected rats with fully established PH reversed the pulmonary artery pressure elevation, structural remodelling of the lung vasculature and right heart hypertrophy [80]. Although PDE-1-selective inhibitors appear to be particularly attractive as novel therapeutics for PH, development of more selective and potent pharmacological PDE inhibitors is awaited.

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA AGONISTS

Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated transcription factor belonging to the nuclear hormone receptor superfamily. Upon ligand activation, PPARγ heterodimerises with the retinoid X receptor (RXR) and binds to PPAR response elements (PPREs) in regulatory promoter regions of their target genes [84]. In that manner, PPARγ regulates diverse physiological processes including cell growth, inflammation, apoptosis and angiogenesis [85, 86], and is described as the 'converging point' of several growth factor signalling pathways (Figure 5.3), suggesting a potential role for PPARγ in the pathogenesis of PH.

In line with this, an abundant expression of PPARγ in the endothelial cells of normal human lung tissue and a reduced lung tissue PPARγ gene and protein expression in the lungs from patients with severe PH, along with the loss of PPARγ expression in their angiogenic plexiform lesions, was observed [87]. Furthermore, ligand-induced activation of PPARγ with thiazolidinedione (TZD) class antidiabetic drugs (pioglitazone, rosiglitazone and troglitazone) significantly reduced PH and pulmonary artery wall thickening in a rat model of hypoxia- and monocrotaline-induced PH [88, 89]. Moreover, these changes were attributed to the inhibition of SMC proliferation with no significant elevation of apoptosis. Further evidence supporting this view has come from transgenic mice after targeted deletion of PPARγ in SMCs. These mice developed spontaneous PH, as indicated by elevated RV systolic pres-

Vasodilatory therapies
PGI2 analogues
NO donors
PDE-5 inhibitors
ETR antagonists
sGC stimulators
Rho-kinase inhibitors
VIP
5-HTR inhibitors
5-HT reuptake inhibitors

Pro-apoptotic therapies
Survivin
Metabolic modulators
PDGFR antagonists
Elastase inhibitors
Rho-kinase inhibitors

Anti-proliferation therapies
PDGFR antagonists
EGFR antagonists
VEGFR antagonists
Multikinase inhibitors
PPARγ agonists
PDE-1 inhibitors

Anti-inflammatory therapies
PGI2 analogues
HMG-CoA reductase inhibitors
NFAT inhibitors

ECM regulation therapies
Elastase inhibitors
MMP inhibitors
PDE-3/4 inhibitors
PDGFR antagonists
ETR antagonists

ATII cells   Inflammatory cells
Alveoli (AT I cells)   Fibroblasts   Endothelial cells
Inflammatory cells   Thrombi   Smooth muscle cells

Figure 5.4 Therapies targeting the vasoconstrictive, pro-proliferative, anti-apoptotic, extracellular matrix (ECM) deregulation and inflammatory components of PH.

sure, RV hypertrophy and increased muscularisation of the distal pulmonary arteries [90]. Thus, PPARγ-mediated events could protect against PH, and PPARγ agonists may prove to be of potential therapeutic benefit in the treatment of pulmonary arterial hypertension.

THERAPIES TARGETING THE ANTI-APOPTOTIC COMPONENT OF PH

The resistance to apoptosis is critical in the pathobiology of PH favouring proliferation and the progression of PH. Since many of the PH patients presenting with disease have profound, fixed pulmonary vascular lesions, reversal of this pathology would be a better therapeutic strategy than mere inhibition of disease progression. In support of this hypothesis, pharmacological inhibitors such as PDGFR inhibitors, serine-elastase inhibitors, simvastatin, Rho-kinase inhibitors and metabolic modulators were shown to reverse established experimental PH by inducing apoptosis in the PA media [5, 39, 47, 90, 91]. In addition, aberrant expression of Bcl-2, the most notable inhibitor of apoptosis, has been well characterised in the lungs of patients with PAH [92]. Recent studies described selective expression of survivin, a novel apoptosis inhibitor, in the PAs of patients with PAH and rats with monocrotaline-induced PAH, but not in the PAs of patients and rats without PAH. Indeed, further exploration of the pathophysiological role of survivin in monocrotaline-induced PAH rats has shown that it causes vascular remodelling and elevated PA pressures. On the other

hand, gene therapy with inhalation of an adenovirus carrying a dominant-negative mutant of survivin reversed established monocrotaline-induced PAH and prolonged survival [93].

THERAPIES TARGETING THE INFLAMMATORY COMPONENT OF PH

A role for inflammation in the development of PH has been suggested based on the finding of inflammatory cells (including macrophages and T and B lymphocytes) and dendritic cells around the plexiform lesions of PAH [94, 95]. Enhanced levels of various cytokines and chemokines such as fractalkine (CX3CL1) and CCL2 (MCP-1) have also been described in severe PAH [44]. This may further contribute to inflammatory cell recruitment and PA SMC proliferation [96, 97]. In this context, NFAT (nuclear-factor-of-activated-T-cells), a master activator of T cells that increases the transcription of multiple inflammatory mediators including cytokines, has become an interesting candidate for investigation in the pathogenesis of PH.

NFAT INHIBITORS

NFAT is a family of transcription factors activated by calcineurin and comprised of four well-characterised members, NFATc1 (NFAT2/c), NFATc2 (NFAT1/p), NFATc3 (NFAT4/x), and NFATc4 (NFAT3) [98]. An elevation in intracellular Ca^{2+}, induced by a variety of mechanisms, increases the activity of the Ca^{2+}-calmodulin-dependent phosphatase, calcineurin. Activated calcineurin binds to and dephosphorylates NFAT, which then translocates to the nucleus, induces cytokine expression and activates T and B cells. Although first identified in activated T cells, NFAT has since been shown to play a role in non-immune cells, including vascular smooth muscle and myocardial cells [99].

Most interestingly, NFAT activation and NFAT nuclear accumulation, as well as increased levels of NFAT-regulated cytokines, were found in both human and experimental models of PAH. Exposure to chronic hypoxia elicited a significant upregulation of NFATc3 in PA SMCs [100], whereas NFATc2 is activated in circulating inflammatory cells and in the remodelled pulmonary arterial wall of PAH patients [101], suggesting that NFAT inhibition can be a powerful therapeutic modality in the treatment of PH. Indeed, pharmacological inhibition of calcineurin activation of NFAT by cyclosporine or genetic ablation of NFATc3 prevents chronic hypoxia-induced arterial wall thickness and right ventricular hypertrophy [100]. In another study, Michelakis and colleagues also found that inhibition of NFAT by VIVIT peptide (which blocks the docking of calcineurin on NFAT) or cyclosporine decreased proliferation and increased apoptosis *in vitro* and *in vivo*; cyclosporine decreased established rat monocrotaline-induced PAH [101]. However, considering the non-specific effects of clinically used NFAT inhibitors (cyclosporine, FK506), more selective and less toxic NFAT inhibitors that directly target NFAT functions are eagerly awaited.

THERAPIES TARGETING THE EXTRACELLULAR MATRIX COMPONENT OF PH

Changes in the extracellular matrix (ECM) underlying the structural and functional abnormalities in the vessel wall are an important component of progressive PH. Early studies involving ultrastructural evaluation of PAs in lung biopsies from patients with PAH showed fragmentation of the internal elastic lamina [102], suggesting a possible role of elastinolytic enzyme in the disease pathogenesis.

SERINE ELASTASE-/MATRIX METALLOPROTEINASE INHIBITORS

Serine elastase inhibitors have been shown to regress experimental PAH [91]. In addition, of the matrix metalloproteinases (MMP), expression of MMP2 and MMP9, which degrade

type IV collagen of basement membranes, increased in the pulmonary vascular bed during both monocrotaline- and hypoxia-induced PH. In accordance, the broad-ranging pharmacological inhibition of MMPs in the monocrotaline model of PH in rats resulted in regression of pulmonary vascular lesions [103], whereas inhibition of MMPs using adenovirus-expressing tissue inhibitor of metalloproteinase 1 (TIMP-1) has been reported to cause both the same and opposite effects [104, 105]. Moreover, several other pharmacological inhibitors such as endothelin antagonists, PDGFR antagonists and PDE inhibitors, which indirectly target either the MMP cascades or endogenous vascular elastases, proved to be beneficial in experimental PH models [47, 83]. However, the clinical use of MMP inhibitors still needs to be explored further.

THERAPIES TARGETING METABOLIC ABNORMALITIES OF PH

Recently, a shift in glucose metabolism from oxidative phosphorylation to glycolysis, significant mitochondrial hyperpolarisation and depressed activity of pyruvate dehydrogenase complex were characterised in PAH mitochondria compared to those of healthy PA SMCs. This metabolic shift suppresses Kv1.5 expression, leading to membrane depolarisation and an elevation of cytosolic K^+ and Ca^{2+}, creating a proliferative, apoptosis-resistant phenotype in the PAH PA SMCs [106]. Most interestingly, the generic drug DCA is an orally available small molecule which, by inhibiting pyruvate dehydrogenase kinase, increases the flux of pyruvate into the mitochondria, thus promoting glucose oxidation over glycolysis and reversing the suppression of mitochondrial apoptosis *in vitro* and *in vivo*. Specifically, DCA treatment reduced pulmonary vascular resistance, pulmonary vascular remodelling and RV hypertrophy with no systemic effects in different models of PH [39, 107]. DCA may therefore prove to be a promising, effective and selective therapy for PAH.

SUMMARY

■ Vasoactive therapy of pulmonary hypertension mitigates further progression of the disease. Approved therapies like endothelin antagonists, prostanoids and phosphodiesterase-5 inhibitors address major pathways which have been shown to contribute to a vasoconstrictor–vasodilator imbalance.
■ New vasodilators are currently under development for the treatment of pulmonary hypertension. Activators of sGC, selective endothelin receptor antagonists, Rho-kinase inhibitors and serotonin antagonists have demonstrated efficacy in preclinical animal models of pulmonary hypertension and are currently under development.
■ Future therapy should also address dysregulated proliferation of cells in the vessel wall and normalise the vessel structure. Increased proliferation, decreased apoptosis, increased migration and metabolic changes have been shown to contribute to pulmonary hypertension.
■ Inhibitors of tyrosine kinases, elastases and pyruvate dehydrogenase normalised vessel structure in animal models of pulmonary hypertension. These compounds are antiproliferative drugs and some of these compounds, such as tyrosine kinase inhibitors, are currently being investigated in clinical trials.

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6

Endothelin receptor antagonists

C. F. Opitz, D. Pittrow

INTRODUCTION

In the last few years, considerable knowledge has been gathered about the pathogenesis of pulmonary arterial hypertension (PAH). An initial vascular insult (sometimes facilitated by genetic susceptibility, often triggered by certain underlying diseases), leads to the end result of pulmonary smooth muscular cell (SMC) and endothelial dysfunction [1]. The typical triad found in the disease comprises vasoconstriction, proliferation of SMC and endothelial cells with remodelling of the pulmonary arterial wall, and thrombosis *in situ* [2, 3]. Many different vascular effectors seem to be involved in the disease process, offering the opportunity for various logical approaches for therapeutic intervention. Indeed, vasoactive mediators such as nitric oxide (NO) and prostacyclin have a confirmed place in therapy, together with antagonists to the activated endothelin (ET) system.

Endothelin-1 (ET-1) has been proven to be a key mediator in the pathogenesis of PAH. Presently, non-selective ('dual', 'mixed') ET_A/ET_B and selective ET_A receptor antagonists are available for therapy and are recommended as first-line therapy in patients with PAH in World Health Organization (WHO) group 1, according to all relevant PAH guidelines [4–6].

