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REVIEW

Pulmonary arterial hypertension: Basic knowledge for clinicians

Hypertension artérielle pulmonaire : connaissances de base pour les cliniciens

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Summary Pulmonary arterial hypertension is a progressive syndrome based on diverse aetiologies, which is characterized by a persistent increase in pulmonary vascular resistance and overload of the right ventricle, leading to heart failure and death. Currently, none of the available treatments is able to cure pulmonary arterial hypertension; additional research is therefore needed to unravel the associated pathophysiological mechanisms. This review summarizes current knowledge related to this disorder, and the several experimental animal models that can mimic pulmonary arterial hypertension and are available for translational research.
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Abbreviations: BMPR2, bone morphogenetic protein receptor type 2; cGMP, cyclic guanosine monophosphate; ET, endothelin; Kv channel, voltage-gated potassium channel; mPAP, mean pulmonary artery pressure; NO, nitric oxide; PAH, pulmonary arterial hypertension; PASMC, pulmonary arterial smooth muscle cell; PDE-5, phosphodiesterase type 5; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; RV, right ventricle/ventricular.

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MOTS-CLÉS
Hypertension artérielle pulmonaire (HTAP) ;
Insuffisance ventriculaire droite ;
Mécanismes physiopathologiques et modèles expérimentaux de l'HTAP

Résumé L'hypertension artérielle pulmonaire (HTAP) est un syndrome progressif caractérisé par une augmentation persistante de la résistance vasculaire pulmonaire et par une surcharge du ventricule droit, ce qui conduit à une insuffisance cardiaque et la mort. À l'heure actuelle, l'HTAP est incurable et, par conséquent, davantage de recherche est nécessaire pour comprendre les mécanismes physiopathologiques associés. Cette revue résume les connaissances actuelles liées à ce trouble et les différents modèles animaux de l'HTAP disponibles pour la recherche translationnelle.
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Background

Pulmonary arterial hypertension (PAH) is a syndrome based on diverse aetiologies and pathogenesis, potentially leading to right heart failure and death. PAH is characterized by excessive pulmonary vascular remodelling, pulmonary arterial obstruction and elevated pulmonary vascular resistance (PVR), which result in a marked increase in right ventricle (RV) afterload. Eventually, the RV is unable to cope with the increase in load and heart failure develops [1].

PAH is defined by a mean pulmonary artery pressure (mPAP) $\geq$ 25 mmHg at rest, and is haemodynamically characterized by the presence of precapillary pulmonary hypertension (PH), which implies a normal pulmonary capillary wedge pressure or left ventricular end-diastolic pressure $\leq$ 15 mmHg, with a PVR > 3 Wood Units [1,2]. So far, there is insufficient evidence to add an exercise criterion to this definition [3].

Pathophysiology

Histopathology

PAH is considered a vasculopathy and, in general, all PAH subgroups (i.e. idiopathic, heritable, drug or toxin-induced, or associated with connective tissue disease, human immunodeficiency virus [HIV] infection, portal hypertension, congenital heart disease or schistosomiasis) and other forms of PH (i.e. PH resulting from lung disease and/or hypoxia) exhibit several arterial abnormalities that are mainly present in the small pulmonary arteries and arterioles [1]. The most common pathological features in PH are medial hypertrophy, local dilation and intimal atheromas, and because they are present in all forms of PH, they are of poor diagnostic value. However, PAH is characterized by constrictive lesions, which include medial hypertrophy and intimal and adventitial thickening, and by complex lesions that include plexiform and dilation lesions, as well as arteritis [4].

Medial hypertrophy is defined by an increase in the diameter of the medial layer, measured between the internal and external elastic lamina, exceeding 10% of the cross-sectional diameter of the arteries. This abnormality appears in all PAH subgroups, and occurs as a result of pulmonary arterial smooth muscle cell (PASMC) proliferation and/or recruitment to the tunica media. This lesion is considered an early event in PAH pathogenesis, but it is usually regarded as reversible [4]. Intimal and adventitial thickening occurs as a result of the proliferation and recruitment of connective tissue cells and, consequently, by the interstitial deposition of collagen, leading to fibrosis. This thickening can be uniform (concentric) or focal (eccentric); the former is often associated with thrombotic events [5]. The presence of plexiform lesions in the vascular compartments is very characteristic of PAH [6], and is a consequence of local and excessive pulmonary arterial endothelial cell proliferation, which leads to the formation of capillary-like channels within the arterial lumen [7]. These lesions are responsible for the expansion and destruction of the arterial wall, as they tend to enlarge into the perivascular space. Fibrin, thrombi and platelets are frequently encountered in these lesions, as well as dilation lesions, which are thin-walled vein-like vessels that are a potential cause of haemorrhages and subsequent fibrosis. The artery wall may also accumulate necrotic and fibrotic tissue and/or be infiltrated with inflammatory cells, leading to arteritis [4].

