The role of disturbed blood flow in the development of pulmonary arterial hypertension: lessons from preclinical animal models

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PULMONARY ARTERIAL HYPERTENSION (PAH) is a fatal and progressive form of pulmonary hypertension (PH) with, so far, unknown origin (37, 56). PAH is characterized by a typical form of pulmonary vascular remodeling, i.e., plexogenic arteriopathy (Fig. 1) (71). While thickening of the medial and adventitial layer of the pulmonary arterioles is seen in many forms of PH (e.g., PH associated with hypoxemia), PAH is additionally characterized by the formation of complex cellular and fibrotic lesions with, at the end of the spectrum, the formation of concentric laminar intima fibrosis and plexiform lesions (Fig. 1) (71, 88). These neointimal lesions cause intraluminal obstruction characterized by apoptotic dysregulation and proliferation of endothelial cells (ECs), smooth muscle cells (SMCs), fibrosis, and inflammation (36, 67). PAH is considered irreversible when these neointimal lesions have formed (67), resulting in increased pulmonary vascular resistance, increased right ventricular (RV) workload, and eventual death due to RV failure (7, 11, 39). The complex pathogenesis of PAH needs to be further explored to identify potential therapeutic targets and improve future treatment possibilities.

According to the typical histopathology, the progressive clinical course, and therapeutic responses, PAH is classified in group I of the clinical classification (Dana Point) for PH (Fig. 1) (70). PAH is subdivided in idiopathic, heritable, drug-induced, and associated with other conditions, including congenital heart disease (CHD). Although the histomorphology of PAH is well described, the pathogenesis of these progressive angioproliferative lesions remains largely unknown.

After three decades of research on the pathobiology of PAH, the focus has shifted from vasoconstriction (38) towards a cancer-like mechanism of angioproliferation (58, 67) in which the role of disturbed pulmonary blood flow is reappreciated as an important trigger in the development of pulmonary vascular remodeling (Fig. 2) (81). In general, disturbed blood flow can be defined as any pattern of flow that is nonuniform and
irregular. Examples are recirculation or swirling flow, reciprocating flow, and turbulent flow. These patterns can occur naturally at specific regions in the vascular system (e.g., branching points) or due to (pathological) changes in either vascular geometries (vessel stenosis or obstruction) or hemodynamic forces (17, 20). Under pathophysiological conditions these changes can lead to specific vascular and cellular responses resulting in remodeling of the vascular wall (17, 20).

Clinical observation has shown that, in patients with CHD who develop PAH, characteristic vascular lesions occur almost exclusively in defects associated with increased pulmonary blood flow, qualifying this increased pulmonary blood flow as a trigger for the induction of neointimal development. Here, increased pulmonary blood flow is regarded an inductor of disturbed blood flow patterns in the pulmonary vasculature. In these patients with increased pulmonary blood flow, additional increased pulmonary arterial pressure, as in nonrestrictive posttricuspid shunts, accelerates the progression of pulmonary vascular remodeling, as an apparent second trigger (81). Disturbed blood flow patterns are likely to be one of the triggers in PAH. Also other triggers, such as genetic predisposition due to bone morphogenetic protein receptor 2 (BMPR2)-signaling defects, drugs, toxins, and inflammation, have been associated with induction or aggravation of the vascular disease process in PAH (36, 55). Apart from the initiating trigger, once PAH has developed, elevated pulmonary arterial pressure in itself leads to altered hemodynamic forces and flow patterns that subsequently may propagate the vascular disease process. Still, questions remain about the exact role of blood flow disturbances in neointimal development and disease progression.

During the past decades animals models have played a crucial part in this process and will continue to give people new insight through PH research in the future. Although much insight has been gained from “historical” PH models, such as hypoxic PH or monocrotaline (MCT)-induced PH, most of these studies are hampered by the fact that these models do not truly reflect the typical features of PAH, i.e., the histopathology and progressive course (73). Indeed, while it is possible to “cure” many forms of PH in animal models, the clinical profile of patients with PAH is more resistant to therapy. Hence, there is an increasing interest in models that more reliably reflect the complex disease PAH. This review will summarize and discuss current knowledge about models of PAH.