EFFECTS OF ENDOTHELIN-1 MEDIATED VIA ET_A AND ET_B RECEPTORS

ET-1 is released principally from endothelial cells that line blood vessels, mainly abluminal, but also from other vascular and non-vascular cells. The majority of its effects are paracrine, the most striking of which is its extremely potent and long-lasting vasoconstrictor action [7]. In addition, ET-1 is profibrotic and involved in the pathogenesis of various diseases, including PAH. Specifically, ET-1 can induce hypertrophy and hyperplasia in various cell types, fibroblast proliferation, extracellular matrix production, inflammation, and neuro-humoral stimulation. Furthermore, it stimulates the generation of other local mediators of vascular tone, including NO, prostacyclins and platelet-activating factors. These factors modulate the effects of ET-1 in the cardiovascular system through their vasorelaxant action and anti-proliferative potential.

When compared with the normal state, circulating plasma ET-1 levels are elevated in a number of diseases such as atherosclerosis, arterial hypertension, heart failure and PAH [8]. Of note, ET-1 plasma levels correlate with parameters of pulmonary haemodynamics [9] and may predict survival in patients with untreated PAH [10].

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Figure 6.1 Schematic illustration of the endothelin system (redrawn with permission from http://www.nature. com/bjp/journal/v153/n6/fig_tab/0707516f1.html). ECE = endothelin converting enzyme; EDRF = endothelium-derived relaxing factor; ET = endothelin; LDL = low-density lipoprotein.

ET_A- VERSUS ET_B-MEDIATED EFFECTS
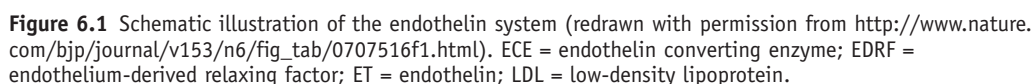
ET-1 acts through two receptor subtypes – ET_A and ET_B. In the vasculature, ET_A receptors are located on (SMCs) and fibroblasts, whereas ET_B receptors are predominantly localised on endothelial cells and, to a lesser extent, on SMCs, fibroblasts and macrophages (Figure 6.1). Recent data suggest that ET_B receptors expressed on SMCs have the ability to couple with ET_A receptors and the former adopt the function of the latter, such that ET_B receptors in heterodimers mediate vasoconstriction similar to ET_A receptors (dimerisation theory) [11, 12]. Furthermore, it has been suggested that selective antagonism of one ET receptor subtype only may result in compensation by the other receptor. This experimental hypothesis has been called 'cross-talk' [13].

However, under normal physiological conditions, the receptor types have broadly opposing functions (Figure 6.1). Activation of ET_A receptors mediates vasoconstriction, proliferation, hypertrophy, cell migration and fibrosis, whereas activation of the endothelial ET_B receptors stimulates the release of potent vasodilators (NO and prostacyclin), exhibits anti-proliferative properties, and prevents apoptosis [14]. Importantly, ET_B receptors on endothelial cells mediate the clearance of circulating ET-1 in the lungs, kidney and liver, with up to 50% of mature ET-1 in healthy subjects and 40% in patients with PAH cleared via

pulmonary ET_B receptors [15]. Endothelial cell ET_B receptor activation also inhibits endothelin converting enzyme-1, the enzyme required to produce mature ET-1 [16].

Alterations in the distribution and number of ET_A and ET_B receptors in conditions such as PAH suggest that their roles in the disease state may differ from those in normal physiology. For example, there are more ET-1 binding sites in the distal pulmonary vessels of patients with PAH, and ET_B receptors are also upregulated [17]. ET_B receptors may not exclusively mediate pulmonary vasodilatation, due to the effects of a subpopulation of ET_B receptors located on SMC and fibroblasts.

Early suggestions that the endothelium might be dysfunctional, resulting in diminished expression or loss of function of the ET_B receptors [13], have recently been challenged by the findings of Langleben and colleagues who observed intact or only modestly reduced ET_B-mediated clearance of ET-1 in patients with pulmonary hypertension of various aetiologies [15]. The authors concluded that the increased ET-1 levels were primarily due to excess synthesis rather than reduced clearance.

PHARMACOLOGICAL APPROACHES TO ENDOTHELIN SYSTEMS

Endothelin receptor antagonists (ETRAs) are usually categorised according to their selectivity for the ET_A or ET_B receptors. To some extent, however, the definition of receptor selectivity is arbitrary, given the wide variation in values obtained using different experimental systems. For example, the ETRA ambrisentan has been reported to have an ET_A:ET_B selectivity ranging from 29:1 for ET-1-mediated contraction in the rat aorta [18] to 4000:1 in myocardial membranes [16].

An indication of functional selectivity can be gained from observations of the effects of different ETRAs on circulating ET-1 levels *in vivo*. For example, sitaxentan (*in vitro* ET_A:ET_B selectivity 6500:1) acutely decreases ET-1 levels in patients with chronic heart failure [19], indicating that ET_B receptors, which play a role in ET-1 clearance, remain functional. In contrast, bosentan and less selective ET_A receptor antagonists (ET_A/ET_B ratio <2000:1) increase plasma ET-1 in healthy volunteers and in patients with heart failure or PAH (Table 6.1). Interestingly, significant increases of ET-l levels occurring 2 hours following ingestion have been reported with ambrisentan (widely reported to be selective for ET_A) suggesting that its functional selectivity may differ from that observed *in vitro* [20]. Whether elevated ET-1 levels seen in ETRA-treated PAH patients have pathophysiological or prognostic significance remains unknown.

EXPERIMENTAL EVIDENCE ON ET RECEPTOR SELECTIVITY: VASODILATATION, REMODELLING AND FIBROSIS

Vasodilatation
Vasodilatation is an important goal of therapeutic intervention for PAH. Theoretically, selective ET_A receptor antagonists should be more effective at achieving this than non-selective ET_A/ET_B receptor antagonists, given the role played by ET_B receptors in both vasodilatation and ET-1 clearance. In animal models of PAH, however, positive dilatory effects have been observed with both selective ET_A receptor blockade as well as non-selective antagonism [21]. The majority of available data are extrapolated from human studies performed on blood vessels in the systemic circulation (reviewed by Opitz *et al*, [22]). Collectively, these studies indicated that:

1. Selective ET_A receptor blockade results in a robust vasodilator response and increased blood flow.
2. Selective ET_B receptor blockade results in vasoconstriction and reduced blood flow.
3. Co-administration of selective ET_A and selective ET_B receptor antagonists attenuates the vasodilator response relative to selective ET_A receptor blockade.

Table 6.1 Effects of bosentan and sitaxentan on ET-1 plasma levels in humans

Author, year	Indication	Design	n	Bosentan dose	interval	ET-1 level
Kiowski, 1995 [81]	CHF	r, pc, db	24	100 → 200 mg i.v. (acute)	1h	↑ >2.0x
Sütsch, 1998 [82]	CHF	r, pc, db	36	1000 mg oral (acute)	3h (day 1)	↑ >2.0x
				(chronic 1000 mg bid (day 14))	3h (day 14)	↑ >1.3x
Weber, 1996 [83]	Healthy volunteers	o		3–2400 mg oral		↑ 2x (oral)
				10–750 mg i.v. (single ascending doses)		↑ 3x (i.v.)
Williamson, 2000 [84]	PAH	o	7	50, 150, 300 mg i.v.	6h	↑ 2x (dose-dependent)
Hiramoto, 2007 [85]	PAH	o	7	62.5 mg (single oral dose)	6h	↑ 2.0x
Author, year	**Indication**	**Design**	**n**	**Sitaxentan dose**		**ET-1 level**
Givertz, 2000 [19]	CHF	o	47	0.5, 3.0, or 6.0 mg/kg	6h	↓ 0.8
Author, year	**Indication**	**Design**	**n**	**Ambrisentan dose**		**ET-1 level**
FDA, 2007 [20]	Healthy volunteers	o	7	5 mg (single oral dose)	2h	↑ 1,6 pg/ml *
			7	10 mg (single oral dose)	2h	↑ 1,1 pg/ml *

* = placebo-subtracted median; ↑ = increase; ↓ = decrease; x = times; CHF = congestive heart failure; db = double-blind; o = open; pc = placebo-controlled; pg = picogram; r = randomized.

However, while these data provide information regarding the effects of receptor selectivity on blood vessel tone in general, they do not provide precise information on how these drugs work in the pulmonary arterial circulation.

Vascular remodelling

Several studies in animal models document that ETRAs, both non-selective and ET$_A$-selective, prevent, attenuate or even reverse vascular remodelling and/or hypertrophy (reviewed in [22]). For example, in a rat model, during a 2-week hypoxia exposure, sitaxentan [23] and bosentan [24] significantly prevented increases in pulmonary artery pressure (PAP) and pulmonary vascular remodelling. During 6 weeks of exposure to hypoxia, both drugs partially reverted pre-established pulmonary vascular remodelling. Interestingly, sitaxentan but not bosentan prevented the increase in ET-1 levels when treatment was initiated early, with hypoxia, but late treatment (2 weeks after initiation of hypoxia) did not affect established ET-1 elevations.

Fibrosis

Extravascular antimitotic and antifibrotic effects of ETRAs may result in greater efficacy in scleroderma than therapies directed exclusively at the vasculature. Data from animal models using either ET_A-selective or non-selective ETRAs demonstrated an amelioration of ET-1-related effects involving the reduction of growth factor expression, extracellular matrix deposition and matrix metalloproteinase (MMP) activity (reviewed in [22]). *In vitro* data with skin fibroblasts suggested that targeting both the ET_A and ET_B receptors is preferable in order to block collagen type I and III production [25]. However, subsequent *in vitro* data using lung fibroblasts indicated that ET-1 induced collagen matrix contraction through the ET_A but not the ET_B receptor [26]. Furthermore, while there is evidence that ET_B receptors are linked to collagen production *in vitro*, *in vivo* animal data with ET_A antagonists have shown that they effectively block the accumulation of collagen I, III and IV [27], normalise pro-collagen I and III mRNA [28] and abolish the effect of ET-1 on pro-collagen metabolism [29]. Likewise, while there is evidence that under certain conditions ET-1 can act as a mitogen *in vitro* through both ET_A and ET_B receptor activation [30], ET_B receptors have been shown to inhibit vascular SMC proliferation *in vivo* [31]. It has been suggested that ET_B receptors may be upregulated on SMCs and fibroblasts in certain disease states such as scleroderma lung disease [32]. However, the spatial distribution of these receptors among different cell types within the lung microcirculation remains unclear, as does the significance of any increased ET_B receptor expression in PAH.

RESULTS FROM CLINICAL TRIALS IN PAH PATIENTS

Currently three ETRAs – bosentan, ambrisentan and sitaxentan are being marketed. They differ in terms of chemistry, pharmacokinetics and drug–drug interaction potential (Table 6.2).

All three drugs have been investigated in relatively large clinical development programmes with adequate randomised placebo-controlled studies in patients with PAH. The characteristics and main outcomes of the pivotal studies are detailed in Table 6.3.

BOSENTAN

Bosentan is an orally active, non-peptidic, non-selective, sulphonamide-class ET_A/ET_B antagonist with twice-daily (bid) dosing. Bosentan was the first ETRA to receive approval for the treatment of patients with PAH in New York Heart Association (NYHA) functional class III (Europe, USA and Canada) and IV (USA and Canada) at a target dose of 125 mg bid.

In two randomised, controlled trials, bosentan was shown to improve exercise capacity, functional class, haemodynamics and time to clinical worsening [33, 34]. Additional open-label, long-term studies in patients with PAH demonstrated persistent efficacy of bosentan over time and potential for improved survival compared with predicted survival [35, 36].

Since these first pivotal studies, significant benefits of bosentan treatment have been shown in separate studies (Bosentan Randomised Trials of Endothelin Antagonist Therapy: BREATHE) in children with PAH [37] (BREATHE-3: idiopathic PAH [IPAH] and congenital heart disease [CHD]), in PAH associated with human immunodeficiency virus (HIV) [38] (BREATHE-4), in patients with PAH and Eisenmenger syndrome [39, 40] (BREATHE-5), and in patients with portopulmonary hypertension [41].