Cellular factors

The main mechanisms responsible for pulmonary vascular dysfunction are the abnormal proliferation of PASMCs and pulmonary arterial endothelial cells, infiltration of inflammatory cells and fibrosis [5]. However, PAH is not only associated with cell proliferation, but also with apoptotic processes, as the imbalance between these two events is the major cause of the narrowing of the pulmonary arteries in PAH [8].

All forms of PAH have in common the migration and proliferation of PASMCs, which in general is accompanied by the migration of fibroblasts and the formation of an extracellular matrix layer. The uncontrolled proliferation of PASMCs ultimately leads to medial hypertrophy, also contributing to the thickening of the intima and adventitia layers of the pulmonary vessels [9]. The formation of an extracellular matrix and myofibroblasts between the endothelium and internal elastic lamina is termed neointima. Another feature characteristic of PAH is the increase in vasa vasorum neovascularization, which mainly affects the adventitia, and can expand to the media [10].

Beyond the intrinsic dysfunction present in both pulmonary arterial endothelial cells and PASMCs in PAH,

recent evidence suggests that crosstalk between these cells may contribute to further impairment. Eddahibi et al. demonstrated that pulmonary arterial endothelial cells constitutively produce and release growth factors that act on PASMCs, which appears to be critical for pulmonary vascular remodelling [11]. Although the majority of mechanisms governing this crosstalk remain to be elucidated, Humbert et al. have discussed whether some of the pathways already known to be involved in PAH pathophysiology are also involved in the crosstalk between pulmonary arterial endothelial cells and PASMCs [12].

In response to growth factors, pulmonary arterial endothelial cell and PASMC can grow, proliferate, migrate or differentiate, and therefore these substances assume a very important role in vascular remodelling processes. One of the most studied growth factors in the scope of PAH is vascular endothelial growth factor, which has been identified as an important mediator in the hypoxia-inducible factor-1α signalling pathway under hypoxic conditions, which eventually culminates in endothelial proliferation and the formation of new blood vessels. Another growth factor that also regulates blood vessel development is fibroblast growth factor-2, which, like vascular endothelial growth factor, is also able to promote the formation of new blood vessels in the presence of low levels of oxygen. Furthermore, platelet-derived growth factor and epidermal growth factor have also been implicated in PAH pathophysiology because of their ability to stimulate PASMC proliferation and survival, contributing to the abnormal PASMC phenotype observed in the distal pulmonary arteries of PAH patients [13,14]. In PAH, if the pulmonary endothelium becomes damaged, these vascular growth factors mentioned above may mediate an exaggerated or persistent response to injury, leading to new vessels or hyperproliferation of the endothelium.

In response to shear stress, hypoxia, inflammation and/or other stimuli, endothelial cells proliferate excessively and generate plexiform lesions [15]. In response to these stimuli, endothelial cells develop an imbalance between proliferative and apoptotic processes, as well as changes at the functional level. Endothelial dysfunction eventually results in a clear imbalance between the production and release of vasoconstrictors/vasodilators, activator/inhibitory growth factors, prothrombotic/antithrombotic mediators and proinflammatory/anti-inflammatory signals [16,17].

In certain forms of PAH (i.e. PAH associated with autoimmune diseases), the inflammatory response plays an important role, as some patients improved both clinically and haemodynamically when administered with immunosuppressant therapy. Indeed, several markers of inflammation, such as interleukin-1b, interleukin-6 and tumour necrosis factor-α, are highly expressed in both PAH patients [18,19] and animal models [20,21], while demonstrating a positive correlation with disease severity and poor outcome. At high concentrations, these cytokines may contribute to the exaggerated contractility and proliferation of vascular cells, thereby promoting the adverse remodelling seen in PAH [22]. Moreover, some inflammatory cells, such as lymphocytes and macrophages, can also be found in plexiform lesions [16].

Some PAH patients exhibit elevated plasma concentrations of fibrinopeptides, along with von-Willebrand factor and plasminogen activator inhibitor type 1, reflecting an abnormal coagulation process and endothelial dysfunction, respectively. Both events are very important in PAH development because they can generate or aggravate in situ thrombosis. Platelets also participate in vasoconstriction and vascular remodelling, as they are able to produce prothrombotic, vasoactive and mitogenic factors [23].

Molecular abnormalities and therapeutic targets

The molecular abnormalities seen in PAH patients are normally associated with increased endothelin (ET)-1 concentrations and decreased nitric oxide (NO) and prostacyclin concentrations, as these factors influence vascular homeostasis, cell survival and proliferation, among other processes, and are key therapeutic targets for the management of PAH.

