Animal Models for the Study of Pulmonary Hypertension: Potential and Limitations

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Abstract

Pulmonary hypertension (PH) is a multifactorial disease, commonly associated with heart failure. Different experimental models have emerged to help in the understanding of the molecular and cellular mechanisms associated with human PH, providing also a useful approach to test experimental therapies for PH treatment. Although there is no ideal animal model that mimics human PH, animal models have clearly provided valuable insights into the characterization of the cellular and molecular pathways underlying PH onset and progression, and have been successfully applied in the discovery of novel therapeutic approaches. In here we summarize the features of the animal models described within the field of PH research, either the physical, chemical and genetic models, emphasizing its advantages and limitations.
1. Introduction

The World Health Organization classified pulmonary hypertension (PH) into five groups which share a mean, resting, pulmonary artery pressure (PAP) $\geq 25$ mmHg. The Group 1 is pulmonary arterial hypertension (PAH), Group 2 is PH associated with left heart disease, Group 3 is PH associated with lung disease and/or hypoxia, Group 4 is PH associated with chronic thromboembolic disease (CTEPH), and Group 5 is PH associated with unclear multifactorial mechanisms (5th World Symposium on PH, Nice, 2013) [1, 2]. Each group reflects specific etiology, pathological and hemodynamic characteristics and therapeutic approaches. However, there are common processes to the pathology of all PH groups. Vasoconstriction, remodeling, thrombosis, and inflammation are the basic mechanisms of pulmonary vascular pathology in PH. Nevertheless, their relevance, origin, and order of appearance may differ depending on the etiology [2-5]. Over the last years, major advances in the understanding of
PH pathogenesis allowed a delay in disease progression, reducing the symptoms and increasing the quality of life of PH patients. Unfortunately, PH remains a disease without cure [6]. The fact that the disease is usually diagnosed in advanced stages difficult its study in humans. Animal model studies have allowed the investigation of the various phases of disease progression, being crucial to understand the pathophysiology of PH, and to test experimental therapies. Furthermore, they provide us advantages in terms of economy, control of the experimental conditions, replicability and drug testing envisioning its safety translation to humans [7, 8].

An ideal PH model should manifest the key clinical, hemodynamic and histopathological features of human PH [7]. Pulmonary hypertension is a complex disease of diverse etiology and so there is no single animal model that accurately reproduces the human disease, even focusing on just one of PH groups [9]. Consequently, a vast list of PH experimental models is currently available (Table 1). Each model has its own characteristics and allows the investigation of specific hypothesis. Some of them are used in the study of different groups of human PH, once they present molecular and pathological features common to those groups [4]. We grouped these models according to the stimuli that result in PH development (physical, chemical, genetic and multiple) and we critically highlight the general advantages and limitations of their use in PH research.

2. Physical Animal Models

The chronic hypoxia model is one of the most used to study PH pathogenesis and treatment. Its pathological features of pulmonary vasoconstriction and vascular medial hypertrophy mimic the ones observed in human PH [10]. Although being a model of Group 3 PH, it is often used to make conclusions regarding Group 1 PH (PAH) [11]. Chronic hypoxia can be induced by exposing animals to normal air at hypobaric pressure or to oxygen-poor air at normal pressure [12]. This decrease in oxygen pressure causes a strong pulmonary vasoconstrictor response that is characteristic of this model [13]. However, there is little evidence of right ventricle (RV) failure, that is usually the main cause of death in PAH patients [10]. Furthermore, the response to hypoxia varies among animal species, making difficult the translation of findings to human [11, 13].

Another described animal model of PH resulting from a physical stimulus involves repeated microembolizations with the injection of synthetic microspheres, such as Sephadex® microspheres to induce chronic emboli. Thus, this model is useful to study chronic thromboembolism PH (Group 4 PH). The possibility of target different-sized
vessels depending on the diameter size of the microspheres used is an advantage of this approach. However, although this model allows moderate PH development, attention should be taken regarding the microspheres material, since no cellular reaction related with the material type is desired [13, 14].