In addition, EARLY (Endothelin Antagonist Trial in Mildly Symptomatic PAH Patients) was the first study specifically designed to evaluate the effects of ETRA treatment in 185 PAH patients in functional class II [42]. The results from this 6-month trial showed a significant reduction in pulmonary vascular resistance (PVR), while the other primary endpoint, the 6-minute walk distance (6MWD), did not reach statistical significance. The secondary endpoint, time to clinical worsening, showed a significant improve-

Table 6.2 Pharmacological and pharmacokinetic characteristics of approved ET receptor antagonists

Parameter	Bosentan	Sitaxentan	Ambrisentan
Structure	Etherocyclic sulfonamide	Amidothiophene-sulfonamide	Diphenly propionic acid
Selectivity ET_A: ET_B	30:1	6500:1	4000:1*
ET plasma levels after administration	↑	↓	↑
Approved daily dosing	125–250 mg	100 mg	5–10 mg
Titration	yes	no	yes
Resorption			
Absolute Bioavailability	~50%	70–100%	high
Food effect on resorption	no	no	no
Time to max plasma concentration (tmax)	3–5 h	1–4 h	1.7–3.3 h
Distribution			
Albumin binding	>98%	>99%	n.r.
Metabolism and excretion			
Terminal elimination half-life	5,4 h	10 h	15 h
Steady state	3–5 d	6 d	3–4 d
Metabolism	hepatic (CYP)	hepatic (CYP)	hepatic (glucunoridation)
p450 cytochromes mainly involved	CYP 2C9 ↑, 3A4 ↑	CYP 2C9 ↓	none
Excretion in urine	<3 %	50–60 %	low
Significant drug–drug interactions	sildenafil, glibenclamide, warfarin, cyclosporin, contraceptives	warfarin, cyclosporin A	none reported‡ (caution with cyclosporin A)

CYP: ↑ = drug induces; ↓ = drug inhibits; ‡ = no relevant interactions for sildenafil, ketoconazole, digoxin or warfarin, however drug interaction potential not well characterised according to US labelling. *Depending on the model used: 29:1 to 4000:1.

ment with bosentan, which translated into a 70% risk reduction. Effects were consistent in PAH patients receiving bosentan as monotherapy and those who were sildenafil-treated and received bosentan as add-on therapy.

In another study of 157 patients with chronic thromboembolic pulmonary hypertension (CTEPH, WHO group 4), bosentan therapy led to significant reductions in PVR and improved dyspnoea score, while the 6MWD remained unchanged over the 6-month study period (BENEFIT; Bosentan Effects in Inoperable Forms of Chronic Thromboembolic Pulmonary Hypertension) [43].

AMBRISENTAN

Ambrisentan is an orally active ET_A-receptor antagonist belonging to the propanoic acid class. Although data describing the selectivity of ambrisentan for the ET_A receptor vary between 29:1 [18] to >4000:1 [16] depending on the assay cited, the drug is considered a selective ET_A receptor antagonist [44, 45]. In the USA and Europe, ambrisentan has been

approved at a dose of 5–10 mg once daily for PAH patients with WHO functional class II or III symptoms to improve exercise capacity and delay clinical worsening.

Results are based on a 12-week, blinded-to-dose (1, 2.5, 5 or 10 mg daily) Phase II study [46] (improvements in 6MWD, functional class, Borg score, quality of life and pulmonary haemodynamics) and two trials, ARIES-1 and ARIES-2 (Ambrisentan in Pulmonary Arterial Hypertension, Randomized, Double-Blind, Placebo-Controlled, Multicenter Efficacy Study) [47]. The long-term follow-up of patients treated with ambrisentan in these two pivotal studies and the open-label extension (ARIES-E, n = 383) showed that 95% were alive at 1 year and 94% were still receiving ambrisentan monotherapy, with sustained efficacy for 6MWD, dyspnoea score and functional class [47, 48].

SITAXENTAN

Sitaxentan sodium, a selective ET_A-receptor antagonist with sulphonamide structure, has been approved for the treatment of PAH patients with WHO functional class III symptoms at an oral dose of 100 mg once daily (European Union, Canada, Australia). The Food and Drug Administration (FDA) has not approved sitaxentan to date: another placebo-controlled study with this agent is currently underway (STRIDE-5, Sitaxentan to Relieve Impaired Exercise in Pulmonary Arterial Hypertension) to provide additional data.

The safety and efficacy of sitaxentan in patients with PAH has been clinically tested in the STRIDE programme [44], including three randomised, placebo-controlled pivotal trials (STRIDE-1 [49], STRIDE-2 [50], and STRIDE-4), two non-controlled studies (Study 211 and STRIDE-6 [51]) and three long-term studies (STRIDE-1X, STRIDE-2X [52] and STRIDE-3).

Sitaxentan significantly improved functional class (STRIDE-1, STRIDE-2, STRIDE-4), 6MWD (STRIDE-1, STRIDE-2), dyspnoea score (STRIDE-1), and haemodynamics (Study 211, STRIDE-1). Improvements in time to clinical worsening could only be demonstrated in a *post hoc* meta-analysis using pooled data from the three pivotal studies [44].

Long-term data are available from a small group of patients, suggesting that efficacy and safety are maintained for up to 12 months [53], as well as data from the extension studies, with mean exposures of 26 weeks (STRIDE-1X [44]) and 44 weeks (STRIDE-2X [52]).

ET RECEPTOR ANTAGONISM AND DRUG EFFICACY

The most frequent clinical endpoint used to assess drug efficacy in PAH has been exercise capacity, assessed by the 6MWD, although its appropriateness as a measure has been debated [54].

6-MINUTE WALK DISTANCE (6MWD)

Studying the evidence where 6MWD was used as a measure of efficacy, the placebo-corrected improvements from baseline to week 12 (BREATHE-1 [34], ARIES-1 and ARIES-2 [47]) or week 18 (STRIDE-2 [50]) were +35, +31/+51 (5/10 mg, ARIES-1), +32/+45 (5/10 mg, ARIES-2) and +31 meters for bosentan, ambrisentan and sitaxentan, respectively (Table 6.3). A direct comparison is difficult, as BREATHE-1 included only patients in functional classes III and IV while ARIES-1 and STRIDE-2 included ≥35% of patients in functional classes I and II. These less ill patients would be expected to show a smaller absolute treatment effect compared to severely ill patients.

TIME TO CLINICAL WORSENING

Significant improvements in the time to clinical worsening have also been reported for all three ETRAs discussed. In BREATHE-1, both the time to- and the incidence of clinical worsening were significantly reduced with bosentan compared with placebo [34]. During

Table 6.3 Characteristics and main outcomes of pivotal studies for approved ET antagonists

	Bosentan		Sitaxentan		Ambrisentan	
	Study 351 (Channick 2001) [33]	BREATHE-1 (Rubin 2002) [34]	STRIDE-1 (Barst 2004) [49]	STRIDE-2 (Barst 2006) [50]	ARIES-1 (Galie 2008) [47]	ARIES-2 (Galie 2008) [47]
Selectivity	Oral ETA/ETB antagonist	Oral ETA/ETB antagonist	Oral selective ETA antagonist	Oral selective ETA antagonist	Oral selective ETA antagonist	Oral selective ETA antagonist
Drugs and dosages in the study	placebo/ 125–250 mg	placebo/250/500 mg	placebo/100/300 mg	placebo/sitax50/sitax100/bos	placebo/5/10 mg	placebo/2.5/5 mg
Settting	US and EUR	US and EUR	US, Can (1 centre)	US and EUR	US and EUR	US and EUR
Dosing regimen	2x/d	2x/d	1x/d	1x/d	1x/d	1x/d
Study details at inclusion/baseline characteristics						
Study duration post randomisation	12 wks	16 wks	12 wks	18 wks	12 wks	12 wks
Inclusion range for age [yrs]	≥18	≥12	≥16–75	12–78	≥18	≥18
Basline 6-min walk distance for inclusion (m)	≥150 and ≤500	≥150 and ≤450	not defined (only second endpoint)	≥150 and ≤450	≥150 and ≤450	≥150 and ≤450
PAH etiology	IPAH (81%), PAH-SSc (19%) in Bos group	IPAH (71%), PAH-SSc (23%), PAH-Lupus (6%)	IPAH (53%), PAH-CTD (24%), PAH-CHD (24%)	IPAH (59%), PAH-CTD (30%), PAH-CHD (11%)	IPAH (63%), PAH assoc. with CTD, HIV, anorexigen (37%)	IPAH (65%), PAH assoc. with CTD, HIV, anorexigen (35%)
WHO functional class	III (100%)	III (90%), IV (10%)	II (33%), III (66%), IV (1%)	II (37%), III (59%), IV (4%)	I (3%) II (32%), III (58%), IV (7%)	I and II (46%), III and IV (54%)
Patient disposition	36 screened, 32 randomized (2:1), no discontinued	Screened: n.r., 213 randomized (1:1:1), 14 discontinued	Screened: n.r., 178 randomized (1:1:1), 12 discontinued	Screened: n.r., 247 randomized (1:1:1:1), 31 discontinued	Screened: 269, 202 randomized (1:1:1), 14 withdrawn, 4 died	Screened: 215,, 192 randomized (1:1:1), 16 withdrawn, 4 died
Males: females [%]	19:81 (bos)	29:71 (bos)	21:79	22:78	16:84	26:74
Mean age [yrs]	52 (33–73) (bos)	49 (13–80)	46 (17–74)	54 (14–78)	53±14 in 5 mg and 49±16 in 10 mg	52±15 in 2.5 mg 50±16 in 5 mg
Mean 6-min walk distance at baseline [m]	360 (±86) (bos)	330 (±74)	398 (±110)	337 (±80)	340±77 in 5 mg and 341±78 in 10 mg	347±84 in 2.5 mg and 355±84 in 5 mg

	54 (±13) (bos)	55 (±16)	54 (±15)	48(± 14)	47±13 in 5 mg and 51±16 in 10 mg	48±14 in both ambri groups
mPAP [mmHg]						
Results at study end in the treatment groups						
6-min walk distance [m]	−6 / 70*	−8 / 27*/ 47**	−13 / 22** / 20**	−6.5 / 17.8 / 24.9 / 23.0	(ca. −8) /corr 31*** /corr 51***	(ca. −10) /corr. 32* /corr. 59***
mRAP [mmHg]	4.9 / −1.3**	n.a.	1 / 0*	n.a.	n.a.	n.a.
mPAP [mmHg]	5.1/ −1.6*	n.a.	0 / −3 / −5***	n.a.	n.a.	n.a.
Cardiac output [L/min]	−0.5 / 0.5*** (CI)	n.a.	CI: 0.0 / 0.3 / 0.4***	n.a.	n.a.	n.a.
PVR [dyn.sec.cm^{-5}]	191 / −223***	n.a.	49 / −221 / −194***	n.a.	n.a.	n.a.
Median change in peak VO$_2$ [ml O$_2$/kg/min]	n.a.	n.a.	0.0 / 0.5 / 3.1**	n.a.	n.a.	n.a.
Improvement in Borg dyspnea index	1.4 / −0.2	0.3 / −0.1 / −0.6	n.r.	0.2 / n.r. / 0.0 / n.r.	ambri combined −0.6 vs PBO	ambri combined −1.1 vs PBO
Improvement in WHO functional class [%]	9 / 43	30 / 43 / 41	15 / 29 / 30	sitax 100mg signif.	pooled ambri: signif.*	pooled ambri 2.5 and 5mg: not signif.
Time to clinical worsening	sign. improved vs pla	sign. improved vs pla	n.r.	sitax 100mg not signif., but trend	Not significant for both groups	ambri signif.*** for both groups
Incidence of clinical worsening [n]	27 / 0*	14 / 5 / 4	5 / 0 / 2	10 / 6 / 4 / 9	6 / 3 / 3	14 / 3 / 3
LFT elevations > 3 × ULN [%]	0 / 6.3	3 / 4 / 14	3/0 / 10	6 / 5 / 3 / 11	3 / 0 / 0	2 / 0 / 0
Peripheral edema [%]	5 / 8	5/8†	17 / 16 / 25	8 / 8 / 11/ 15	10 (pla), 27 (5mg), 28 (10mg)	11 / 3 (2.5mg) / 10 (5mg)

Statistics vs placebo: * = P <0.05; ** = P <0.01; *** P <0.001; n.a. = not applicable (i.e., not done in the study); n.r. = not reported; bos = bosentan group only; CI = cardiac index; CHD = congenital heart disease; CTD = connective tissue disease; corr., placebo-corrected; mPAP = mean pulmonary artery pressure; PVR = pulmonary vascular resistance

Table updated from Opitz CF, Ewert R, Kirch W, Pittrow D. Inhibition of endothelin receptors in the treatment of pulmonary arterial hypertension: does selectivity matter? *Eur Heart J* 2008; 29:1936-1948.