Prostanoids

Prostacyclin (or prostaglandin I2) and thromboxane A_2 belong to the prostanoid family, and are produced from arachidonic acid metabolites. The former is a potent vasodilator and inhibitor of platelet activation, while thromboxane A_2 has opposite effects [1]. Prostacyclin is produced in vascular endothelial cells, and acts on both systemic and pulmonary vascular smooth muscle cells, as well as on circulating platelets and other cells, via the cyclic adenosine monophosphate pathway [24]. Prostacyclin plays an important role in antiproliferative, antithrombotic, antimitogenic and immunomodulatory activity [24]. In PAH patients, the expression of prostacyclin synthase in the pulmonary arteries is reduced, and therefore the production of prostacyclin in endothelial cells is decreased [25]. In fact, patients with PAH have reduced endogenous prostacyclin [25]. Therefore, as shown in Fig. 1, prostacyclin analogues are an established PAH therapy, which mimic the prostacyclin signalling pathway and are able to induce vasodilation while inhibiting platelet activation [24].

Endothelin-1

This 21-amino acid vasoactive peptide is expressed in several mammalian tissues in different types of cells, and is responsible for the regulation of vascular tone. ET-1 exerts its effects through interaction with two types of receptors: ET receptor type A (ET_A) and ET receptor type B (ET_B), which belong to the G-protein-coupled receptor family. In PASMCs, when activated, both receptors have a vasoconstrictor effect, while in pulmonary arterial endothelial cells, ET_A is not expressed, and the activation of ET_B leads to vasodilatation [26]. Endothelial cell dysfunction usually leads to ET-1 overexpression, which results in vasoconstriction and reduced synthesis of NO and prostacyclin, worsening the vasoconstrictor response. The upregulation of ET-1 is also involved in inflammatory responses and increased fibrosis. In PAH patients, ET-1 clearance by the pulmonary vasculature is reduced, and ET-1 plasma concentrations are elevated and correlate with PAH severity and prognosis [27,28]. Therefore, ET receptor antagonists are used as treatment for PAH (Fig. 2). So far, several types of ET receptor antagonists have been identified, which differ in their selectivity for ET_A and ET_B receptors [29]. Therapies targeting this pathway can be

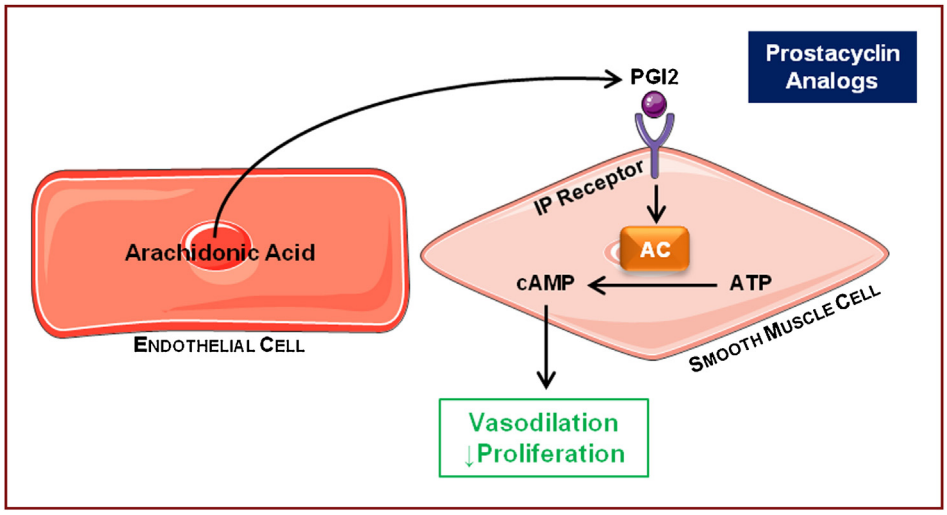

Figure 1. Prostacyclin (PGI2) signalling pathway. PGI2 is produced from arachidonic acid metabolites in endothelial cells, and acts on smooth muscle cells through prostacyclin receptor (IP) mediation, which leads to an increase in cyclic adenosine monophosphate (cAMP) concentration, resulting in vasodilation and reduced proliferation. Therefore, one of the pulmonary arterial hypertension-specific therapies is the use of prostacyclin analogues, as they have the ability to mimic prostacyclin signalling in smooth muscle cells. AC: adenylyl cyclase; ATP: adenosine triphosphate.