The surgical models, on the other hand, are designed to mimic the increased blood flow and pressures imposed on the RV in Group 1 PH. There are two main surgical methods used until today: pulmonary artery banding (PAB) and aorto-caval shunt. The PAB consists in a constriction imposed in the pulmonary artery, which leads to an increased afterload in the RV that drives the hypertrophic response. Thus, it allows separate the cardiac disease from the pulmonary disease, which is not present in this model. Given this, PAB does not replicate the human pathology entirely, but is useful to understand the mechanisms of RV dysfunction, already pointed out as the main determinant of prognosis [11, 15]. The aorto-caval shunt is a volume overload method which displays similar RV hypertrophy when compared with PAB. This model can be combined with the monocrotaline (MCT) model, leading to more severe disease development [4, 11, 16]. The main disadvantages of these surgical methods are related with the fact that they require highly technical skills and are usually associated with a high percentage of animal death [11]. Although not being as used as the chronic hypoxia model, these models are still common. Recently, a novel model of pulmonary artery banding emerged related with an easier method of constricting the pulmonary artery. This new method resulted in a significantly lower surgical mortality and revealed significantly more signs of RV dysfunction [17].

3. Chemically-Induced Animal Models

Chemically-induced PH models can offer advantage in terms of application simplicity and costs. Amongst these, the MCT animal model is the most broadly used to study PH, in particular the pathophysiology and therapeutic application in the Group 1 PH [8, 11, 18]. Indeed, for more than one decade, most studies on therapy of PAH have employed the MCT model [19]. As recently reviewed [20], the administration of the alkaloid MCT affects both the lungs and the heart, modulating primarily biological processes associated with the vascular remodeling and inflammation, two key pathological features of human PH. However, an important drawback is that the response to MCT is variable among species, strains and even animals [13]. The most common specie used in the MCT model is the rat because it is the one that best develops PAH features after the drug injection [21, 22].
MCT effects require conversion to an active form (MCT pyrrole) in the liver by cytochrome P450, which makes the model dependent on the animals-based metabolic differences [4]. For instance, mice must be injected with the MCT pyrrole active form and not MCT itself. However, the disease development is far less extensive, stagnating in an acute lung injury [22]. Other animals less used are dogs [23] or pigs [24], which can replicate human PAH more successfully than rodent models. Still, these kinds of animals are more expensive and the disease takes longer to develop [11]. In spite of the limitations of the MCT model, it is largely used once, in comparison with the other models, it is reproducible, less expensive and does not need particular technical skills [25]. Furthermore, it mimics human PH in terms of hemodynamic and histopathological severity, and high mortality [26].

<table>
<thead>
<tr>
<th>Experimental models</th>
<th>Animal species</th>
<th>Pathological findings</th>
<th>Advantages</th>
<th>Limitations</th>
<th>PH group</th>
<th>References</th>
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<tbody>
<tr>
<td>Physical stimuli</td>
<td></td>
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</tbody>
</table>
| Chronic hypoxia         | Guinea pig, mouse, pig, rat, sheep | Chronic hypoxia exposure results in pulmonary vasoconstriction, muscularization of non-muscular arterioles, increased media thickness and matrix deposition | • Widely used  
• Simple implementation | • Hypoxia response is variable among animal species | 1/3       | [27-30]      |
| Vascular obstruction    | Dog, pig, rat, sheep | Pulmonary arteries embolization caused by intravenous administration of synthetic microspheres | • Useful to study chronic pulmonary thromboembolism  
• Possibility of target different-sized vessels depending on the diameter size of the microspheres | • Possibility of cellular reaction with the microsphere material | 4        | [14, 30-34]  |
### Increased blood flow

<table>
<thead>
<tr>
<th>Animal Models</th>
<th>Surgical Procedure</th>
<th>Description</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog, pig, rat, sheep</td>
<td>Surgical formation of a left-to-right shunt</td>
<td>Causes an increase in pulmonary blood flow, leading to PH with vascular remodeling</td>
<td>Useful to mimic some congenital heart diseases and study the RV response to increased pressure and flow</td>
<td>Requires highly technical skills and Generally associated with a high percentage of animal death</td>
<td>[30, 35-37]</td>
</tr>
</tbody>
</table>