### Clinical Classification Group

<table>
<thead>
<tr>
<th>Clinical Classification Group</th>
<th>Characteristics of arteriopathy</th>
<th>Histological examples</th>
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<tr>
<td>1. Pulmonary arterial hypertension&lt;br&gt;Pulmonary veno-occlusive disease or pulmonary capillary hemangiomatosis</td>
<td>- Medial hypertrophy&lt;br&gt;- Muscularization of arterioles&lt;br&gt;- Cellular proliferation of intima layer&lt;br&gt;- Concentric laminar intimal fibrosis&lt;br&gt;- Plexiform lesions&lt;br&gt;- Fibrinoid necrosis</td>
<td><img src="image1.png" alt="Histological example 1" /></td>
</tr>
<tr>
<td>2. PH due to left heart disease</td>
<td>- Medial hypertrophy&lt;br&gt;- Muscularization of arterioles and veins&lt;br&gt;- non-obstructive intimal fibrosis&lt;br&gt;- moderate intima fibrosis veins</td>
<td><img src="image2.png" alt="Histological example 2" /></td>
</tr>
<tr>
<td>3. PH due to lung disease or hypoxia</td>
<td>- Large arteries mostly normal&lt;br&gt;- Medial hypertrophy&lt;br&gt;- Muscularization of arterioles&lt;br&gt;- similar changes to lesser extent in small pulmonary veins</td>
<td><img src="image3.png" alt="Histological example 3" /></td>
</tr>
<tr>
<td>4. Chronic Thromboembolic PH (CTEPH)</td>
<td>- Mild medial hypertrophy&lt;br&gt;- Eccentric intimal fibrosis&lt;br&gt;- Recanalization of lumen&lt;br&gt;- recent thrombi rare</td>
<td><img src="image4.png" alt="Histological example 4" /></td>
</tr>
<tr>
<td>5. PH with unclear multifactorial mechanisms</td>
<td>- Muscularization of arterioles and veins (fibrotic lung disease, tumors)&lt;br&gt;- non-obstructive intimal fibrosis (fibrotic lung disease, tumors)&lt;br&gt;- vascular granulomas (sarcoidosis, tuberculosis)&lt;br&gt;- enlargement of bronchial arteries (bronchiectasis)</td>
<td><img src="image5.png" alt="Histological example 5" /></td>
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</table>

Fig. 1. Correlate of the Dana Point 2008 clinical classification with the characteristics of the pulmonary vascular arteriopathy. Group 1, pulmonary arterial hypertension (PAH), is characterized by formation of concentric laminar intima fibrosis and plexiform lesions. Typical examples of these lesions are presented in the column on the right. PH, pulmonary hypertension. Figure adapted from Wagenvoort and Mooi (87).
development, particular emphasis will be put on models of increased pulmonary blood flow and the current knowledge of the role of disturbed flow patterns in the development of pulmonary vascular remodeling in PAH.

**Animal Models of PAH: General Concept**

Animal models remain to play a crucial role in studying both new biomolecular pathways as well as investigating new treatment effects in PAH. Although material obtained from patients, usually when the lungs are explanted, is valuable, the use of animal models allows for evaluation of disease development and analysis of processes in early stages of the disease, as well as testing of reversibility. However, as for any preclinical model, the pathophysiological mechanisms and clinical course have to be comparable to the human disease that it is supposed to mimic.

For PAH this means that an ideal model would include the following: a) an initiation or trigger(s) of pulmonary vascular remodeling that mimics the human situation, e.g., an increase in or redistribution of pulmonary blood flow; b) pulmonary vascular remodeling that represents the development of complex obliterator neointimal lesions of the small intra-acinar arteries, and media hypertrophy of the smaller preacinar arterioles; c) a progressive disease development that leads to RV dysfunction and eventually death.

In addition, the model has to be viable in an experimental setting regarding both functional (hemodynamic and histological assessment) and biomolecular analysis, as well as having a workable period to disease progression.

To date no such ideal model for PAH exists. However, some recent animal models may resemble more closely both pathophysiological mechanisms and the clinical course of human PAH compared with more historical animal models of PH.

**Development of Pulmonary Vasculature: How Do Animals Compare to Humans?**

In choosing a proper animal model for PAH research, first consideration has to been given to possible anatomical and developmental differences between specific animal species and humans. These differences could have consequences for disease progression and severity, and therapeutic response.

In humans the pulmonary parenchym undergoes six developmental stages: embryonic, pseudoglandular, canalicular, sacular, alveolar, and vascular maturation (93–95). Of these changes only alveolar development and vascular maturation continue after birth (93, 94). Comparable with humans, rats and mice have little alveolar development at birth, whereas, for instance, lambs have already well-developed alveoli at birth. Such differences are likely to be of importance when studying changes in pulmonary blood flow and vascular remodeling perinatally. Rat lungs on the other hand show quite similar alveolar and capillary surface development after birth compared with humans (95). Mice have less pulmonary blood vessel walls and more alveolar space compared with rats (24, 95), and presumably less distal supernumerary arteries, which might explain the mild forms of vascular remodeling found in most murine PH models (28).
The pulmonary vasculature is lined by ECs, which are the first structure to perceive changes in hemodynamic forces. The endothelium has an important function in controlling vasomotor tone, regulating permeability, maintenance of homeostatic balance, and immunity. As the pulmonary vascular tree branches and the size of the pulmonary arteries decreases, the EC phenotype progresses from pulmonary artery ECs towards pulmonary microvascular ECs, which differ in their permeability and mechanical properties (17). These differences may reflect in the location-specificity of the pulmonary vascular histomorphological changes observed in pulmonary PAH. In the larger preacinar arterioles (100–500 µm) only increased medial wall thickness and intima proliferation is observed, whereas complex neointimal lesions progressively develop in the normally nonmuscular arterioles (<100 µm), the so-called intra-acinar arteries (Fig. 3) (88). Laminar concentric intimal fibrosis and plexiform lesions are predominantly formed at distal dichotomous branching points and branching points of supernumerary arteries, respectively (18, 70, 91) (Fig. 3).