ARIES-1 [47], the differences between ambrisentan and placebo with respect to clinical worsening did not reach statistical significance; however, this was demonstrated in the ARIES-2 trial and in the combined 5 mg group [47]. In a *post hoc* meta-analysis pooling 512 patients from STRIDE-1, STRIDE-2 and STRIDE-4, significant improvements in time to clinical worsening were seen in patients treated with sitaxentan 100 mg daily compared with placebo [44]; this is in contrast to the individual STRIDE-1 [49] and STRIDE-2 [50] studies, where statistical significance was not reached.

HAEMODYNAMICS

Since ET_A and ET_B receptors counter-regulate vascular tone, variations in receptor selectivity could result in different haemodynamic profiles. The haemodynamic changes from baseline to week 12 for bosentan and sitaxentan are shown in Table 6.3. Despite differing study populations, both drugs equally reduced PVR by an average of 220 dynes.sec.cm^{-5} following a 3-month treatment period; comparable to the decrease of 226±202 dynes.sec.cm^{-5} reported for ambrisentan [46]. From these clinical data, no ETRA seems to have distinct haemodynamic advantages over another.

SURVIVAL

There is no definitive study proving a survival benefit for any ETRA, owing to the fact that long-term, placebo-controlled studies are perceived as ethically unjustifiable. Therefore, survival rates for new PAH therapies are generally compared with survival rates extrapolated from the dataset of a historical cohort of untreated patients [55].

For patients enrolled in the two placebo-controlled bosentan trials and subsequently followed-up for a mean of 2.1 ± 0.5 years, survival estimates were 96% and 89% at 12 and 24 months, compared with a predicted survival of 69% and 57%, respectively [35]. At the end of 12 and 24 months, 85% and 70% of patients, respectively, remained on bosentan monotherapy. Another retrospective analysis of 103 consecutive IPAH patients treated with first-line bosentan therapy reported overall survival estimates of 92, 89 and 79% at 1, 2 and 3 years, respectively, compared with a predicted survival of 71, 61 and 51% at these time-points [36] (85% and 70% on monotherapy at 12 and 24 months, respectively). In this group, 44% of patients received additional intravenous epoprostenol therapy during follow-up.

For ambrisentan, an integrated analysis of 383 PAH patients in ARIES-l, ARIES-2 or ARIES-E reported a one-year survival of 95% [48]. During long-term, open-label treatment (mean 1.7 years), of the 64 PAH patients treated with ambrisentan, survival in the IPAH subgroup was 89% (67% on monotherapy) compared with a predicted survival of 66% [56].

Survival data for sitaxentan are available from the STRIDE-2X study for 92 patients with PAH treated with sitaxentan 100 mg/day [52]. At 1 year, survival estimates were 96% for the entire PAH group with no difference in the subgroup of patients with PAH and connective tissue disease. Additional PAH therapies had been added during this period in 17% of the patients. In summary, from these data, differential effects on survival with any of the ETRAs discussed cannot be inferred.

COMPARATIVE TRIALS

A unique dataset is provided by the STRIDE-2 trial, in which 245 patients were randomised to placebo, sitaxentan (50 or 100 mg q.d.) or bosentan (62.5 mg bid for 1 month followed by 125 mg bid) [57]. The bosentan arm was, however, open-label and included only as a comparator arm (events were rater-blinded). At 18 weeks both the sitaxentan 100 mg and bosentan arms showed significant increases in 6MWD, the primary endpoint. Improvements in

Table 6.4 Frequent side-effects of the three ETRAs in PAH patients according to labelling

Side-effect	Sitaxentan	Bosentan	Ambrisentan
ALT/AST elevations	>3 × ULN: 7% for sitax 100 mg/d-treated patients (*n* = 887) vs 5% of PBO-treated patients (*n* = 155). >5 × ULN: 4% (36/887) for sitaxentan 100 mg/d vs 0.6% in the PBO group (1/155). Elevation of cases of symptomatic hepatitis has occurred in patients receiving Thelin 100 mg once daily. One fatal case has been reported with an initial dosage of sitaxentan of 600 mg/day.	>3 × ULN: 8 integrated PBO-controlled studies (6 other than PAH): 11.2% of the bosentan vs 1.8% of the PBO-treated patients. In PAH: 11.6% for bosentan 125 mg bid, and 14.3% for bosentan 250 mg twice daily >8 × ULN: 2.1% for bosentan 125 mg bid vs 7.1% for 250 mg twice daily.	>3 × ULN: 0.8% for ambri vs 0.2% PBO >8 × ULN: 0.2% for ambri vs 0% for PBO
Peripheral oedema	9%	PBO-controlled studies: 4.7% vs 1.4% PBO BREATHE-5 study: 18.9% vs 5.9% PBO BREATHE-4 study: 31% (no PBO) RAPIDS-1, -2: 14% vs 5% PBO	17% (PBO-adjusted 6%)
Headache	15%	PBO-controlled studies: 15.8% vs 12.8% PBO BREATHE-5 Study: 13.5% vs 11.8% PBO BREATHE-4 study: 19% (no PBO)	15% (PBO-adjusted 1%)
Decreased haemoglobin	7% (PBO-adjusted 4%)	5.6% vs 2.6% plac	7% (PBO-adjusted 3%)

Source: Package leaflets (Summary of Product Characteristics, SmPC) of Tracleer, Thelin and Letairis. ALT = alanine aminotransferase; AST = aspartate aminotransferase; PBO = placebo.

functional class (secondary endpoint) were observed with sitaxentan 100 mg ($P = 0.04$). Time to clinical worsening did not improve with either treatment.

After 18 weeks, patients were entered into the extension study STRIDE-2X where patients who received sitaxentan (100 mg) or bosentan during STRIDE-2 continued on their respective therapies in an open-label fashion [52]. Patients receiving sitaxentan 50 mg daily during STRIDE-2 had their dosages increased to 100 mg daily, and the patients on placebo were assigned to sitaxentan (100 mg daily) or bosentan. Results of *post hoc* analyses (of pre-specified endpoints) for patients treated for up to 1 year (bosentan: *n* = 84, sitaxentan

100mg: n = 92) revealed differences between the treatment arms, with better outcomes for the sitaxentan-treated patients when compared with bosentan therapy in parameters such as risk of discontinuation of monotherapy (30% vs 43%, hazard ratio 0.58; 95% CI 0.35–0.97) and elevation of AST (aspartate aminotransferase) and/or ALT (alanine aminotransferase) levels >3 × ULN (upper limit of normal) (6% vs 14%). No significant differences between both treatment regimens were observed for risk of clinical worsening (34% vs 40%, hazard ratio 0.73; 95% CI 0.45–1.21) or survival at 1 year (96% vs 88%, hazard ratio 0.34; 95% CI 0.11–1.10) [52].

ETRAs AND SAFETY

CLINICAL SIDE-EFFECTS

ETRAs are generally well tolerated. However, owing to their vasodilatory properties, peripheral oedema, headache and palpitations can occur. Table 6.4 provides an overview on the incidence of those side-effects that have the greatest relevance in everyday clinical care.

ABNORMAL LIVER FUNCTION TESTS AND OPTIONS IN CASE OF HEPATOTOXICITY

The most clinically important side-effects reported with ETRA therapy are dose-dependent liver function abnormalities. These present as elevated ALT/AST and/or bilirubin levels and are seen more frequently with the sulphonamide-class ETRAs. Since these changes are a marker for potentially serious liver injury, serum aminotransferase levels (and bilirubin, if aminotransferase levels are elevated) must be measured prior to initiation of treatment and then monthly thereafter. It has been reported that bosentan inhibits the bile salt export pump, which may lead to cholestatic liver injury due to the intracellular accumulation of bile salts, while increasing bile salt-independent bile flow [58, 59]. While the incidence of hepatotoxicity in the placebo-controlled trials was highest with bosentan (Tables 6.2 and 6.4), the inclusion criteria and control group characteristics of the underlying studies should be taken into account: in the ARIES-1, ARIES-2 and STRIDE-2 studies, patients with liver enzyme elevations >1.5 × ULN at baseline were excluded, while in STRIDE-1 and BREATHE-1 patients with elevations up to 3 × ULN were eligible. Similarly, the incidence of hepatic aminotransferase elevations >3 × ULN in the control groups varied between 0 and 6% (Table 6.3).

    Consequently, drug surveillance programmes, named TRAX (TRAcleer eXcellence Post-Marketing Surveillance Programme) for bosentan, TOPS (Thelin Outcomes for Patients Surveillance) for sitaxentan and VOLT (VOLibris Tracking) for ambrisentan had/have to be conducted in the first years after introduction for all ETRAs. In the USA, the marketed drugs (bosentan, ambrisentan) can only be prescribed within the framework of specially restricted distribution programmes.

    It is a useful finding that in case of elevated transaminases, a switch to another ETRA may be an option. The STRIDE-6 study [51] aimed to explore the potential use of sitaxentan in PAH patients who had previously discontinued bosentan treatment (13 patients owing to 'safety issues'; 12 with aminotransferase elevations; and 1 with rash). Among the 12 patients with liver enzyme elevations on bosentan treatment, only one individual re-developed this side-effect during 12 weeks of sitaxentan therapy. In a recent study with a similar 'switch' design, 36 patients who previously discontinued bosentan (n = 31), sitaxentan (n = 2), or both (n = 3) due to elevations in hepatic aminotransferases were enrolled (at baseline, 69.4% of patients were receiving prostanoid and/or sidenafil therapy) and treated with ambrisentan. No patient had an aminotransferase >3 × ULN that required ambrisentan discontinuation, one patient had a transient aminotransferase >3 × ULN that resolved following a temporary dose reduction, and no additional aminotransferases >3 × ULN were observed with long-term treatment (median exposure 102 weeks), despite dose increases to 10 mg

once daily in more than half of the patients. Significant improvements in 6MWD and other efficacy assessments were observed [60].

It is likely that chemical properties of the drugs, the pharmacokinetics or drug–drug interactions or patient characteristics may influence the incidence and severity of the side-effects rather than differences in ET receptor selectivity.

DECREASED HAEMOGLOBIN

Due to an as yet incompletely identified mechanism, potentially related to vasodilatation and subsequent fluid shift producing haemodilution, all ETRAs are associated with a (usually modest) dose-dependent and partially transient reduction in haemoglobin levels. Decreases in haemoglobin were not related to haemolysis, bone marrow depression or risk of bleeding. They occur in about 5–7% of patients, irrespective of the ETRA used. Haemoglobin (and haematocrit) reductions are likely to be a dose-dependent class effect of the ETRAs. For all three agents these symptoms typically do not require discontinuation of therapy or dose adjustment and are usually not dose-dependent (up to the approved doses).

PERIPHERAL OEDEMA

There has been speculation as to whether peripheral oedema occurs more frequently as a drug-specific, ET_B-mediated side-effect. However, looking at the pivotal ETRA studies (Table 6.4) suggests that the incidence of leg oedema is related to patient characteristics (as can be derived from the large variance in the placebo groups), and is not a significant drug-related effect. Notably, a warning label has been issued by the FDA for ambrisentan based on post-marketing reports of fluid retention occurring within weeks of starting ambrisentan [45].