### Pulmonary artery banding

<table>
<thead>
<tr>
<th>Animal Models</th>
<th>Description</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse, rat, goat</td>
<td>Pulmonary artery banding</td>
<td>Leads to progressive pulmonary artery stenosis and RV hypertrophy</td>
<td></td>
<td>[8, 38, 39]</td>
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### Chemical stimuli

#### Monocrotaline

<table>
<thead>
<tr>
<th>Animal Models</th>
<th>Description</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>Dog, pig, rat, sheep</td>
<td>A single MCT injection induces PH characterized by vascular remodeling, increased muscularization, vascular inflammation, and RV hypertrophy</td>
<td>The most broadly used PH animal model, Simple implementation (one single injection), Relatively inexpensive</td>
<td>MCT response is variable among species, strains and animals</td>
<td>[24, 25, 30, 40-43]</td>
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#### α-Naphthylthiourea

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<thead>
<tr>
<th>Animal Models</th>
<th>Description</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Repeated injections induce pulmonary vascular remodeling associated with PH development and RV hypertrophy. ANTU-related PH could mimic chemotherapy associated pulmonary vascular changes</td>
<td>Simple implementation, Relatively inexpensive</td>
<td>Mimic only a few features of human PH, not being commonly used models</td>
<td>No defined group [44, 45]</td>
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</table>

#### Bleomycin

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<thead>
<tr>
<th>Animal Models</th>
<th>Description</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse, rabbit, rat</td>
<td>Bleomycin administration leads to pulmonary fibrosis development with increased lung inflammation and muscularization. Useful to study PH in interstitial lung diseases</td>
<td>Simple implementation, Relatively inexpensive</td>
<td>Mimic only a few features of human PH, not being commonly used models</td>
<td>3 [46-49]</td>
</tr>
</tbody>
</table>
### Group B Streptococcus

Pig, sheep

Group B Streptococcus exposure induces vasoconstriction in persistent PH of the newborn

- Simple implementation
- Relatively inexpensive
- Mimic only a few features of human PH, not being commonly used models

1” (Persistent pulmonary hypertension of the newborn) [50-52]