Historical PH Models

Historically the most widely used animal models of PH have been chronic hypoxia and the MCT-induced PH rodent models. Although these models have added enormously to the understanding of the mechanisms of pulmonary vascular remodeling in PH, they are truly limited by the lack of typical complex vascular neointimal lesions and severe disease phenotype seen in PAH (73). Still, these models form a basis for other newer animal models where additional hits (triggers) result in characteristic pulmonary vascular lesions resembling human disease, e.g., the MCT/increased flow model (28, 73) or the hypoxia/Sugen model (79). For more knowledge about historical models the reader is referred to reviews published recently (29, 57, 73).

Models of Increased Pulmonary Blood Flow

Early chronic studies in large animals have shown that, when either one lung (i.e., left pulmonary artery) (19, 24, 33) or a single lobe (13, 59, 69) is directly connected to the aorta, the pulmonary arterial blood flow is increased. This results in chronic hypoxia, leading to vasoconstriction and increased vascular tone. Over time, this leads to the development of pulmonary arterial hypertension. The specific lesions seen in pulmonary hypertension throughout the pulmonary vascular tree are shown in Fig. 3. Pre-acinar pulmonary arteries mainly display a phenotype of media hypertrophy (A) and neointimal formation (B), whereas the intra-acinar arteries have typical occlusive lesions such as concentric intima fibrosis (C) and plexiform lesions (D).
which induces both high pulmonary blood flow and increased pulmonary arterial pressure, complex neointimal lesions develop comparable with human disease (Fig. 4). Unfortunately, since extensive morphometric analysis was not conducted in these studies it is difficult to interpret the exact magnitude of this remodeling.

Many later animal studies inducing increased pulmonary blood flow have used restrictive shunts resulting in increased muscularization of the pulmonary arteries but no complex neointimal lesions (10, 25, 26, 60, 62, 89).

An explanation for the relatively mild form of vascular remodeling found in these “increased-flow only” models is that the shunts used in these models were restrictive shunts with no significant flow-induced rise in pulmonary arterial pressure (10, 25, 26, 60, 62, 89). This is in analogy to the clinical observation that patients with untreated pretricuspid shunts, such as atrial septal defect, in general develop advanced pulmonary vascular remodeling only in 5–15% of the cases and only after two to three decades. In contrast, patients with untreated nonrestrictive, posttricuspid shunts, such as ventricular septal defects, develop advanced complex neointimal lesions in 1–2 yr in 80–100% of the cases (81).

Whether longer duration of the increased pulmonary blood flow in these animal models (months/years instead of weeks) or an associated increase in pulmonary arterial pressure by a nonrestrictive shunt would have induced advanced pulmonary vascular lesions can only be speculated. However, the absence of advanced vascular remodeling in these restrictive-flow models makes them less suitable to study the typical features of human PAH and to extrapolate the therapeutic effects to patients with PAH (64, 78).

Despite the lack of relevant endpoints, the high-flow low-pressure models allow to study the role of various pathways known in human PH. First, several studies have reported that systemic-to-pulmonary shunting in young lambs and piglets results in alterations of the endothelin pathway (Table 1) (9, 10, 60, 62, 89).
Increasing pulmonary blood flow, which can occur via a number of mechanisms, is a prerequisite for the development of pulmonary neointimal lesions (8, 21, 84). Interestingly, in an experimental study Nishimura and colleagues showed that, when MCT was administered after induction of the av-shunt, pulmonary vascular remodeling was less pronounced. This could possibly be explained by the fact that, due to the increased blood flow through the av-shunt, the concentration of metabolized monocrotaline pyrrole (after activation in the liver) is lower in the venous system reaching the pulmonary circulation compared with nonshunted rats.

**Neointimal lesions in models with increased pulmonary blood flow.** Are the vascular lesions seen in “double-hit” models comparable with those in human disease (Fig. 5)?