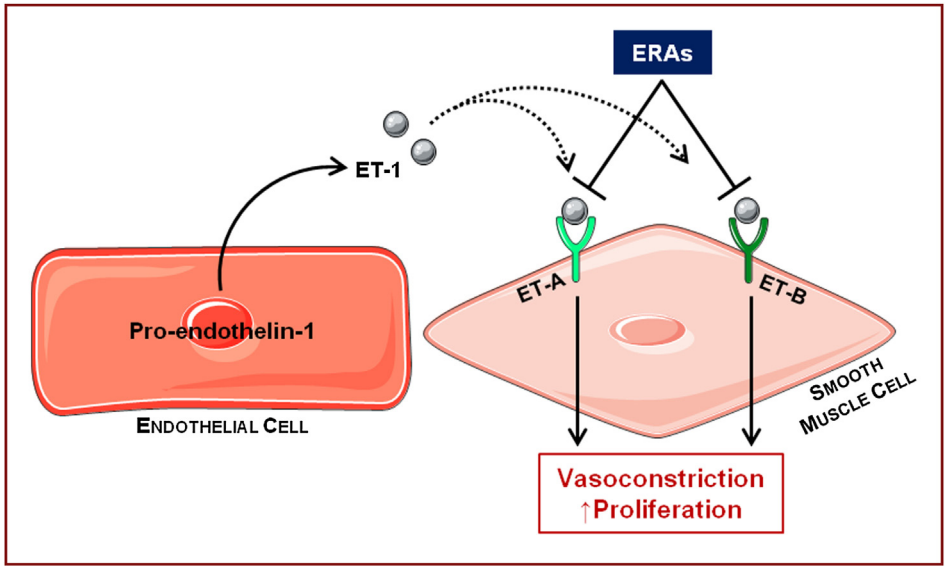

Figure 2. Endothelin (ET)-1 signalling pathway. ET-1 is released from endothelial cells and acts on smooth muscle cells through the interaction of two types of receptors (ET-1 receptor type A [ET-A] and ET-1 receptor type B [ET-B]), both of which mediate vasoconstriction and proliferation. The use of endothelin-1 receptor antagonists (ERAs) is one of the therapeutic strategies to attenuate pulmonary arterial hypertension, because ERAs can block the ET-1 receptor, albeit with different selectivity, and stop the signalling that leads to further vasoconstriction.

dual, if they block both receptors, or selective if they only block ET_A.

Endothelial nitric oxide

NO is a 30 Da lipophilic gaseous molecule that can be synthesized in mammalian tissues via activation of one of the three NO synthase isoforms, which have the ability to catalyse the formation of NO from L-arginine in a two-step reaction. NO is a vasodilator that modulates several physiological processes, and is also capable of inhibiting leukocyte adhesion, platelet aggregation, thrombus formation and vascular proliferation [24]. Endothelial NO synthase can be activated either by G-protein-coupled receptor signal transduction, which increases intracellular calcium concentrations and, subsequently, concentrations of calcium-calmodulin, Akt signalling, vascular endothelial growth factor and hormonal stimuli (e.g. oestrogen and insulin) [26]. In both animal models and humans with PH, decreased pulmonary vascular endothelial NO synthase activity is observed, along with loss of NO bioavailability, which is linked to impaired endothelium-dependent and -independent vasodilatation, increased PASMC mitogenesis and platelet aggregation [26,30].

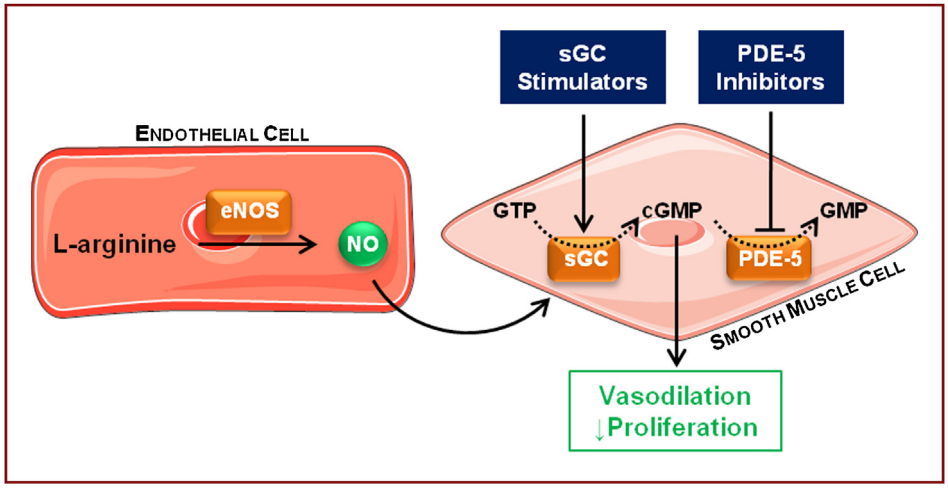

Figure 3. Nitric oxide (NO) signalling pathway. The formation of NO from L-arginine is catalysed by endothelial NO synthase (eNOS). NO is released from endothelial cells, and acts in smooth muscle cells, where it stimulates soluble guanylyl cyclase (sGC) to increase cytoplasmic cyclic guanosine monophosphate (cGMP), which exerts vasodilator and proliferative effects. Phosphodiesterase-5 (PDE-5) is one of the enzymes responsible for the hydrolysis of cGMP, and for that reason it is one of the main therapeutic targets in pulmonary arterial hypertension. Through the use of sGC stimulators and PDE-5 inhibitors, we are able to stimulate the production of cGMP and to slow down its hydrolytic breakdown, respectively, making it available to exert its beneficial effects. GMP: guanosine monophosphate; GTP: guanosine-5′-triphosphate.

Soluble guanylate cyclase stimulators were recently approved for the treatment of PAH, as they are able to restore NO levels (which are decreased in PAH) and the associated beneficial properties [31], such as vasodilatation and reduced proliferation (Fig. 3).