ETRAS AND PULMONARY HYPERTENSION SUBGROUPS

Most ETRA trials included remarkably similar patient groups, mainly patients from WHO group 1. However, the spectrum of PAH is not fully represented in these trials since most patients studied had idiopathic or familial PAH (>50%) or PAH associated with connective tissue disease (20–30%). Other disease entities have rarely been included, like congenital heart disease (CHD, <10%) or HIV (<5%). Some forms of PAH such as portopulmonary hypertension or PAH in children, have even been systematically excluded from the pivotal trials.

CONNECTIVE TISSUE DISEASE

Patients with PAH in association with connective tissue disease (PAH-CTD) such as systemic sclerosis, lupus erythematosus or mixed connective tissue disease have a particularly poor prognosis [61]. Importantly, this subgroup has been included in several trials evaluating ETRAs.

The BREATHE-1 trial included 47 patients with systemic sclerosis (22%) [34]. In contrast to patients with IPAH, bosentan did not significantly increase 6MWD. However, the decline in walking distance of 40 m at 16 weeks in the systemic sclerosis placebo group ($n = 14$) was prevented by bosentan (+3 m; $n = 33$).

For ambrisentan, comparable efficacy with respect to functional capacity, measured by 6MWD, was described for 19 patients (30%) with PAH associated with collagen vascular disease, when compared with IPAH patients [46].

A *post hoc* analysis of 42 patients with PAH-CTD enrolled in the STRIDE-1 study [62] showed that during the 12-week placebo-controlled phase, sitaxentan (pooled 100 and

300 mg groups) increased the placebo-subtracted 6MWD by 58 m ($P = 0.027$) improved haemodynamics as well as certain domains within the quality-of-life assessment. Notably, in contrast to the bosentan data, sitaxentan not only prevented deterioration of exercise capacity but significantly improved 6MWD (+20 ± 52 m; $P = 0.037$) when compared with baseline. In another *post hoc* meta-analysis pooling 512 patients from STRIDE-1, STRIDE-2 and STRIDE-4, a subgroup of 129 patients with PAH-CTD was analysed. Within this subgroup the 39 patients treated with sitaxentan 100 mg daily showed a significantly improved 6MWD (+38 m; $P = 0.0419$), compared with placebo [44]. This effect was not seen with sitaxentan 50 mg or 300 mg daily in this PAH subgroup.

Long-term outcomes in PAH associated with connective tissue disease

Retrospective analyses have been published examining the long-term effects of ETRAs in patients with PAH associated with CTD. In two randomised, controlled studies investigating bosentan in PAH [33, 34], 66 patients with PAH-CTD were randomised to receive either bosentan ($n = 44$) or placebo ($n = 22$). The 44 patients on bosentan were stable in 6MWD at the end of the study, while the placebo patients had deteriorated: the placebo-subtracted difference was 22 m (non-significant). Subsequently, in an open-label, long-term extension study (1.6 ± 0.9 years), survival rate in the 64 patients receiving bosentan was 86% after 1 year and 73% after 2 years [63]. These outcome data are comparable to the 81% and 71% survival rates at 1 and 3 years, respectively, seen among 45 patients with PAH associated with scleroderma, treated with bosentan (mono- or combination therapy), as detailed in the Royal Free Hospital registry. These findings compare favourably to the 68% and 47% 1- and 2-year survival, respectively, in an historical cohort of 47 patients in the same institution treated with conventional therapy [64].

Within the STRIDE-2X study, 52 patients with PAH-CTD (scleroderma $n = 38$; overlap syndrome $n = 9$; lupus $n = 5$) suggested a differential response to sitaxentan versus bosentan, in that the risk of discontinuation at 1 year was 24% for sitaxentan and 57% for bosentan (compared to 32% and 37%, respectively, for the non-CTD group of 123 patients). Furthermore, survival at 1 year in patients with PAH-CTD was 96% with sitaxentan and 80% with bosentan [52].

OTHER PULMONARY HYPERTENSION SUBGROUPS

HIV-ASSOCIATED PAH

Among the randomised, controlled trials, only ARIES-1 and ARIES-2 [47] included patients with PAH and HIV, representing ≤5% of the population studied. Open-label studies [38, 65, 66] reported the effects of bosentan in patients with HIV-associated PAH, showing an improvement in all efficacy parameters, including 6MWD, functional class and haemodynamic variables. The rate of liver toxicity was comparable to those in other PAH subgroups.

PORTOPULMONARY HYPERTENSION

The use of ETRAs in this patient population is controversial due to the risk of liver toxicity. However, bosentan has been used successfully in these patients, improving haemodynamics, symptomatic state and survival when compared to historical controls [41, 67]. In Europe, only bosentan is approved for patients with Child A liver disease, while it is not recommended in Child B and C patients.

CONGENITAL HEART DISEASE AND PAH

Bosentan has been studied in patients with PAH and CHD (Eisenmenger syndrome) showing improvements of pulmonary vascular resistance and 6MWD [39]. These positive effects

persisted for up to 40 weeks [40]. In a series of 14 patients with Eisenmenger syndrome on sitaxentan, with initiation of treatment an improved pulmonary to systemic vascular resistance (PVR/SVR) ratio consistent with pulmonary selectivity was observed, and treatment was well tolerated, with no significant decrease in resting SaO_2 at early or late-term follow-up [68]. To date, no data on ambrisentan in CHD have been published.

CHILDREN

In general, treatment recommendations for PAH are similar in children when specific studies are lacking. Bosentan has been studied in this paediatric population with positive results and survival rates around 80–90% at 1 year [37, 69, 70]. The drug is available in a dedicated paediatric formulation with specific dosing regimens.

Apart from patients with PAH, ETRAs have been evaluated in patients with other forms of pulmonary hypertension (PH), especially in chronic thromboembolic disease (WHO Group 4) and in patients with lung disease and hypoxia (WHO Group 3).

CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION (CTEPH)

Only following careful evaluation for potential surgical therapy can certain patients with CTEPH become candidates for targeted medical treatment. Non-controlled studies suggested beneficial effects of bosentan treatment in this patient population [71–73]. The BENEFIT trial is the only randomised, controlled trial so far demonstrating safety and efficacy of bosentan in patients with CTEPH [43]. Bosentan significantly reduced pulmonary vascular resistance at 16 weeks although exercise capacity (6MWD), functional class and time to clinical worsening did not improve. So far, none of the ETRAs has been approved in CTEPH.

PAH ASSOCIATED WITH COPD OR FIBROSIS

In patients with PH and chronic obstructive lung disease or idiopathic pulmonary fibrosis no randomised controlled trials have been performed with ETRAs and these drugs are not approved in this indication.

DRUG METABOLISM, DRUG INTERACTIONS AND COMBINATION THERAPY

Bosentan, ambrisentan and sitaxentan have divergent pharmacological and pharmacokinetic characteristics, resulting in clinically important differences with respect to drug metabolism, drug interactions and their potential for use in combination therapy (Table 6.2). Of interest are the interactions of bosentan with sildenafil, a frequently used combination therapy, where sildenafil plasma levels are reduced by about 50% while bosentan concentrations rise by approximately 50% [74, 75]. Theoretically, sub-therapeutic sildenafil levels as well as increased bosentan-related liver toxicity may result. However, in clinical practice, this combination is well tolerated and appears effective [76]. No such interaction with sildenafil has been described for sitaxentan or ambrisentan.

Other important co-medications in patients with PAH are vitamin K antagonists. Bosentan and sitaxentan have different effects on the doses of these oral anticoagulants. Bosentan partially *induces* the cytochrome P450 system, thereby increasing warfarin metabolism and the required dose [77]. By contrast, sitaxentan *inhibits* the liver isoenzyme CYP2C9. Thus, combining sitaxentan and warfarin in healthy volunteers can lead to a 2.4-fold increase in exposure to warfarin, requiring a substantial reduction in dose (~80%) at initiation of therapy to avoid bleeding complications [78]. No such interaction occurs with ambrisentan,

however: according to the US labelling, the drug interaction potential of ambrisentan *'has not been well characterised'* [45].

TREATMENT RECOMMENDATIONS ACCORDING TO CURRENT GUIDELINES

A number of authoritative guidelines summarise the current knowledge and provide treatment recommendations for PAH: worldwide by the Third World Symposium on PAH in Venice [5] (updated at the Fourth World Symposium on Pulmonary Hypertension in Dana Point 2008); in the US by the American College of Chest Physicians (ACCP, updated 2007) [6]; in Europe by the European Society of Cardiology (ESC [4], updated in the pocket guidelines 2006); and in individual countries, for example, by the German Society of Cardiology (DGK 2006 [79]) or the National Pulmonary Hypertension Centres of the UK and Ireland [80].

While these guidelines differ from each other in some aspects, they are remarkably similar with respect to ETRAs. Firstly, the three available agents (if ambrisentan is already mentioned), are generally considered as a class, as has been proposed at the World Symposium in Dana Point and no drug is explicitly preferred over another. The only exemption is the most recent UK guidelines, where individual ETRAs have been ranked with respect to treatment preference [80]. Secondly, ETRAs – and the phosphodiesterase-5 inhibitor sildenafil – are first-line oral therapies in PAH WHO functional class III. Thirdly, the body of evidence from clinical studies is limited in functional classes II and IV. However, ambrisentan has also been approved in functional class II and bosentan has achieved this status based on the results of the EARLY study [42]. Finally, combination therapy is becoming more important, as there is an obvious medical need to combine drugs with different mechanisms of action to achieve treatment goals and to prevent or delay clinical deterioration.

Owing to their oral availability and good tolerability, ETRAs are often part of such a combination regimen. Several large-scale multicentre trials are either planned or already underway in order to further evaluate the role of ETRAs in various treatment regimens. Examples are: COMPASS-2 (Effects of Combination of Bosentan and Sildenafil versus Sildenafil Monotherapy on Morbidity and Mortality in Symptomatic Patients with PAH) – bosentan added to sildenafil: COMPASS-3 – sildenafil added to bosentan: FREEDOM-C – oral treprostinil with ETRA and/or phosphodiesterase-5 inhibitor: SERAPHIN (Study with an Endothelin Receptor Antagonist in Pulmonary Arterial Hypertension to Improve Clinical Outcome) – ACT 064992 (ETRA) in symptomatic PAH: STRIDE-5 – sitaxentan vs placebo and vs combination with sildenafil: Pfizer A1481243 – sildenafil added to bosentan.

In addition to these randomised, controlled trials, registries can provide clinically important information derived from large patient populations. With respect to the clinical value of ETRAs in the treatment of patients with PAH, important data are expected from the investigator-initiated registry (since May 2007) *Comparison of endothelin receptor antagonist therapy in routine care* (CompERA, http://compera.org/) in the European Union.

SUMMARY

- ■ Endothelin receptor antagonists are an effective and generally well-tolerated treatment option for patients with symptomatic PAH.
- ■ They represent a major advance within the available therapeutic armamentarium for this severely compromised patient population and are therefore first-line therapies for PAH in WHO functional class (FC) III, and increasingly in FC II as well.
- ■ The importance of ET receptor selectively is still under intense debate. In recent years, the focus of development has been on ET_A-selective agents, but additional clinical data are needed for definitive conclusions.

- In clinical practice, chemical or pharmacological drug properties are of relevance when selecting agents for the individual patient including: potential for serious drug–drug interactions, convenience of dosing schedule, and rates of limiting side-effects.
- There are ongoing study programmes and registries examining the effects of combination therapies including ETRAs which will provide important information for clinical practice.