In human PAH irreversible neointimal lesions are comprised of both ECs and SMCs with a reduction in apoptotic markers and increase in inflammatory cells (43). In flow models, these obliterative lesions are comprised of cells that stain positive for the EC markers VEFG-R2 (90), eNOS (84), von Willebrand Factor (vWF) (59), CD31⁺ (47), and for SMCs staining positive for α-smooth muscle actin (Figs. 4 and 6 and Table 1) (21, 47, 84, 90). Longitudinal studies using the av-shunt or unilateral pneumonectomy combined with MCT in rats have shown that these neointimal lesions start to form 1–2 wk after increased pulmonary blood flow (Fig. 4) (21, 51, 52, 90). In these experimental models the exact role of apoptotic or proproliferative state of these vessels during vascular remodeling is fairly unknown. In addition, questions still remain where in the smaller pulmonary vasculature these occlusive lesions are most prominent (i.e., at branching points of larger arteries or at more distal locations).

Interestingly, White and colleagues have reported in their model that, when MCT administration is combined with unilateral pneumonectomy in younger rats, more complex plexiform-like lesions are formed (Fig. 4) (90). The authors suggested that younger rats were more prone to vascular proliferation after injury. These plexiform-like lesions stained positive for vWF, vascular endothelial growth factor receptor 2

<table>
<thead>
<tr>
<th>Species</th>
<th>Trigger</th>
<th>PAH Lesions</th>
<th>Progressive Disease</th>
<th>Findings</th>
<th>Ref. No.</th>
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</thead>
<tbody>
<tr>
<td>Rat</td>
<td>SU-5416 + hypoxia</td>
<td>+</td>
<td>–</td>
<td>Apoptosis</td>
<td>1, 77</td>
</tr>
<tr>
<td>Rat</td>
<td>ETBr deficiency</td>
<td>–</td>
<td>–</td>
<td>None</td>
<td>41</td>
</tr>
<tr>
<td>Rat</td>
<td>ETBr deficiency + MCT</td>
<td>+</td>
<td>–</td>
<td>ETB</td>
<td>40</td>
</tr>
<tr>
<td>Pig</td>
<td>BT shunt</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Tissue Factor</td>
</tr>
<tr>
<td>Rat</td>
<td>MCT + BT shunt</td>
<td>+</td>
<td>+</td>
<td>TGF-β, ACE</td>
<td>76</td>
</tr>
<tr>
<td>Rat</td>
<td>MCT + AoC shunt</td>
<td>+</td>
<td>+</td>
<td>Egr-1, mast cells</td>
<td>20, 81, 84</td>
</tr>
<tr>
<td>Rat</td>
<td>mBT shunt + MCT</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>47</td>
</tr>
<tr>
<td>Rat</td>
<td>MCT + pneumectomy</td>
<td>+</td>
<td>+</td>
<td>Oxidative stress</td>
<td>21</td>
</tr>
</tbody>
</table>

PAH, pulmonary arterial hypertension; BT shunt, classical Blalock-Taussig shunt (subclavian artery-pulmonary artery anastomosis); mBT shunt, modified Blalock-Taussig shunt = goretex conduit for subclavian artery to pulmonary artery; AoC shunt, aortacaval shunt; MCT, monocrotaline; ET, endotheli; TGF, transforming growth factor; ACE, angiotensin-converting enzyme; Egr-1, early growth response protein-1.
(VEGFR-2), and α-smooth muscle actin and, as shown by microangiography, to be part of the pulmonary vasculature. However, in this MCT/pneumonectomy model, unlike the MCT/av-shunt model, the effects of unilateral pneumonectomy have to be taken into consideration, including compensatory proliferation (up to 35%) of the remaining lung parenchyma itself (15).

Increased pulmonary blood flow and inflammation. Inflammation has been suggested to play a role in PAH as well as in the development of flow-induced neointimal formation in PAH patients with systemic-to-pulmonary shunts (32, 80, 86). Pro-inflammatory cytokines and other inflammatory cells, including mast cells (31), macrophages, lymphocytes (32), and dendritic cells (22), have all been linked to flow and nonflow PAH (Table 1). Accumulation of inflammatory cells in experimental PAH has also been linked to increased oxidative stress (22), which is also seen in pulmonary vessels of PAH patients (14).

In animal models of increased pulmonary blood flow inflammation has also been suggested to play a role in neointimal development (8, 22, 80). Clearly, the effect of MCT administration in vascular inflammation has to be taken into account in these experimental models (29), and questions remain whether inflammation can be seen as the additional trigger for neointimal development in the increased pulmonary blood flow/MCT models.

Still, reducing inflammation such as recently shown by mast cell stabilization or inhibition of chymase can attenuate the development of pulmonary vascular remodeling (8, 50). These results justify future exploration of the possible role of anti-inflammatory therapy for PAH.