Phosphodiesterase

Phosphodiesterase enzymatic activity is implicated in the endogenous degradation of cyclic adenosine monophosphate and cyclic guanosine monophosphate (cGMP) [32]; currently, 11 phosphodiesterase isoforms are known in mammalian tissue [33]. More specifically, in the setting of PAH, phosphodiesterase type 5 (PDE-5) has gained some interest because it was found in elevated concentrations in PASMCs, platelets and myocytes. PDE-5 regulates cGMP bioactivity via hydrolysis of cGMP to GMP, and the allosteric binding of cGMP to PDE-5, which induces a conformational change to the structure of PDE-5, and positively feeds back to promote cGMP metabolism [26]. In the setting of PAH, expression of PDE-5 is increased in both smooth muscle cells and RV myocytes [34,35], which is associated with decreased levels of NO, pulmonary vascular dysfunction and impaired RV lusitropy [36]. As the vasodilator activity of NO in PASMCs is achieved through the upregulation of cGMP, and because its metabolism depends on the activation of phosphodiesterases, phosphodiesterase inhibitors are used as a therapy for PAH, as they are able to slow down the hydrolytic breakdown of cGMP, and thus have vasodilator effects [24] (Fig. 3).

Mitochondrial dysfunction and voltage-gated potassium channels (Kv channels)

In PAH, similarly to cancer, smooth muscle cell mitochondria have suppressed glucose oxidation and increased cytoplasmic glycolysis, along with inhibition of the enzyme pyruvate dehydrogenase [37]. The decreased pyruvate metabolism leads to the inhibition of the Krebs cycle and electron transport chain complexes, along with a decrease in its downstream mediators, such as reactive oxygen species and α-ketoglutarate. The reduced production of reactive oxygen species inhibits membrane Kv channels, and leads to an increase in intracellular calcium [38]. These alterations result from a variety of stimuli, such hypoxia or anorexigens [39], and contribute to the activation of the hypoxia-inducible factor-1 alpha [40] and the nuclear factor of activated T cells (NFAT) [41], both of which lead to the downregulation of Kv channel expression and a reduction in several mitochondrial factors and enzymes, contributing to the suppression of glycolysis and to the remodelling process in smooth muscle cells that is characteristic of PAH.

Some Kv channels are downregulated in the smooth muscle cells of PAH patients [42], and therefore gene transfer of these types of channels has been used in animal models, as an experimental strategy to prevent and/or attenuate PAH [43]. Moreover, the inhibition of pyruvate dehydrogenase through dichloroacetate administration seems to reverse mitochondrial abnormalities and Kv channel dysfunction, leading to regression of pulmonary vascular remodelling [37,44].

Serotonin and Rho proteins

Serotonin (5-hydroxytryptamine) is a vasoconstrictor that is also capable of promoting PASMC hypertrophy and hyperplasia [1]. While the serotonin transporter facilitates the induction of proliferation, by carrying serotonin into PASMCs, the serotonin$_{1B}$ receptor mediates vasoconstriction, both of which contribute to PAH pathogenesis [45]. PAH patients usually present elevated plasma concentrations of serotonin [46].

Several cellular functions, such as contraction, migration, proliferation and apoptosis, are regulated by Rho

proteins, especially Rho protein A, and Rho kinases have been implicated in PAH vasoconstriction and vascular remodelling [47]. The signalling pathway involving these proteins is directly involved in serotonin transporter-mediated PASMC proliferation and platelet activation during PH progression [48]. In fact, serotonin-induced proliferation requires the activation of Rho kinase by serotonin$_{1B/D}$ receptors, enabling the nuclear translocation of extracellular signal-regulated kinases and the activation of the GATA4-dependent transcriptional pathway that is involved in promoting cell proliferation [49]. The involvement of serotonin in the pathophysiology of PAH was first described in association with appetite-suppressant drugs, which are also known to induce PAH [50].

Genetic mutations

If not associated with other clinical conditions or induced by toxins, PAH can be either idiopathic or heritable. This disease segregates an autosomal dominant trait with a markedly reduced penetrance, as only 10—20% of individuals who carry the mutation will develop PAH [51]. The bone morphogenetic protein receptor type 2 (*BMPR2*) gene encodes for a serine/threonine receptor kinase that belongs to the transforming growth factor-β family, and is considered to be the major factor responsible for the development of 75% of heritable PAH, and approximately 25% of idiopathic PAH [52,53]. Mutations in *BMPR2* cause aberrant signal transduction in PASMCs, resulting in an imbalance between apoptosis and proliferation in favour of the latter [54]. The pathway downstream of BMPR2 activation is heavily coordinated by the SMAD cascade, which begins with the phosphorylation of SMAD1, SMAD5 and/or SMAD8, which bind to SMAD4 and enter the nucleus to drive transcription [55]. SMAD proteins also have the ability to regulate microribonucleic acid splicing via a non-transcriptional mechanism [56]. Thus, this signalling pathway regulates the smooth muscle differentiation state either by direct transcriptional regulation of genes involved in the maintenance of a differentiated and contractile state or by regulation of microribonucleic acid [57].