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7

Gene and stem cell therapy in pulmonary arterial hypertension

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INTRODUCTION

The pathological changes seen in pulmonary arterial hypertension (PAH) were initially thought to be due mainly to an imbalance between vasodilation and vasoconstriction in the pulmonary vasculature, however more recently arterial remodelling has also emerged as an important area of investigation [1, 2]. The mechanisms underlying these particular changes have been linked to cell proliferation and to inhibition of cell apoptosis involving several cell types including endothelial, smooth muscle and fibroblasts [1, 2]. Although major advances have been made in recent years in the therapeutic options available to treat PAH patients, at this time, PAH therapies do not address or alleviate the pulmonary vascular remodelling process.

Severe pulmonary hypertension in adults is considered by some to be a neoplastic, angio-proliferative disease [3, 4]. In the plexiform lesions of pulmonary hypertension, increased endothelial cell growth has been described [2, 5] and angiogenesis-related molecules detected. Monoclonal expansion of endothelial cells has been observed, with somatic cell mutations similar to those found in neoplastic disease reported [6]. In the plexiform lesions endothelial cells appear abnormal with the loss of tumour suppressor genes and lack of apoptotic cells [7].

The concept of an adult lung structure maintenance programme (LSMP) and a loss of homeostatic control following activation or injury of the pulmonary vasculature has been put forward as a possible mechanism leading to uncontrolled angiogenesis ([7], Figure 7.1).

In chronic lung disorders, including PAH, pulmonary vascular cell apoptosis has been suggested as a mechanism involved in beginning the process of 'misguided angiogenesis' [2, 7]. The lungs of patients with idiopathic pulmonary arterial hypertension (IPAH) have a lower number of apoptotic cells than both normal and emphysematous lungs, and it has been suggested that 'apoptosis-resistance' might have a pivotal role in both endothelial- and smooth muscle cell-based pulmonary vascular lesions [2].

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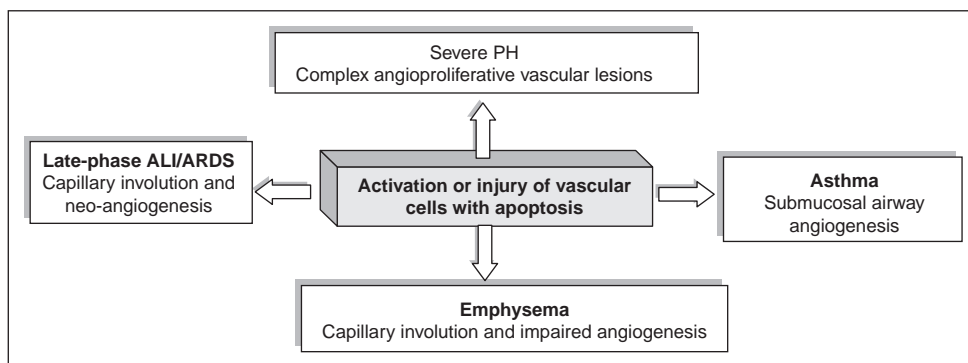
Figure 7.1 Effective and ineffective angiogenesis in the pathogenesis of chronic lung diseases (with permission from [7]). ALI = acute lung injury; ARDS = acute respiratory distress syndrome; PH = pulmonary hypertension.

In chapter 5, recent developments in anti-proliferative approaches for the treatment of pulmonary hypertension and therapies targeting the vasoconstrictive, pro-proliferative, anti-apoptotic, extracellular matrix deregulation and inflammatory components of pulmonary hypertension were discussed in detail (Table 7.1). Alongside this, the potential of both gene therapy and stem cell therapy to induce a reversal of PAH-associated vascular structural damage and to facilitate regeneration of normal pulmonary microvasculature has become a focus of recent investigations. Advances in understanding some of the genetic and molecular mechanisms associated with PAH has opened new avenues of research. Recognised as an area of new therapeutic potential, several promising concepts currently being explored to increase therapeutic options for patients with PAH are outlined below.

GENE THERAPY

One major advance in understanding the cellular and molecular mechanisms associated with PAH has been the identification of mutations in the gene for bone morphogenetic protein receptor 2 (BMPRII) in patients with familial IPAH [1, 8].

Bone morphogenetic proteins (BMPs) regulate growth, differentiation and apoptosis in several cell lines, including mesenchymal and endothelial cells [8]. In normal pulmonary artery smooth muscle cells, activation of BMPRII-associated pathways leads to a suppression of both proliferation and apoptosis [9].

The combined effect of cell proliferation and reduced apoptosis is thought to be important in PAH cellular pathogenesis [8]. BMPRII is a member of the transforming growth factor-β (TGF-β) receptor family. Interrupting, or decreasing, BMPRII expression leads to a decrease in BMP signalling and altered cellular responses to BMP, resulting in abnormal cell proliferation [2, 8]. Early endothelial cell apoptosis has been proposed as a mechanism for the development of apoptosis-resistant endothelial cells in plexiform lesions [2, 9] and altering BMPRII function can lead to edothelial cell apoptosis.

Support for this hypothesis comes from transgenic mice expressing a dominant-negative kinase domain mutant BMPRII, in vascular smooth muscle. The mice demonstrated increased pulmonary vascular remodelling, and pulmonary hypertension. Hence, in this model, the loss of BMPRII signalling in smooth muscle produced a pulmonary hypertensive phenotype [10].

However, around 80% of patients who carry the BMPRII gene mutations never develop PAH, leading to the supposition that additional external factors may be involved, including

Table 7.1 Future non-stem cell therapy options (see chapter 5 for a full discussion and details)

Therapies targeting:
Vasoconstrictive component of PH
Rho kinase inhibitors
Vasointestinal peptide
Serotonin receptor-/serotonin reuptake inhibitors
Soluble guanylate cyclase activators/stimulators
Kv channel activators
Pro-proliferative component of PH
Platelet-derived growth factor receptor antagonists
Epidermal growth factor receptor antagonists
Vascular endothelial growth factor receptor antagonists
Fibroblast/insulin growth factor receptor antagonists
Multi-kinase inhibitors
Phosphodiesterase inhibitors
Peroxisome proliferator-activated receptor (PPAR) gamma agonists
Anti-apoptotic component of PH
Inflammatory component of PH
Nuclear factor of activated T cell inhibitors
Extracellular matrix component of PH
Possible role of elastinolytic enzyme
Serine elastase-/matrix metalloprotease inhibitors
Metabolic abnormalities of PH
Dichloroacetate (DCA)
PH = pulmonary hypertension; Kv = voltage gated.

other genetic mutations and external risk factors like human immunodeficiency virus 1 (HIV-1) infection, or anorexigens [1] (Figure 7.2).

A range of cellular abnormalities, including excessive vasoconstriction and impaired production of vasodilators, have been associated with PAH and may also play a role in promoting disease development. Pulmonary endothelial dysfunction leading to the production of vasodilators including nitric oxide (NO) and prostacyclin, as well as over-production of the vasoconstrictors thromboxane A2 and endothelin-1, has been reported [1, 11]. Other cellular abnormalities under investigation include impaired potassium channels and altered expression of the serotonin transporter in smooth muscle cells, together with enhanced matrix production in the adventitia [12].

Survivin, a member of the inhibitor of apoptosis protein (IAP) family is expressed in most cancer cells but not in most normal adult cell types, and has been investigated as a potential cancer drug target [13]. It is known to confer protection from apoptosis and a potential role in the pathogenesis of PAH has been investigated [14, 15].

Immunohistochemistry performed on lung tissue from patients with or without PAH showed that Survivin was expressed in the pulmonary arteries of six patients with PAH but not in three control patients without PAH [15]. In an animal model, over-expression of Survivin in an adenovirus vector (carrying a phosphorylation deficient Survivin mutant with dominant-negative properties) reversed established monocrotaline (a plant alkaloid)-induced PAH in rats and prolonged survival by 25% [15]. In this model, the Survivin mutant lowered pulmonary vascular resistance, right ventricular hypertrophy and pulmonary arterial medial hypertrophy.

The results of a study designed to investigate the efficacy of angiogenic gene therapy with vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase
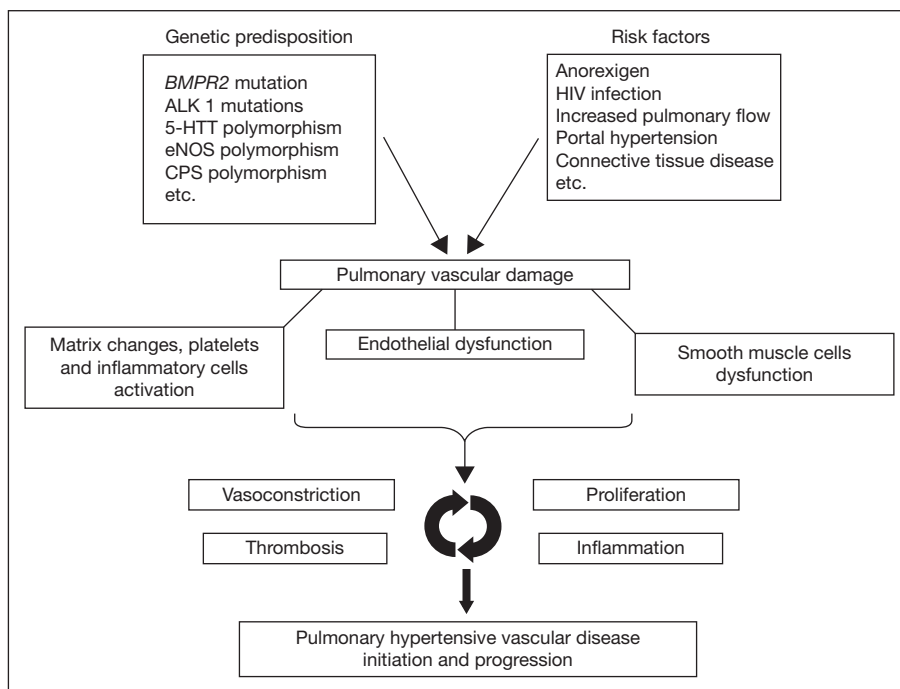
Figure 7.2 Pulmonary arterial hypertension: potential pathogenetic and pathological mechanisms (with permission from [1]). 5-HTT = serotonin transporter gene; ALK 1 = activin receptor-like kinase 1; *BMPR2* = bone morphogenetic receptor protein 2 gene; CPS = carbonyl-phosphate synthetase gene; eNOS = nitric oxide synthase gene.

(eNOS) in established PAH have been reported [16]. The authors used a 'reversal' model, in which gene therapy was delayed until 3 weeks after monocrotaline administration, when PAH and associated vascular remodelling had developed in the animal model. The three-dimensional architecture of the rat pulmonary microcirculation was assessed to determine any microvascular regeneration and improvement in pulmonary haemodynamics. The authors reported that although both eNOS and VEGF gene transfer reduced the progression of PAH, only eNOS significantly reversed established PAH in the rat which was in part achieved by inducing regeneration of the pulmonary microcirculation [16].

STEM CELL THERAPY

Progenitor cells are primitive bone marrow (BM) cells with the ability to proliferate, migrate and differentiate into a variety of mature cell types [17]. Endothelial progenitor cells (EPCs) mature into the cells that line the lumen of blood vessels and may be important in endothelium maintenance with respect to both re-endothelialisation and neovascularisation [17]. Preclinical evidence suggests that, through a combination of angiogenesis and vasculogenesis, BM-derived cells stimulate neovascularisation in different tissues [18].