### Genetic stimuli

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Species</th>
<th>Description</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ang-1 overexpression</td>
<td>Rat</td>
<td>Transgenic rats overexpressing Ang-1 develop increased pulmonary arterial muscularization and vascular occlusion</td>
<td>Useful to study the role of specific pathways in PH development and progression</td>
<td>May not sum up all the complex features of PH</td>
</tr>
<tr>
<td>IL-6 overexpression</td>
<td>Mouse</td>
<td>Mice overexpressing IL-6 develop PH with increased pulmonary arterial muscularization and RV hypertrophy. IL-6 effects are augmented by hypoxia</td>
<td>Expensive models</td>
<td></td>
</tr>
<tr>
<td>S100A4/Mts1 overexpression</td>
<td>Mouse</td>
<td>Approximately 5% of transgenic mice overexpressing the calcium binding protein S100A4/Mts1 develop pulmonary arterial changes resembling human plexogenic arteriopathy</td>
<td></td>
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<tr>
<td>5-HTT overexpression</td>
<td>Mouse</td>
<td>5-HTT overexpression leads to PH development with pulmonary arterial remodeling and RV hypertrophy. Increased hypoxia-induced remodeling</td>
<td></td>
<td></td>
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<tr>
<td>Model</td>
<td>Effect</td>
<td>Advantages</td>
<td>Disadvantages</td>
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<tr>
<td>TGF-α overexpression</td>
<td>TGF-α overexpression leads to disruption of pulmonary vascular development and induction of severe PH and vascular remodeling characterized by abnormally extensive muscularization of small pulmonary arteries</td>
<td>• Useful to study the role of specific pathways in PH development and progression</td>
<td>• May not sum up all the complex features of PH • Expensive models</td>
<td></td>
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<tr>
<td>Mouse</td>
<td></td>
<td>1 [57]</td>
<td></td>
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<tr>
<td>TNF-α overexpression</td>
<td>Mice overexpressing TNF-α develop chronic lung inflammation, pulmonary emphysema, severe PH, RV hypertrophy</td>
<td></td>
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<td></td>
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<tr>
<td>Mouse</td>
<td></td>
<td>1 [58]</td>
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<tr>
<td>Apolipoprotein-E knockout</td>
<td>Apolipoprotein-E knockout mice develop PH with increased pulmonary arterial muscularization and RV hypertrophy. Useful to study insulin resistance and obesity as risk factors for PH development</td>
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<tr>
<td>Mouse</td>
<td></td>
<td>1 [59]</td>
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<tr>
<td>BMPRII knockout</td>
<td>Loss of BMPRII signaling leads to an increase in media smooth muscle thickness and muscularization of small pulmonary arteries</td>
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<tr>
<td>Mouse</td>
<td></td>
<td>1 [60]</td>
<td></td>
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<tr>
<td>Neprilysin knockout</td>
<td>Neprilysin knockout mice present severe PH characterized by muscularization of the distal pulmonary arteries, thickening of the proximal media and adventitia and RV hypertrophy in response to hypoxia</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mouse</td>
<td></td>
<td>1 [61]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>Species/Model Description</td>
<td>PH Characteristics</td>
<td>Advantages</td>
<td>Disadvantages</td>
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<tr>
<td>VIP knockout</td>
<td>Mouse VIP knockout mice develop moderately severe PH with pulmonary vascular remodeling, increased muscularization of the pulmonary arteries and RV hypertrophy. The condition is associated with increased mortality</td>
<td>- Useful to study the role of specific pathways in PH development and progression</td>
<td>- May not sum up all the complex features of PH</td>
<td></td>
</tr>
<tr>
<td>Fawn-hooded rat</td>
<td>Rat Rat strain with a disorder characterized by a deficient serotonin uptake into platelets and with immature developed lungs and a reduced number of alveoli, PH development</td>
<td></td>
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<tr>
<td>Broiler chicken</td>
<td>Chicken Broiler chicken strain is characterized by PH development, possibly by a mitochondrial dysfunction</td>
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<tr>
<td>Multiple stimuli</td>
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<tr>
<td>SU5416 + chronic hypoxia</td>
<td>Mouse, rat VEGFR-2 blockade coupled with chronic hypoxia leads to PH development with complex plexiform-like lesions formation</td>
<td>- Mimic more accurately human PH than single stimuli models, once they exhibit more severe PH and/or vascular lesions (neointimal and plexiform lesions)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Athymic + SU5416</td>
<td>Rat Athymic rats treated with VEGFR-2 blocker develop severe PH with vascular remodeling</td>
<td></td>
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<tr>
<td>Athymic + MCT</td>
<td>Rat Athymic rats treated with MCT develop severe PH with a greater number of mast cells and severer histopathological changes, such as thickening of the alveolar wall</td>
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</table>
Table 1: Animal models of pulmonary hypertension.

<table>
<thead>
<tr>
<th>Model</th>
<th>Species</th>
<th>Description</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT + pneumonectomy</td>
<td>Rat</td>
<td>Monocrotaline administration coupled with pneumonectomy leads to PH development with additional neointimal lesions formation</td>
<td>- Mimic more accurately human PH than single stimuli models, once they exhibit more severe PH and/or vascular lesions (neointimal and plexiform lesions) - Can be expensive and/or require technical skills</td>
</tr>
<tr>
<td>Young age + MCT + pneumonectomy</td>
<td>Rat</td>
<td>Monocrotaline administration with pneumonectomy in young rats leads to PH development with plexiform-like lesions formation</td>
<td>1 [74]</td>
</tr>
<tr>
<td>Endothelin receptor-B deficiency + MCT</td>
<td>Rat</td>
<td>Monocrotaline administration in endothelin receptor-B deficiency rats leads to acceleration of PH progression, enhances the appearance of cellular and molecular markers related with PH pathobiology and develops neointimal lesions</td>
<td>1 [75]</td>
</tr>
</tbody>
</table>

Table 1: Animal models of pulmonary hypertension.

5-HTT : Serotonin Transporter; Ang-1 : Angiopoietin-1; BMPRII : Bone Morphogenetic Protein Receptor Type II; IL-6 : Interleukin-6; TGF-α : Transforming Growth Factor-α; TNF-α : Tumor Necrosis Factor-α; VEGFR-2 : Vascular Endothelial Growth Factor Receptor-2; VIP : Vasoactive Intestinal Peptide.