Another two PAH predisposing genes are *ALK1*, which codes for the activin-like kinase type I receptor that is present in endothelial cells, and *endoglin*; these genes are most common in patients displaying hereditary haemorrhagic telangiectasia [58,59]. Interestingly, all the genes mentioned above encode proteins involved in the transforming growth factor-β signalling pathway, which may be a trigger for pulmonary vascular remodelling, as this signalling pathway controls growth, differentiation and apoptosis in different cell types [60].

Recently, Ma et al. identified the gene *KCNK3* as a novel player in PAH pathogenesis. *KCNK3* encodes a distinct family of mammalian non-voltage-dependent potassium channels that seem to have a crucial role in the regulation of resting membrane potential and pulmonary vascular tone. In PAH, KCNK3 is important in the vascular remodelling process, as it is able to prevent or attenuate apoptosis. Moreover, the presence of loss-of-function mutations can lead to vasoconstriction, through the depolarization of the resting membrane potential [61].

The right ventricle

The integrity of right ventricular (RV) function, rather than the degree of vascular injury, is the major determinant of prognosis in PAH [62]. The abnormal changes that occur in the pulmonary arteries of PAH patients, at first lead to vessel narrowing and/or obstruction, which then results in a progressive increase in PVR and mPAP [63].

In a healthy heart, the RV, which differs anatomically from the left ventricle, is able to adapt and respond to an increase in load with an increase in contractility, because its thin wall, crescent shape and greater compliance give it the ability to adapt rapidly to changes in volume and pressure load [62].

In PAH patients, the RV copes initially with increased afterload, with enhanced contraction and concentric RV remodelling, while right atrial pressure remains normal. The rise in ventricular pressures increases diastolic and systolic stretch on the RV wall, which firstly leads to an increase in muscle mass — adaptive hypertrophy — as a result of increased protein synthesis and cardiomyocyte size. However, if the pressure overload is maintained, the RV cannot sustain the adaptive hypertrophy, and eventually dilates without any increase in RV contractility despite further increases in load, reaching a state called uncoupling of the RV [64].

The mechanisms involved in further adaptation of the RV and the decline in its contractility are poorly understood, but are thought to be associated with an imbalance between oxygen supply and demand [65], increased chronic sympathetic activation [66], oxidative and nitrosative stress, immune activation and cardiomyocyte apoptosis [64].

The increase in ventricular volume may also lead to tricuspid regurgitation, resulting in further RV volume overload and functional decline. The latter is accompanied by an increase in RV contraction time and ventricular asynchrony, together with a reduction in RV stroke volume, leading to underfilling of the left ventricle [67]. Impaired left ventricular filling in concert with RV dysfunction contributes to the evident decline in cardiac output seen in severe cases of PAH and, if not interrupted, this circle of events ends in right heart failure and death [63].

Animal models of PAH

In order to better understand the pathophysiological mechanism and remodelling process behind PAH, and to search for novel therapeutic agents, a variety of animal models have been developed and characterized.

These experimental in vivo models mimic certain histological and molecular features seen in PAH pathophysiology in humans; these include endothelial dysfunction, muscularization of previously non-muscular arterioles and increased medial thickness of normally muscularized arterioles, in situ thrombosis and the appearance of plexiform lesions [68]. Several techniques are available to induce PAH-associated alterations in animals, such as chemical agents [69,70], genetic manipulation [71,72], environmental factors [73] and surgical procedures [74] (Table 1).

Currently, monocrotaline administration and chronic exposure to hypoxia are the most widely used models of

Table 1 Experimental animal models of pulmonary arterial hypertension.

Animal model	Species	Histological features	Advantages	Disadvantages
Monocrotaline	Sheep; dog; rat	Medial hypertrophy; muscularization of non-muscular arteries; vascular inflammation	Severe PH; RV failure; predictable and reproducible	Toxic stimulus; no plexiform lesions
Monocrotaline + pneumonectomy	Rat	Medial hypertrophy; muscularization of non-muscular arteries; neointima formation	Severe PH; RV failure; proliferation of endothelial cells	Toxic stimulus; difficult manipulation
BMPR2 knockout	Mouse	↑ Muscularization	Well-suited to study the genetic factors that contribute to PH	Homozygous knockouts die in utero
Fawn-hooded rat	Rat	↑ Muscularization	Well-suited to study the genetic factors that contribute to PH	Presence of systemic hypertension
Overexpression of *S100A4*	Mouse	Plexiform lesions	Presence of plexiform lesions	Only 5% of S100A4 overexpressing mice develop PH
Chronic hypoxia + SU-5416	Rat	Medial hypertrophy; muscularization of non-muscular arteries; neointima formation; plexiform lesions	Physiological stimulus; proliferation of endothelial cells	Not clear which group it mimics
Schistosomiasis	Mouse	Perivascular inflammation; medial thickening; formation of plexiform-like lesions	Comparable to human schistosomiasis associated with PAH	Not significant PH
Left-to-right shunt	Sheep; pig; dog; rat	Medial hypertrophy; ↑ VSMC proliferation; intimal proliferation; plexiform lesions can be seen	Imitate the formation of plexiform lesions in human severe PAH	Sophisticated surgical approaches; pathological alterations can appear at a late stage
Closure of the ductus arteriosus	Fetal and newborn lambs	Medial hypertrophy; muscularization of non-muscular arteries; adventitial fibrosis	Relatively large size of the fetal lamb; uterine surgical intervention is well tolerated	Sophisticated surgical approaches; pathological alterations can appear at a late stage