In summary, in flow models that combine a trigger such as vascular injury (MCT) with either pneumonectomy or an av-shunt, increased pulmonary blood flow specifically induces severe, characteristic pulmonary vascular remodeling, including neointimal development, perivascular inflammation, and plexiform-like lesions with remarkable similarities with human plexogenic arteriopathy (Figs. 4 and 5). In addition increased flow leads to more pronounced increases in pulmonary artery pressure, RV hypertrophy, and mortality.

Role of Disturbed Flow on Vascular Remodeling: Lessons To Be Learned from the Systemic Circulation

In systemic vessels, blood flow, and the shear stress it inflicts, has been demonstrated to play a critical role in vascular
homeostasis and vessel remodeling. Shear stress imposed upon the luminal surface of the EC is defined as the tangential force per unit area caused by flowing blood (46). Here, the concept is that physiological laminar flow (seen in the straight part of a healthy vessel) has atheroprotective effects due to a physiological shear stress with a definite direction resulting in endothelial homeostasis (17, 20). Changes in flow patterns (e.g., swirling flow, turbulent flow, or low flow) lead to changes in shear stress (e.g., reciprocating shear stress, a high shear stress gradient, or reduced shear stress). These changes in shear stress are able to induce endothelial changes, specifically at predilection points (e.g., arterial branching points) where blood flow patterns are disturbed rather than laminar (4, 16, 17, 20).

In pathophysiological settings (such as increased blood flow, vascular stenosis, or surgical intervention) disturbed blood flow at these predilection sites is amplified, resulting in vascular remodeling due to upregulation of proatherogenic and prothrombogenic genes in ECs, EC proliferation and inflammation, and SMC modulation (16, 17, 20). A vicious cycle is made when the consequential changes in vessel wall geometry at these sites (due to vessel wall proliferation) further disturb flow patterns (and thus shear stress) resulting in propagation of vascular remodeling (17, 20). In addition, besides changes in local flow patterns, other triggers such as oxidative stress, inflammation, hypercholesterolemia, and advanced glycation end products can result in endothelial dysfunction, thereby accelerating the process of vessel wall remodeling in the systemic vasculature (17).

Can these concepts of vascular remodeling triggers in systemic vessels be translated to the pulmonary vasculature? Of course, one needs to take into account that the pulmonary endothelium differs from its systemic counterpart in several aspects, for instance, 1) angiogenic rather than vasculogenic development, 2) a transition from a low-flow/high-resistance environment towards a high-flow/low-resistance environment at birth, and 3) the molecular heterogeneity of pulmonary ECs within the pulmonary vascular bed (3, 4). Nevertheless, several concepts of hemodynamic changes in systemic vessels may also apply to the pulmonary circulation, supported by various observations.

First, pathophysiological triggers that result in disturbed flow patterns in systemic vascular remodeling (e.g., increased pulmonary blood flow and/or increased pressure) are also seen to induce pulmonary vascular remodeling in PAH. For instance, as in systemic vessels, increased blood flow induces characteristic vascular lesions in pulmonary arterioles, as illustrated in patients with CHD and systemic-to-pulmonary shunt.

Also as in systemic vessels, in PAH pulmonary vascular remodeling is specifically found at specific branching points where flow is disturbed (swirling or reciprocating flow) and shear stress is altered (18, 91). Conceptually, different branching points (dichotomous vs. supernumerary arteries) could then induce different flow pattern alterations, thereby inducing different forms of pulmonary vascular remodeling. This would explain why laminar concentric intimal fibrosis is described to form predominantly at dichotomous branching points and plex-
iform lesions predominantly at the origin of supernumerary arteries (Fig. 2) (18, 91).

Finally, as in systemic vessels, additional triggers (such as increased pressure, genetic predisposition, inflammation) may add to EC injury, thereby accelerating the process of vascular remodeling.

Still, it is yet unclear how changes in flow patterns (i.e., shear stress) exactly induce endothelial changes in the pulmonary vasculature ultimately leading to advanced pulmonary vascular remodeling in PAH. Several mechanotransduction pathways linked to changes in pulmonary blood flow have been suggested to be involved in the pathobiology of PAH. These include direct induction of proliferation through vascular endothelial growth factor (VEGF) (27), platelet-derived growth factor (79), transforming growth factor-β (56) pathways, apoptosis-resistance of ECs (65), induction of inflammatory processes like mast cells (8), macrophage or lymphocyte (32) recruitment, differentiation of ECs into mesenchymal cells (6), and direct damage to the EC barrier and autocrine effects (77).

This concept of disturbed flow patterns leading to neointimal development may also play a role in other animal models in which characteristic neointimal lesions have been reported. These models will briefly be described below.