4. Genetic Animal Models

In the past few years, numerous genetic animal models have emerged in the field of PH research [11, 13, 30]. The transgenic and knockout models allow the evaluation of the effect of overexpressing or downregulating a specific gene in the susceptibility to the development of PH. The high diversity of these models reflects the different molecular pathways underlying PH development [4, 9]. Even though there is not a clear separation, we can group the genetic models by the main processes that they interfere with: vascular tone and inflammation/vascular remodeling. Independently of the group, the most common specie used is the mouse, which is harder to handling in the experimental procedures, such as hemodynamic evaluation [11].
Endothelin (ET)-1 is a potent vasoconstrictor that drives PH development and progression, by acting in its receptors (ET\textsubscript{A} and ET\textsubscript{B}). ET\textsubscript{A} is expressed mainly in pulmonary artery smooth muscle cells (PASMCs) and is related to PASMC proliferation and vasoconstriction. On the other hand, ET\textsubscript{B} is expressed in pulmonary artery endothelial cells (PAECs) and PASMCs. Activation of ET\textsubscript{B} in the PAECs causes vasodilatation \textit{via} the release of nitric oxide and prostaglandin, while stimulation of ET\textsubscript{B} in the PASMCs causes vasoconstriction [76]. Nevertheless, both heterozygote ET-1 knockouts and ET-1 overexpressers transgenic models fail to alter pulmonary vascular pressures \textit{per se} [4]. Yet, ET\textsubscript{B} receptor knockout mice have the vasodilatory effect of this receptor blunted and is linked with enhanced appearance of cellular and molecular markers related with PH pathobiology and development of neointimal lesions when in combination with MCT [75]. Serotonin (5-HT) is also an important regulator of vascular tone associated with PH pathogenesis [3]. Genetic models with 5-HT related alterations greatly contributed to the current knowledge of this mediators’ role. Consistently, tryptophan hydroxylase 1 (involved in 5-HT synthesis) knockout mice [77], 5-HTT (5-HT transporter) knockout [78] and 5-HT1B (5-HT receptor) knockout [79] attenuate hypoxia-induced PH, while 5-HTT gene overexpressing mice present a more severe form of the disease [56].

Inflammation is a key feature of PH pathogenesis, being already a therapeutic target [80, 81]. Genetic models are particularly useful for studying the effect of specific cytokines in PH pathophysiology. Among others, TNF-\(\alpha\) [58] and TGF-\(\alpha\) [57] overexpression is linked to PH. But, by far, the best studied models are the ones that target IL-6 related pathways. In fact, it is documented an increase in serum expression of IL-6 in patients with PAH, which positively correlates with the mortality rate [82, 83]. The mouse model of IL-6 overexpression was first implemented by Steiner \textit{et al.} [54]. They found RV hypertrophy, an increased muscularization throughout the entire pulmonary vascular bed and the formation of occlusive neointimal angioproliferative lesions composed of endothelial cells and T-lymphocytes. Consistently, Savale \textit{et al.} [84] found, in an IL-6 knockout model, diminished susceptibility to hypoxia-induced PH. They reported a decrease in media thickening of pulmonary vessels in IL-6 deficient mice and also a role of IL-6 in PASMC migration. Altogether, the IL-6 overexpressing mouse seems to be a model that resembles many of the pathologic features of PH. As a matter of fact, IL-6 has been proposed to regulate several pathways that are implicated in PH. It is believed that IL-6 drives the hyperproliferative state of PASMCs, modulates several pro- and anti-apoptotic factors [54] and the BMP signaling cascade [85]. Since a mutation of the \textit{BMPRII} gene that encodes for the bone morphogenetic protein receptor II was discovered to be a
principal mutation in hereditary PAH [86], there have been attempts to create BMPRII-deficient mice. However, the complete deletion of the gene is incompatible with life and heterozygotes do not develop adequate disease severity [11]. This problem was overcome by the appearance of smooth muscle-specific transgenic mice expressing a dominant-negative BMPRII under control of a tetracycline gene switch system [60]. There is consistent evidence of the disease development in this model, namely an increase in RV systolic pressure, RV hypertrophy, an increase in muscularization of small pulmonary arteries and some blood flow changes [60, 87]. The BMPRII ligands are also targets of research in the field of PAH. BMP-2 and BMP-4 are the most important factors of this class and act in opposite ways in response to hypoxia: BMP-2 knockout mice have increase severity while the opposite happens with BMP-4 knockout mice [88].