BMPR2: bone morphogenetic protein receptor type 2 gene; PAH: pulmonary arterial hypertension; PH: pulmonary hypertension; RV: right ventricle; *S100A4*: S100 calcium-binding protein A4 gene; SU-5416: vascular endothelial growth factor receptor inhibitor; VSMC: vascular smooth muscle cells.

PH in translational research, because of their good reproducibility and well-described histopathology.

Toxic stimuli

Monocrotaline is a pyrrolizidine alkaloid extracted from *Crotalaria spectabilis* seeds. When administered to rats, it is metabolized by several oxidases present in the liver, producing the reactive bifunctional cross-linking compound monocrotaline pyrrole. Because this compound has a short half-life, and the pulmonary circulation represents the first major vascular bed after the liver, its toxic effect concentrates on pulmonary vessels, without major effects in the systemic circulation [68].

After monocrotaline injection, rats undergo a severe inflammatory reaction, followed by endothelial cell death

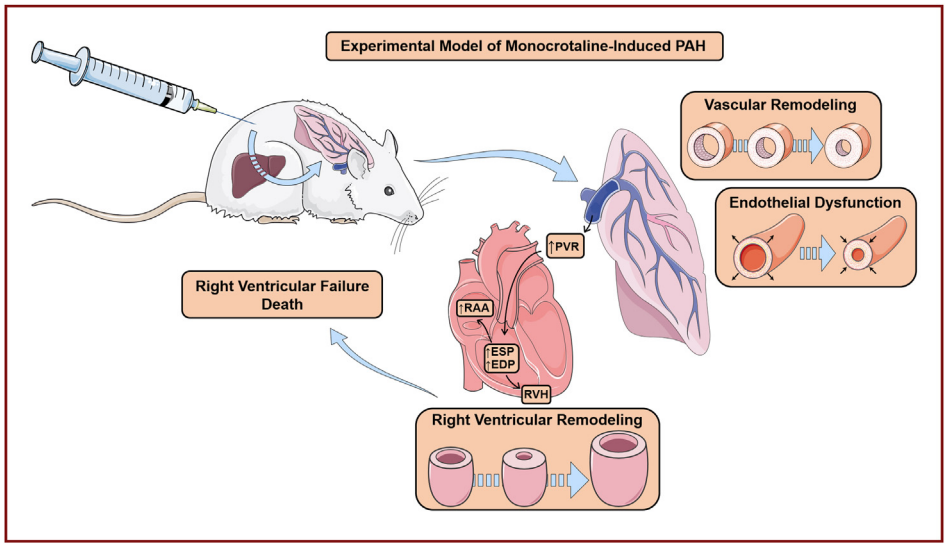

Figure 4. Experimental model of monocrotaline-induced pulmonary arterial hypertension (PAH). After monocrotaline injection, rats undergo vascular remodelling and endothelial dysfunction, which lead to a progressive increase in pulmonary vascular resistance (PVR) and, ultimately, to right ventricular hypertrophy (RVH) and increased RV systolic and diastolic pressures, as well as an increase in right atrial area (RAA). Due to the progressive increase in PVR, RV function deteriorates over time and, eventually, the animal dies of RV failure. ESP: end-systolic pressure; EDP: end-diastolic pressure.

and the loss of small peripheral arteries as well as an increase in the alveoli/arteries ratio. Generally, in the first 2 weeks no clinical disorder can be noticed, whereas in the following 2—3 weeks, the animal's state begins to deteriorate as a result of progressive thickening of the media and muscularization of non-muscularized arteries, along with adventitial proliferation [75]. As depicted in Fig. 4, these abnormalities lead to a progressive increase in mPAP and PVR, ultimately leading to RV hypertrophy and an increase in RV systolic pressure (from 25 to 60 mmHg). At this stage, animals show impaired breathing and cyanotic mucus membranes, acquire a hunched posture and are visibly sick. After 3—4 weeks of monocrotaline administration animals develop severe PH, with compensatory RV hypertrophy caused by the increase in PVR. With the progressive increase in PVR, RV function deteriorates, and eventually the animals die [76]. However, because of the different pharmacokinetics of monocrotaline in different rat strains, and even between individuals, differences in time of onset and extent of toxic effects can be seen [75].