### Other Neointimal Animal Models

In addition to flow-associated PAH animal models, other models (mostly rodents) have also been reported to develop specific obliterative lesions, some with great resemblance to neointimal lesions in human PAH (Fig. 7). Murine models are of interest since they allow one to investigate the relevance of single genes using genetic manipulation. The main disadvantage of murine models, however, is the mere mild degree of PH and RV dysfunction that usually is achieved in mice compared with other animal models (28). In addition, possible differences in the vascular bed of mice and humans have to be taken into consideration when comparing these data, as described above.

**Sugen/hypoxia rat model.** In both idiopathic PAH as well as PAH associated with congenital systemic-to-pulmonary shunts, VEGF is strongly expressed in plexiform lesions in end-stage disease (27).

<table>
<thead>
<tr>
<th>Experimental model</th>
<th>Characteristics of arteriopathy</th>
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<tr>
<td><strong>Increased flow models:</strong></td>
<td></td>
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<tr>
<td>• short term or small systemic-to-pulmonary shunt in rats, pigs, sheep</td>
<td>• media hypertrophy and muscularization of arterioles</td>
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<tr>
<td><strong>Other models:</strong></td>
<td></td>
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<tr>
<td>• hypoxic model rat / mouse</td>
<td>• media hypertrophy and muscularization of arterioles</td>
</tr>
<tr>
<td>• MCT model in rats</td>
<td></td>
</tr>
<tr>
<td><strong>Increased flow models:</strong></td>
<td>Media hypertrophy</td>
</tr>
<tr>
<td>• MCT + aortocaval shunt in rats</td>
<td>• muscularization + obliterative lesions (SMCs/ECs), perivascular inflammation.</td>
</tr>
<tr>
<td>• MCT + pneumonectomy</td>
<td>• neointimal proliferation of arterioles; muscularization + obliterative lesions arterioles</td>
</tr>
<tr>
<td>• Chronic systemic-to-pulmonary shunt in rats, pigs, sheep, dogs</td>
<td></td>
</tr>
<tr>
<td><strong>Other models:</strong></td>
<td></td>
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<tr>
<td>• Sugen 5416 + chronic hypoxia</td>
<td>• muscularization + obliterative lesions (ECs)</td>
</tr>
<tr>
<td>• S100A4/Mts1 overexpression in mice</td>
<td>• muscularization + obliterative lesions (SMCs) + perivascular inflammation</td>
</tr>
<tr>
<td>• S100A4/Mts1 overexpression + HSV-68 in mice</td>
<td>• obliterative lesions (ECs) + perivascular inflammation</td>
</tr>
<tr>
<td>• IL6 overexpression + with hypoxia in mice</td>
<td></td>
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<tr>
<td><strong>increased flow models:</strong></td>
<td></td>
</tr>
<tr>
<td>• systemic-to-pulmonary shunt (prolonged increased flow + pressure) in dogs, sheep</td>
<td>• plexiform lesions with cellular vessel obstruction, dilatation and recanalization</td>
</tr>
<tr>
<td>• MCT + pneumonectomy in young rats</td>
<td>• perivascular proliferation with lesions containing ECs</td>
</tr>
<tr>
<td><strong>Other models:</strong></td>
<td></td>
</tr>
<tr>
<td>• Sugen 5416 / hypoxia + prolonged normoxia</td>
<td>• plexiform like lesions containing obliterative lesions (SMCs/ECs and aneurysm like lesions</td>
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Fig. 7. Summary of the main histopathological changes found in several experimental models used to study pathophysiological mechanism of PAH. Models with only increased blood flow typically show only increased medial wall thickness in the time frame studied. Double-hit models with histology resembling human pathology (e.g., increased flow + additional hit) typically progress into advanced neointimal lesions with obliteration of the vascular lumen within the time frame studied. Severe prolongation of increased pulmonary blood flow can induce plexiform lesions, as is also observed in other double-hit models using experimental stimuli.
Taraseviviene-Stewart et al. have shown that combining VEGFR2 inhibition, using the compound Sugen-5416, with chronic hypoxia results in PAH with characteristic obstructive neointimal lesions in the arterioles, increased mean pulmonary artery pressure, and RV hypertrophy (75, 76). In addition, when these rats were subjected to normoxia for a longer period of time (up to 14 wk), more complex neointimal lesions did form (1). However, unlike in irreversible human PAH, the survival rate of Sugen-5416/hypoxia rats has been shown to be close to 100% even during long follow-up (1). Apparently the degree and magnitude of arteriopathy in these rats is unable to induce RV failure, which can raise the question whether the type of arteriopathy is representative for the human setting.