The protein S100A4/Mst1 is part of a family of calcium-binding proteins whose functions are related with cell proliferation, differentiation, cytoskeletal dynamics and apoptosis [4, 8]. Interestingly, the transgenic mice overexpressing S100A4/Mst1 model was initially developed aiming the study of S100A4/Mst1 role in metastatic cancer [89]. However, it was observed that approximately 5% of the S100A4/Mst1 overexpressing mice develop pulmonary vascular remodeling similar to that observed in PH [55]. Thus, the importance of this model in PH study is related with the presence of pulmonary vascular changes resembling human plexiform lesions, which is a feature absent in the majority of the PH models. Noteworthy, although the majority of the PH models show increased male susceptibility, contrary of what is observed in human PH; two genetic animal models (mice overexpressing the protein S100A4/Mts1 and mice overexpressing the serotonin transporter) showed a PH development female gender specific [90]. The fact that genetic models may not sum up all the complex features of PH, once they focus in the study of particular pathways is a limitation [11]. Furthermore, genetically modified mice are expensive, which may cause a limitation in the number of samples [91].

5. Animal Models Involving Multiple Stimuli

Animal models that involve chronic hypoxia and MCT models have been developed aiming more severe PH and/or vascular lesions such as neointimal and plexiform lesions [92]. These occlusive lesions, which result from smooth muscle and endothelial cell proliferation, are hallmarks of Group 1 PH, being major contributors for the high pulmonary vascular resistance in PAH [93, 94]. However, they are absent in the most common single stimuli animal...
models. Those models that combine multiple insults result in more severe PH than single stimuli, suggesting that the pathogenesis of PH requires several insults [11]. SU5416 is a small molecule inhibitor of the vascular endothelial growth factor receptor-2 (VEGFR-2). Given that VEGF is important for normal endothelial cell function, its blockade was expected to induce endothelial cell dysfunction, stimulate apoptosis-resistant endothelial cell proliferation and consequently cause PH [93]. Indeed, the SU5416/chronic hypoxia model is the most used multiple stimuli model to study both PH pathogenesis and treatment. Interestingly, this model demonstrated resistance to some drugs commonly used in PAH patients, being thus refractory to treatment as it is verified in most PAH patients [8, 93, 95]. However, a limitation of SU5416/chronic hypoxia model is the absence of perivascular inflammation, a key feature of human PAH [10, 11]. Another relevant multiple model consists in MCT administration coupled with pneumonectomy, which add to the MCT model vascular characteristics the presence of neointimal lesions [73]. Nevertheless, it requires technical skills associated with the experimental procedure.

6. Conclusion and Future Perspectives

The complexity of the molecular mechanisms underlying PH pathogenesis and the diverse etiology that characterizes this disease makes the implementation of animal models a truly demanding task. Although a “gold” animal model in PH research does not exist, there are several animal models available nowadays, each one presenting specific features of the disease, allowing the investigation of key aspects of the disease. These models are important not only for the discovery and exploration of the molecular pathways underlying disease pathogenesis but also for the assessment of therapeutic suitability to treat PH patients.

However, caution should be taken when translating data from animal models to the human context considering the different aspects of the disease in animal models. So, efforts should continue to be done in the development of animal models that more exactly mimic the features of each group of human PH. The current trend is the use of two animal models, such as MCT and chronic hypoxia, to demonstrate that data obtained are model independent and to facilitate data translation to the human clinical set. The simultaneous use of distinct PH animal models to test an experimental therapy is expected to continue and even increase once raises the hypothesis of its suitability for PH patients.
Acknowledgements

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Conflicts of Interest

The authors report no conflicts of interest.

References