In humans, one of the main characteristics of PAH is the presence of several vessels with neointimal remodelling. As this does not usually happen in the pulmonary arteries of monocrotaline-treated animals, monocrotaline treatment can be combined with pneumonectomy, as the alteration in haemodynamics caused by the surgical procedure extends the monocrotaline injury, leading to neointimal formation in the pulmonary arterioles [77].

Physiological stimuli

In humans, idiopathic PH can also arise as a consequence of congenital heart defects [78], such as the presence of systemic-to-pulmonary shunts. Therefore, the surgical creation of this type of channel in animals (e.g. aortocaval shunt in rats [79]; anastomosis of the left subclavian artery to the pulmonary arterial trunk in piglets [80]) can mimic some of the congenital heart diseases that lead to PH, through the increase in pulmonary blood flow and vascular resistances [80]. The abnormalities seen in this model are medial hypertrophy and excessive proliferation of smooth muscle cells in pulmonary arteries and arterioles, progressing to intimal proliferation and complete obliteration of small arteries in the later stages of the disease [73].

Chronic hypoxia is one of the most widely used animal models of PH, and is usually combined with the vascular endothelial growth factor receptor type 2 antagonist, SU-5416. Patients from the Group 1 classification of PH (PAH) possess a unique vasculopathy, marked by the appearance of plexiform lesions that develop as a result of the uncontrolled proliferation of endothelial cells, and eventually obliterate the vascular lumen. This unique PAH hallmark results not only from endothelial dysfunction but also from alterations in vascular endothelial growth factor function, which is known to be an essential endothelial survival factor [15]. In fact, a few studies [81,82] have already demonstrated that the pharmacological inhibition of vascular endothelial growth factor receptor type 2 in combination with chronic hypoxia results in severe PAH with a vasculopathy similar to in humans, including the formation of neointima.

Persistent PH of the newborn is a very specific subgroup of PAH, characterized by systemic arterial hypoxemia secondary to increased PVR, caused by the diversion of venous blood through the fetal channels (e.g. ductus arteriosus and foramen ovale) into the systemic circulation [83]. This pathology can be surgically recreated using intrauterine models (usually fetal lambs) to elucidate the mechanism underlying this particular form of PH. The histopathological features of this model include the muscularization of normally non-muscularized pulmonary arteries and fibrosis deposition — features also seen in the human syndrome. For this reason, this model has been widely used for the study of

the pathophysiology and treatment of persistent PH of the newborn [84].

Genetic stimuli

Nowadays, there are several genetically altered animal models that mimic PH, which are well-suited to the study of the genetic factors that contribute to PH expression and development.

From the age of 4 weeks, fawn-hooded rats develop PH, as a result of their inherited platelet storage disorder that is characterized by deficient serotonin uptake into platelets [85]. These rats also show an abnormal oxygen sensing mechanism in the mitochondria of smooth muscle cells, which leads to a deficiency in reactive oxygen species, normoxic HIF-1α activation and ultimately to reduced Kv channel function [40]. Approximately only 68% of fawn-hooded rats show an increase in muscularization and RV systolic pressure; however, this process can be accelerated and aggravated by exposure to increased altitude [71]. At birth, the fawn-hooded rats have immature lungs, with a decreased lung weight and alveolar number that persists into adulthood, accounting for the increased pulmonary resistances. Beyond PH, these rats also have systemic hypertension, which may be a confounding factor in the interpretation of results [86].

One of the most studied genes in the setting of familial PAH is the *BMPR2* gene that belongs to the transforming growth factor-β family [87]. Homozygous *BMPR2*$^{(-/-)}$ knockout mice die in the uterus; on the other hand, the heterozygous *BMPR2*$^{(+/-)}$ mice develop normally and show no pathological phenotype [88]. Therefore, a conditional and tissue-specific (PASMCs) knockout was created to overcome the homozygous lethality, and to produce a similar PAH phenotype. These transgenic mice were created using a cell-specific promoter and a dominant-negative *BMPR2* gene that causes an increase in muscularization [72] as a result of the aberrant signal transduction that produces an imbalance between apoptosis and proliferation in favour of the latter [54].

Lastly, approximately 5% of transgenic mice overexpressing S100A4 — a calcium-binding protein — develop plexiform lesions with intimal hyperplasia of the pulmonary arteries, leading to their occlusion, and mimicking the plexogenic vasculopathy seen in PAH [89]. These mice therefore offer a good model for studying the development of these particular lesions.

Concluding remarks

In short, continuing research is needed to deepen our knowledge of PAH pathophysiology and to develop novel therapeutic strategies, also directed at RV dysfunction secondary to PAH, an important mortality predictor.

Although, nowadays, several other techniques are available for basic and clinical research, these and other animal models continue to be of great importance in translational research, as they are capable of exhibiting several characteristics similar to the human pathology.

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Disclosure of interest

The authors declare that they have no competing interest.

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