In addition, it is unclear how in these rats VEGFR2 inhibition, as additional “trigger” to hypoxia, leads to the induction of neointimal lesions. Sugen-5416 injection or hypoxia alone both do not result in oblitative lesions of the intra-acinar vessels (75). Unfortunately, little is known about the exact role is of Sugen-5416 in this process of SMC and EC proliferation and vascular occlusion. For instance, besides VEGFR2 inhibition, Sugen-5416 is also known for its inhibitory effect on other tyrosine kinases (66, 92). It is possible that other kinases also play a role in the development of vascular remodeling in the 5416/hypoxia model. In other words, in this model, the trigger is already interfering with the pathobiological mechanisms of PAH. This hampers biomolecular analyses of PAH and evaluation of treatment effects using this Sugen/hypoxia (normoxia) model. Indeed, Moreno-Vinasco et al. have shown that, when comparing gene expression of Sugen/hypoxia rat lungs with human PAH lung tissue, only one similar hit comes up (Fyn: a protein-tyrosine kinase oncogene) (45).

**Endothelin B receptor-deficient rats**. Ivy et al. have reported in rats that endothelin B receptor deficiency combined with MCT results in the development of neointimal lesions, increased RV hypertrophy, and reduced cardiac output compared with MCT alone (40). The vascular lesions in this model are comprised of cells staining positive for both EC and SMC markers and show similarities with human PAH lesions as well as those in other PAH models. However, when endothelin B receptor-deficient rats were subjected to hypoxia instead of MCT (41), these neointimal lesions were not seen even though PH did occur.

Other models of potential interest are the cell-specific BMPR2 loss-of-function models in mice (35), IL-6 transgenic mice (72), and mice overexpressing the calcium-binding protein S100A4Ms (30) (Fig. 7). In these models, however, incomplete disease penetrance and the need for additional hits may limit its use.

### Treatment Effects in Neointimal Animal Models

Currently PAH patients are treated, in addition to supportive medication, with either 1) calcium channel blockers or 2) one or a combination of the following agents: prostacyclin analogs, endothelin receptor antagonists, and phosphodiesterase-5 inhibitors. Despite improvements with these treatments, PAH still remains a fatal disease (39). The general thought is that this is related to the severity of pulmonary vascular remodeling, which is considered irreversible once neointimal lesions have formed. Therefore, future treatments should focus targeting on reversal of these so-called irreversible neointimal lesions, something that has not been accomplished so far.

In general, although some positive effects have been described (predominantly in prevention studies), to date no cure for animals with established advanced lesions has been described. Nevertheless, several interesting “proof of principle”

**Table 2. Summary of the therapeutic effects in animal models mimicking PAH**

<table>
<thead>
<tr>
<th>Species</th>
<th>Trigger</th>
<th>PAH Lesions</th>
<th>Therapy</th>
<th>Strategy</th>
<th>Findings</th>
<th>Ref. No.</th>
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<tr>
<td><strong>Trigger flow only</strong></td>
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<tr>
<td>Pig</td>
<td>mBT shunt</td>
<td>–</td>
<td>Bosentan</td>
<td>Prevent</td>
<td>↓ Media hypertrophy</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>Sitaxsentan</td>
<td>Prevent</td>
<td>↓ Media hypertrophy ↓ PVR</td>
<td>65</td>
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<tr>
<td></td>
<td></td>
<td>–</td>
<td>Sildenafil</td>
<td>Prevent</td>
<td>↓ pPA</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>Losartan</td>
<td>Prevent</td>
<td>↓ PVR</td>
<td>63</td>
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<td><strong>Trigger flow + second hit</strong></td>
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<td>Rat</td>
<td>MCT + AoC shunt</td>
<td>+</td>
<td>Cromolyn</td>
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<td>↓ Occlusion</td>
<td>10</td>
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<td></td>
<td></td>
<td>+</td>
<td>EPO</td>
<td>Prevent</td>
<td>↓ mPA</td>
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<tr>
<td></td>
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<td>+</td>
<td>Trepostinil</td>
<td>Prevent</td>
<td>↓ mPA</td>
<td>85</td>
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<td>+</td>
<td>Prostacyclin, aspirin</td>
<td>Prevent</td>
<td>↓ RV capillary myocyt ratio</td>
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<td></td>
<td>MCT + pneumectomy</td>
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<td>Quinapril</td>
<td>Prevent</td>
<td>↓ Occlusion score, pPA</td>
<td>51</td>
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<td></td>
<td></td>
<td>+</td>
<td>DHEA</td>
<td>Prevent</td>
<td>↓ Occlusion score, pPA</td>
<td>34</td>
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<tr>
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<td>+</td>
<td>Idem</td>
<td>Reverse</td>
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<tr>
<td></td>
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<td>+</td>
<td>Rapamycin</td>
<td>Reverse</td>
<td>No effect</td>
<td>49</td>
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<tr>
<td></td>
<td></td>
<td>+</td>
<td>Idem</td>
<td>Prevent</td>
<td>↓ Occlusion score, pPA</td>
<td>48, 83</td>
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<td></td>
<td></td>
<td>+</td>
<td>Simvastatine</td>
<td>Reverse</td>
<td>Similar but attenuated effect</td>
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<tr>
<td>Rat</td>
<td>SU-5416 + hypoxia</td>
<td>+</td>
<td>Simvastatine</td>
<td>Prevent</td>
<td>↓ pPA</td>
<td>78</td>
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<td></td>
<td></td>
<td>+</td>
<td>Nifedipine, cyclofosfamide, taxol, thalidomide, VEGF, Ibesartan, PPAR-γ agonist, lisinopril</td>
<td>Prevent</td>
<td>No effect</td>
<td>78</td>
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</table>