Over the last decade, cell transplantation for cardiac regeneration has been investigated as a novel mechanism to treat heart failure (outlined in [19]). The viability, safety and potential efficacy of this novel therapy in humans has been investigated in several preclinical and early phase clinical trials using EPC delivery, or mobilisation, for the treatment of post-angioplasty restenosis and acute myocardial infarction (MI) [18].
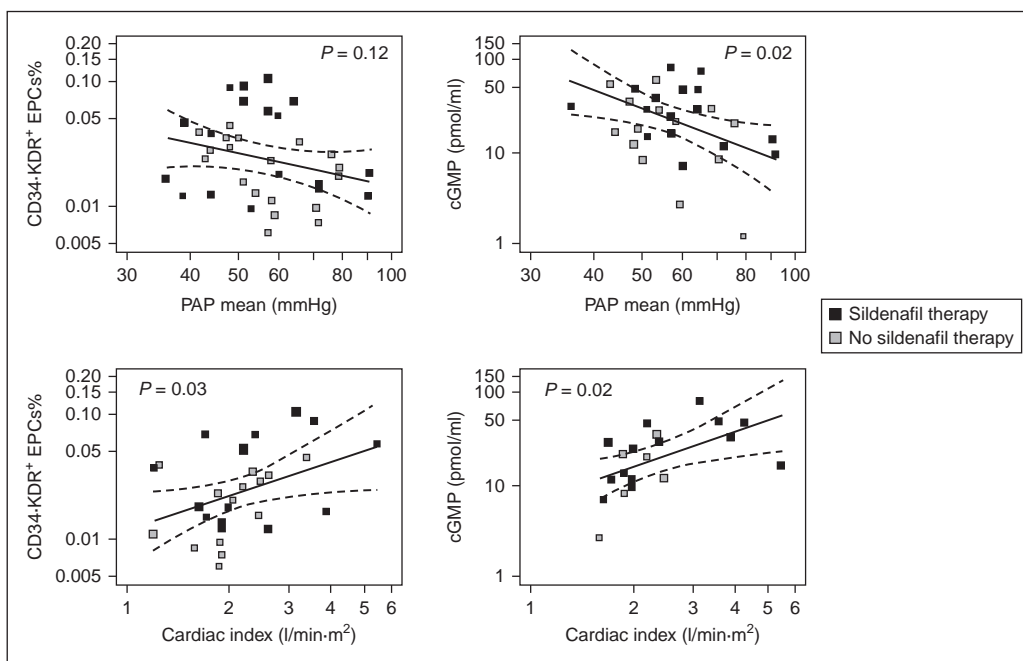
Figure 7.3 Correlation between haemodynamic parameters (mean pulmonary arterial pressure [PAP] or cardiac index) and cGMP levels or circulating CD34-KDR+ cells in patients with IPAH. Parameters were log transformed (with permission from [20]).

Following on from the promising research on cell transplantation for cardiac disease including MI, the potential role of EPC therapy in treating PAH patients has also been under investigation [18]. The administration of stem cells derived from BM has been proposed as a possible treatment for PAH. Several groups have reported the beneficial effects of stem cell-based therapies in the treatment of PAH when used in animal models involving the administration of monocrotaline to induce selective pulmonary endothelial injury in the animal models.

The baseline number and function of circulating EPCs in patients with PAH have been investigated and the hypothesis that PAH patients are deficient in circulating EPCs, thus potentially contributing to endothelial dysfunction and disease progression, has been explored [20]. Comparing the number of circulating EPCs and the function of cultured peripheral blood mononuclear cells (PBMCs) in patients with Eisenmenger syndrome or IPAH with healthy control subjects has demonstrated elevated levels of inflammatory mediators, indexes of NO synthesis, and asymmetric dimethylarginine (known to affect EPC numbers). These levels were directly proportional to EPC numbers for both Eisenmenger syndrome patients and IPAH patients. In IPAH patients, EPC numbers were related to pulmonary haemodynamic parameters, and treatment of these patients with the phosphodiesterase inhibitor sildenafil was associated with a dose-dependent rise in EPC numbers (Figure 7.3). However, further studies are needed to assess the potential therapeutic implications of chronic pharmacologically-induced elevated levels of EPCs [20, 21].

Bone marrow-derived, rat endothelial-like progenitor cells (ELPC) transfected with eNOS were transplanted in rats with monocrotaline-induced PAH [22]. The transplanted cells prevented, and also reversed, PAH by re-establishing connections between proximal and distal pulmonary arteries. Fluorescently labelled ELPCs were detected engrafted into the

endothelium of the injured lung. Compared with the control group, ELPCs were able to prevent the development of pulmonary hypertension seen at 3 weeks in rats with untreated monocrotaline-induced PAH (as assessed by changes in the right ventricle systolic pressure: 48 ± 3 mmHg vs. 31.5 ± 0.95 mmHg; $P < 0.001$) [22]. Additionally, using this animal model, administration of eNOS-transfected ELPCs, but not ELPCs alone, demonstrated significant reversal of established disease at day 35 vs. day 21 (31 ± 2 mmHg vs. 50 ± 3 mmHg, respectively; $P < 0.005$). Compared with animals receiving monocrotaline alone, the injection of eNOS-transduced ELPCs improved survival ($P < 0.02$) [22].

The effect of bone marrow-derived cells (BMDCs) on pulmonary hypertension (PH) induced in mice by either monocrotaline or exposure to chronic hypoxia has also been examined [23]. Quantitative real-time polymerase chain reaction (PCR) was used to detect lung cell engraftment and lung eNOS expression measured and compared with control animals treated with irradiated BMDCs. In this animal model, mice injected with BMDCs (harvested from femurs and tibias of donor mice treated with 5-fluorouracil) significantly attenuated PH as assessed by reductions in right ventricular systolic pressure (20 ± 1 mmHg vs. 27 ± 1 mmHg; $P \leq 0.01$), right ventricle weight/left ventricle + septum weight ratio (0.29 ± 0.02 vs. 0.36 ± 0.01; $P \leq 0.03$), and percentage of muscularised vessels (26.4% vs. 33.5%; $P \leq 0.05$). A number of BMDCs $> 2.5 \times 10^6$ was needed to reduce right ventricular (RV) pressure and hypertrophy, and no additional benefit was noted by doubling the number of BMDCs (Figure 7.4). Compared to controls, eNOS protein levels were decreased in lungs from mice given monocrotaline, but not in mice given both monocrotaline and BMDCs. No alterations in lung eNOS protein levels were found in chronically hypoxic mice [23].

Following monocrotaline-induced PAH in rats, intratracheal administration of syngeneic rat mesenchymal stem cells (rMSCs) decreased pulmonary arterial pressure and pulmonary vascular resistance and improved endothelium-dependent responses [24]. In the same study, treatment with rMSCs also decreased the monocrotaline-induced RV hypertrophy. The authors report that since immuno-labelled cells were not detected in the wall of pulmonary vessels the positive effects might be due to a paracrine effect from transplanted rMSCs in lung parenchyma.

The efficacy of MSCs over-expressing eNOS and transplanted intravenously into rats with monocrotaline-induced PAH has also been investigated [25]. Intravenous administration of MSCs over-expressing eNOS was reported to reduce RV impairment caused by PAH. Additionally, survival time of rats receiving MSCs/eNOS was significantly longer than in the non-treatment rats (19.67 ± 3.11 days vs. 10.89 ± 3.63 days; $P < 0.05$).

Human umbilical cord blood-derived EPCs transfected with adrenomedullin (a potent vasodilator peptide) have been transplanted to immunodeficient nude rats. In this model, the hybrid cell–gene therapy allowed non-viral, gene transfer into EPCs based on the phago-cytosing action of EPCs [26]. Transplantation of EPCs alone resulted in a 16% decrease in pulmonary vascular resistance. In comparison, transplantation of adrenomedullin DNA-transduced EPCs gave a 39% decrease in pulmonary vascular resistance. Monocrotaline-treated rats transplanted with adrenomedullin-expressing EPCs had a significantly higher survival rate than those given culture medium ($P < 0.001$) or EPCs alone ($P < 0.05$). Comparing previous results the authors suggest that autologous EPCs may be more efficacious for pulmonary vascular repair than xenogenic cells [27, 28].

The results of a prospective, randomised trial investigating feasibility, safety and clinical outcome of intravenous infusion of autologous EPCs in patients with IPAH have been reported [29]. The study was designed to compare the effects of EPC transplantation plus conventional therapy with conventional therapy alone and the primary endpoint was change in the 6-min walk distance. After 12 weeks of follow-up, the mean difference between the two groups was 42.5 m (95% CI 28.7–56.3 m; $P < 0.001$). Patients in the cell infusion group had significant improvement (vs. conventional therapy) in mean pulmonary artery pressure (-4.5 vs. -0.4 mmHg; $P < 0.001$), pulmonary vascular resistance (-185.4 vs. -27.8
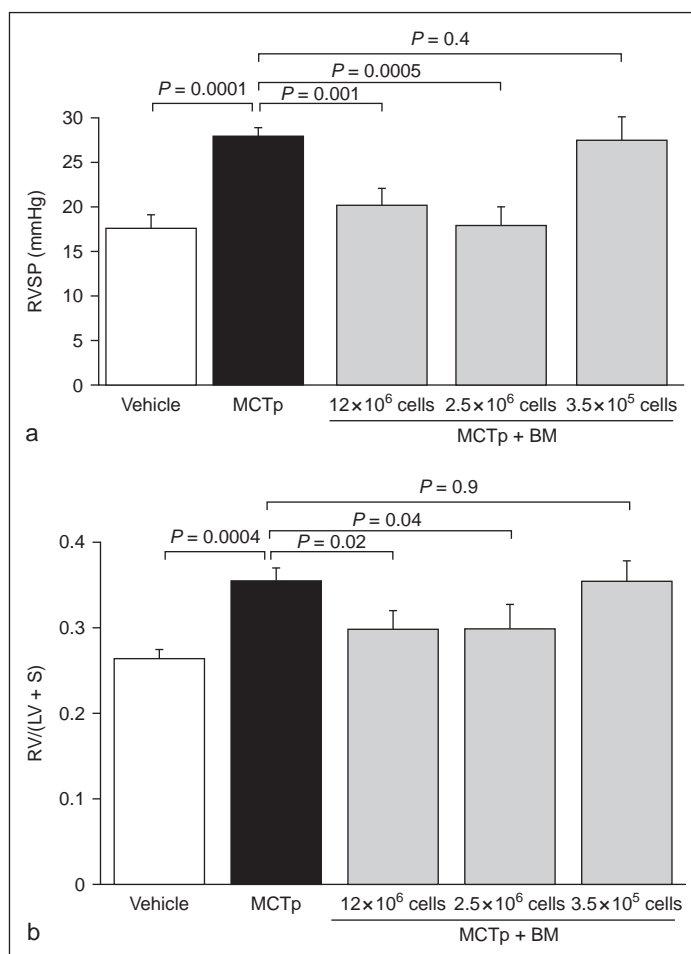
Figure 7.4 Effect of the number of bone marrow-derived cells on the increases in right ventricular systolic pressure (RVSP) and right ventricle to left ventricle + septum weight [RV/(LV+S)] after MCTp injection: 12×10^6 cells (2 male donor mice for 1 female recipient), 2.5×10^6 cells (1 male donor mouse for 1 female recipient) or 3.5×10^5 cells (1/2 male donor mouse sample for 1 female recipient). Values are means $\pm$ SEM for 5 animals in each group (with permission from [23]).

dyne·s·cm^{-5}; $P <0.002$), and cardiac output (0.38 vs. 0.06 l/min; $P <0.021$). The intervention was well tolerated with similar numbers of adverse events reported for each treatment group [29].

These results support the beneficial effects reported for eNOS gene transfer [16] and together with the promising results from the monocrotaline-induced PAH animal models have led to the initiation of a phase I clinical trial. The Pulmonary Hypertension: Assessment of Cell Therapy (PHACeT) trial [NCT00469027] is designed to establish the safety of autologous progenitor cell-based gene therapy delivery of eNOS in patients with severe PAH refractory to conventional treatment. Primary endpoints are related to the tolerability and safety of injection of genetically engineered progenitor cells in patients with severe PAH. Secondary endpoints will investigate potential efficacy by assessing changes in haemodynamic pressures, patient perceived quality of life, and exercise capacity. This open-label,

dose-escalating study is the first clinical study combining gene and cell therapy in cardio-vascular disease and the estimated study completion date is in 2011.