PA, pulmonary artery pressure; PVR, pulmonary vascular resistance; EPO, erythropoietin; DHEA, dehydroepiandosterone; VEGF, vascular endothelial growth factor; PPAR-γ, peroxisome proliferator-activated receptor-γ; RV, right ventricular; pPA, pulmonary artery pressure; mPA, mean pulmonary artery pressure.
studies may direct future research for development of novel therapies (Table 2).

In MCT/flow PAH rats inhibition of the vasoconstrictor tromboxane, a key player of vascular remodeling in PAH, using the prostacyclin analog treprostinil, resulted in a reduction in pulmonary artery pressure, although neointimal development was not reduced (85). Similar resistance to vascular remodeling improvement was seen when using aspirin or iloprost (82), suggesting a vasodilatory effect rather than an antiproliferative effect of the drug.

Other studies have shown that angiotensin-converting enzyme inhibition using Quinapril attenuated neointimal formation of the intra-acinar vessels (50). In the future, novel treatments in PAH may shift more towards targeting angiogenesis and inflammation. Indeed, in MCT/flow PAH models simvastatin (also known for its anti-inflammatory properties) (47), mast cell stabilization (via Cromoly) (8), dehydroepiandrosterone (known to have antioxidant and anti-inflammatory effects) (34), rapamycin (an antiangiogenic agent) (48), and erythropoietin (known for endothelial repair by endothelial progenitor cell mobilization) (83) have shown to attenuate, or in part reverse, neointimal formation and/or increased pulmonary artery pressure. However, caution has to be taken when directly extrapolating these data to the human setting as illustrated in a recent randomized controlled trial in PAH patients in which simvastatin as add on therapy did not show a positive effect on 6 min walking distance (42). Unfortunately, the clinical endpoint of this study, 6-min walk test, was not assessed in animal models. Indeed, most studies in animal models of PAH lack the use of clinical relevant endpoints, such as exercise capacity, although these endpoints have been proven useful in studies addressing RV therapeutic strategies (7, 12).

Possible Novel Pathophysiological Pathways in PAH

The pathogenesis of the complex pulmonary vascular lesions is likely a multifactorial process, necessitating system biology approaches to identify novel targets (2). Using a microarray analysis, van Albada et al. showed that increased pulmonary blood flow specifically induced the expression of WNT-signaling genes and several other transcription factors, including activating transcription factor-3 and early growth response protein-1 (21, 80). Also, in the last years many new putative pathways have been discovered in nonneointimal models that could potentially affect PAH development. Examples are the peroxisome proliferator-activated receptor-γ pathway in pulmonary artery EC in mice (5), tyrosine kinase inhibitors in hypoxic PH or MCT-induced PH (68), soluble guanylate cyclases (23), and the possible role of micro-RNAs in PH (54). Although promising pathways, their roles have yet to be established in more clinically relevant neointimal models of PAH (67, 73).

Concluding Remarks

PAH is still a progressive disease with typical lesions characterized by neointimal formation, obliteration, and plexiform lesions. The pathogenic mechanisms underlying the formation of these progressive pulmonary vascular lesions is still poorly understood but might be the key to curative treatment.

With PAH research shifting from a concept of mere vasoconstriction towards a mechanism of angioproliferation, the role of disturbed pulmonary blood flow is seen as an important instigator (both clinically and experimentally) in plexogenic arteriopathy and PAH development. Further investigation in the role of disturbed blood flow can provide us with relevant clues to further unravel the complex pathogenesis of PAH. In this process, PAH models of disturbed pulmonary blood flow that more closely resemble the human trigger and histopathology are well suited for further investigation. With the use of these models, new pathways are deemed to emerge, which may lead to new therapeutic targets for patients with PAH in the future.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise are declared by the authors.

AUTHOR CONTRIBUTIONS

M.G.D., B.B., M.A.J.B., and R.M.F.B. drafted manuscript; M.G.D. and B.B. prepared figures; M.G.D., B.B., M.A.J.B., and R.M.F.B. edited and revised manuscript; M.G.D., B.B., M.A.J.B., and R.M.F.B. approved final version of manuscript.

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