Although offering great therapeutic potential, some caution is advised while research in this area is at an early stage. Investigations into the pathobiology of pulmonary vascular remodelling in chronic obstructive pulmonary disease (COPD) have shown the presence of vascular progenitor cells (VPCs) in the endothelial surface and the intimal space of pulmonary arteries of patients with COPD [30]. The number of progenitor cells was associated with the response to hypoxic stimulus but also with the enlargement of the arterial wall, suggesting that VPCs might be involved in mechanisms of pulmonary vessel repair and remodelling in COPD.

Bone marrow-derived human mesenchymal stem cells (MSC) are progenitor cells that can differentiate into mesenchymal tissues including cartilage and bone, are easily expanded *ex vivo* and readily transduced by viral vectors. MSCs have been shown to engraft in bleomycin-injured lung, to adopt an epithelial cell-like phenotype and to decrease lung inflammation and collagen deposition in mice [31]. However, an enriched population of murine marrow stromal cells expanded *in vitro* for cell therapy studies, was found to be chromosomally unstable, and may have contributed, in part, to tumour formation after systemic injection [32]. In contrast, no abnormalities were detected in human MSCs after 6 or more passages *in vitro* [32].

SUMMARY

It is recognised that PAH has a complex disease profile involving multiple interactions between different biochemical pathways and cell types. The exact nature of these interactions remains to be wholly elucidated. Much effort is focused on understanding more fully the cellular and molecular processes involved, and exactly how they give rise to the pathological changes seen in PAH.

The potential for both gene therapy and stem cell therapy to reverse established PAH, has become a major focus of investigation. A number of promising areas of research focus on potential mechanisms to induce apoptosis of abnormal vascular cells that are obstructing blood flow, and also promoting the regeneration of normal pulmonary microvasculature.

Although at an early stage, future research in these areas will be assisted by clearly identifying the patient population that will benefit most from the novel treatments. Large-scale randomised clinical trials accurately designed to determine the efficacy of these novel therapeutic options in the treatment of patients with PAH are also needed.

Although at this time the characterisation of EPCs remains incomplete, early promising results in other cardiovascular disease areas suggest that the field of cell therapy has much to offer as a potential for treating PAH patients. Promising results from animal models of PAH have led to the initiation of a phase I clinical trial: the PHACeT trial. The results from this first clinical study to combine gene and cell therapy in cardiovascular disease, will help determine the usefulness of this therapeutic strategy in the clinical setting.

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Abbreviations

5-HT	5-hydroxytryptamine (serotonin)
5-HTR	serotonin receptor
5-HTT	serotonin transporter
6MWD	6-minute walk distance
AA	ascending aorta
AC	adenylate cyclase
ACCP	American College of Chest Physicians
AIR	Aerosolised Iloprost Randomised (study)
AKT/PKB	protein kinase B
ALI	acute lung injury
ALK 1	activin receptor-like kinase 1
ALPHABET	Arterial Pulmonary Hypertension and European Beraprost Trial
ALT	alanine aminotransferase
AMP	adenosine monophosphate
ANP	atrial natriuretic peptide
APAH	acquired pulmonary arterial hypertension
AR	amphiregulin
ARDS	acute respiratory distress syndrome
ARIES	Ambrisentan in Pulmonary Arterial Hypertension, Randomized, Double-Blind, Placebo-Controlled, Multicenter, Efficacy Study
AST	aspartate aminotransferase
ASTAMI	Autologous Stem Cell Transplantation in Acute Myocardial Infarction
AT	anaerobic threshold
ATP	adenosine triphosphate
BAD	Bcl-XL/Bcl-2-associated death
BENEFIT	Bosentan Effects in Inoperable Forms of Chronic Thromboembolic Pulmonary Hypertension
bid	twice daily
BM	bone marrow
BMDC	bone marrow-derived cell
BMI	body mass index
BMP	bone morphogenetic protein
BMPRII	bone morphogenetic protein receptor 2
BMPR2	bone morphogenetic protein receptor 2 gene
BNP	brain natriuretic peptide
BREATHE	Bosentan Randomised Trials of Endothelin Antagonist Therapy
CaM	calmodulin
cAMP	cyclic adenosine monophosphate

cGMP	cyclic guanosine monophosphate
CHD	congenital heart disease
CHF	congestive heart failure
CI	cardiac index
COMBI	Combination Therapy of Bosentan and Aerosolised Iloprost in Idiopathic Pulmonary Arterial Hypertension (trial)
COMPASS	Effects of Combination of Bosentan and Sildenafil versus Sildenafil Monotherapy on Morbidity and Mortality in Symptomatic Patients with PAH (trial)
COPD	chronic obstructive pulmonary disease
CPET	cardiopulmonary exercise testing
CPS	carbonyl-phosphate synthetase gene
CREB	cyclic-AMP-responsive element-binding protein transcription factor
CT	computed tomography
CTD	connective tissue disease
CTEPH	chronic thromboembolic pulmonary hypertension
DCA	dichloroacetate
DGK	German Society of Cardiology
EARLY	Endothelin Antagonist Trial in Mildly Symptomatic PAH Patients (study)
eNOS	endothelial nitric oxide synthase gene
EC	endothelial cell
ECE	endothelin-converting enzyme
ECG	electrocardiogram
ECM	extracellular matrix
EDRF	endothelium-derived relaxing factor
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ELPC	endothelial-like progenitor cell
eNOS	endothelial nitric oxide synthase
EPC	endothelial progenitor cell
ERK	extracellular signal-related kinase
ESC	embryonic stem cell
ESC	European Society of Cardiology
ET	endothelin
ETR	endothelin receptor
ETRA	endothelin receptor antagonist
FC	functional class
FDA	Food and Drug Administration
fen-PAH	fenfluramine-associated pulmonary arterial hypertension
FGF	fibroblast growth factor
FPAH	familial pulmonary arterial hypertension
FREEDOM	Following Rehabilitation, Economics and Everyday Dialysis Outcome Measurements (trial)
GARD	Global Alliance Against Chronic Respiratory Diseases
GTP	guanosine triphosphate
HB-EGF	heparin-binding EGF-like growth factor

HHT	hereditary haemorrhagic telangiectasia
HIV	human immunodeficiency virus
IAP	inhibitor of apoptosis protein
IGF	insulin growth factor
IGFR	insulin growth factor receptor
IGFBP	IGF-binding protein
IGFR	insulin growth factor receptor
II	heme-iron
IKK	IK B kinase
ILD	interstitial lung disease
IPAH	idiopathic pulmonary arterial hypertension
IRS	insulin receptor substrate
IVS	interventricular septum
Kv	voltage gated K+ channel
LAM	lymphangioleiomyomatosis
LDL	low-density lipoprotein
LFT	liver function test
LSMP	lung structure maintenance programme
LV	left ventricule/ventricular
LVEF	left ventricular ejection fraction
MAPK	mitogen-activated protein kinase
MCT	monocrotaline
MCT-PAH	monocrotaline-induced PAH
MEK	protein kinase that phosphorylates the ERK gene product
MI	myocardial infarction
MLCK	myosin light chain kinase
MLCP	myosin light chain phosphatase
MMP	matrix metalloproteinase
MNC	mononuclear cell
MPA	main pulmonary artery
mPAP	mean pulmonary artery pressure
MPD	myeloproliferative disorders
MRI	magnetic resonance imaging
MSC	mesenchymal stem cell
MSCT	multislice computed tomography
mTOR	mammalian target of rapamycin
NFAT	nuclear-factor-of-activated-T-cells
NF-KB	nuclear factor-kappa B
NIH	National Institutes of Health
NO	nitric oxide
NYHA	New York Heart Association
p90RSK	p90 ribosomal S6 kinase
PA	pulmonary artery
PACES	Pulmonary Arterial Hypertension Combination Study of Epoprostenol and Sildenafil
$PaCO_2$	partial pressure of carbon dioxide in the blood
PaO_2	arterial oxygen pressure
PAH	pulmonary arterial hypertension
PAH-CTD	PAH in association with connective tissue disease

PAOP	pulmonary artery occlusion pressure
PA SMC	pulmonary artery smooth muscle cell
PAP	pulmonary arterial pressure
PBMC	peripheral blood mononuclear cell
PBO	placebo
PCH	pulmonary capillary haemangiomatosis
PCR	polymerase chain reaction
PCO_2	carbon dioxide partial pressure
PCWP	pulmonary capillary wedge pressure
PDE-5	phosphodiesterase-5
PDGF	platelet-derived growth factor
PDGFR	platelet-derived growth factor receptor
pGC	particulate guanylate cyclase
PG12	prostacyclin
PH	pulmonary hypertension
PHACeT	Pulmonary Hypertension: Assessment of Cell Therapy (trial)
PHIRST	Phosphodiesterase Type-5 Inhibitor Tadalafil in the Treatment of Patients with PAH
PKB	protein kinase B (or AKT)
PKG	cGMP-dependent protein kinase
PI3K	phosphatidylinositol 3-kinase
PLC-γ	phospholipase C γ
PlGF	placental growth factor
PPAR	peroxisome proliferator-activated receptor
PPH	primary pulmonary hypertension
PPHN	persistent pulmonary hypertension of the newborn
PPRE	PPAR response element
PV	pulmonary vessel
PVOD	pulmonary veno-occlusive disease
PVR	pulmonary vascular resistance
PVSMC	pulmonary vascular smooth muscle cell
RA	right atrium
RAP	right atrial pressure
REPAIR-AMI	Reinfusion of Enriched Progenitor Cells And Infarct Remodeling in Acute Myocardial Infarction
REVEAL	Registry to Evaluate Early and Long Term PAH Disease Management
rMSC	rat mesenchymal stem cell
ROCK	rho-kinase
RPA	right pulmonary artery
RT-PCR	reverse transcription polymerase chain reaction
RV	right ventricule/ventricular
RVEF	right ventricular ejection fraction
RVFAC	right ventricular fractional area change
RVSP	right ventricular systolic pressure
RXR	retinoid X receptor
SaO_2	saturation of oxygen
SC	subcutaneous

SERAPHIN	Study with an Endothelin Receptor Antagonist in Pulmonary Arterial Hypertension to Improve Clinical Outcome
SERT	serotonin transporter
sGC	soluble guanylate cyclase
SMC	smooth muscle cell
sPAP	systolic pulmonary artery pressure
SR	sarcoplasmic reticulum
SSRI	selective serotonin reuptake inhibitor
STAT	signal transducers and activators of transcription
STEP	Iloprost Inhalation Solution Safety and Pilot Efficacy Trial in Combination with Bosentan for Evaluation in Pulmonary Arterial Hypertension
STRIDE	Sitaxsentan to Relieve Impaired Exercise in Pulmonary Arterial Hypertension (study)
SUPER-1	Sildenafil Use in Pulmonary Arterial Hypertension (trial)
SvO_2	mixed venous oxygen saturation
SVR	systemic vascular resistance
TAPSE	tricuspid annual plane systolic excursion
TDI	tissue Doppler imaging
TE	thromboembolic
TGF	transforming growth factor
tid	three times a day
TIMP-1	tissue inhibitor of metalloproteinase-1
TOPCARE-AMI	Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction
TOPS	Thelin Outcomes for Patients Surveillance
Tph1	tryptophan hydroxylase 1
TPR	total pulmonary resistance
TR	tricuspid regurgitation
TRAX	TRAcleer Excellence Post-Marketing Surveillance Programme
TRIUMPH	Trepostinil Sodium Inhalation Used in the Management of Pulmonary Artery Hypertension (trial)
TRP	transient receptor potential
TZD	thiazolidinedione
ULN	upper limit of normal
VA/Q	alveolar ventilation/perfusion ratio
VD/VT	ventilatory dead space fraction of the tidal volume
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
VIP	vasointestinal peptide
VOLT	VOLibris Tracking
VPC	vascular progenitor cell
WHO	World Health Organization
WR	work rate

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