

# Animal Models of Pulmonary Hypertension; Going Beyond Classical Models of Induction

Esraa M. Zeidan<sup>1\*</sup>, Mohamed M. A. Khalifa<sup>1</sup>, Ashraf Taye<sup>2</sup>

<sup>1</sup> Department of Pharmacology and Toxicology, Faculty of Pharmacy, Minia University, El-Minia, Egypt

<sup>2</sup> Department of Pharmacology and Toxicology, Faculty of Pharmacy, South Valley University, Egypt;

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## Abstract

Pulmonary hypertension (PH) is a life threatening pulmonary vascular disease, characterized by elevated pulmonary arterial pressure (PAP), pulmonary vascular remodeling and right sided heart hypertrophy. Due to the emergence of right ventricular (RV) failure, PH, in spite of recent therapeutic improvements, continues to be a fatal condition. Although there are already many animal models of pulmonary hypertension accessible in research, improvement in RV function and effective remodeling reversal remain a therapeutic challenge. The right utilization of traditional and novel PH animal models is crucial for understanding the underlying pathological mechanisms to reverse severe phenomenon. This review gives a general summary of the various models for PH induction, along with each method's benefits and drawbacks.

## Keywords

Animal models, Chronic hypoxia, Monocrotaline, Sugen, Pulmonary hypertension

## Introduction

Pulmonary hypertension (PH) is severe progressive lung disorder characterized by pulmonary vasoconstriction and pulmonary vascular remodeling that subsequently culminates into right heart failure and death. According to the 6<sup>th</sup> World Symposium on Pulmonary Hypertension (WSPH) hemodynamic definitions, two criteria are essential for the diagnosis of PH: mean pulmonary artery pressure (mPAP) higher than 20 mmHg and increased peripheral vascular resistance (PVR)  $\geq 3$  Wood Units (WU) [1]. Thus, the inclusion of PVR in the definition of pre-capillary PH is a crucial indicator of pulmonary vascular disease (PVD), as it accounts for the relation between CO, mPAP and the pulmonary arterial wedge pressure (PAWP); so that  $PVR \times CO = (mPAP - PAWP)$  [1].

PH is multifactorial disease, clinically classified into 5 major groups (**Figure 1**) based on its pathological characteristics [2]. Rare forms of PH include; group (I) PH, pulmonary arterial hypertension (PAH) and group (IV) PH due to obstruction of pulmonary artery, primary as a result of chronic thromboembolic PH (CTEPH), while the more common forms of PH include; group (II) PH due to left sided heart dysfunction, group III PH due to chronic lung disease and/or hypoxia and group (V) PH as a result of miscellaneous factors [2-4].

Each type of PH has an incidence that is linked to the underlying causative disorders, and variability in prevalence across groups is mostly due to co-morbidities, demographic factors, and assumptions about right atrial pressure measurement [5]. In EGYPT, Idiopathic pulmonary arterial hypertension (PAH) is the most common cause of pulmonary hypertension, according to the first registry of PH patient's study [6]. However, according to global registries, group II PH caused by left heart

disease, has the largest incidence ratio and is linked to a poor prognosis and high morbidity ratio in patients with heart failure. Although there are several causes for PAH and PH, the clinical symptoms are mostly the same including clinical signs of heart failure, progressive breathlessness, fatigue and syncope [7].

## 1-Physical manifestations, evaluation, and histopathology of PH

Although PAH and PH have many different origins, all of their clinical symptoms are identical. The most typical symptoms include clinical indicators of heart failure, increasing dyspnea, exhaustion, and syncope. Most often, echocardiography and right cardiac catheterization are used to first indicate the diagnosis, which is then integrated with clinical aspects to permit precise estimation for effective treatment. Depending on the clinical presentation, pulmonary hypertension might be estimated [8, 9]. Moreover, in the context of histopathological changes, pulmonary vasculopathy manifests as medial hypertrophy, intimal fibrosis, and thickening of the adventitial layer, which result from continuously high pulmonary artery pressures. As well concentric intimal lesions are also visible in severe pulmonary hypertension [10].

## 2-Pathogenesis of PH

Regardless of the etiology, PH is caused by restricted blood flow in a pulmonary artery, and the initial hypothesis that pulmonary vasoconstriction mechanisms are the primary etiology of PH has

\* Correspondence: Esraa M Zeidan

Tel.: 01092700562

Email Address: Ph\_esraamohammed@yahoo.com

been extended to include pulmonary vascular remodeling [3, 11, 12].

The vasoconstriction mechanism is a compensatory response that limits blood flow to affected alveoli in order to maintain ventilation-perfusion matching [13]. The initial mechanism of the vasoconstriction is triggered by mitochondrial signaling followed by modulation of the voltage-gated calcium and potassium ion channels, particularly in the pulmonary vascular smooth muscle cells [14-16].

However, medial enlargement, adventitial thickening, intimal fibrosis, thrombotic lesions, inflammatory cells recruitment, and plexiform lesions describe the pulmonary vascular remodeling that underpins PH development [17]. Many studies have shown that all types of vascular wall cells, such as fibroblasts, pulmonary artery smooth muscle cells, and pulmonary artery endothelial cells, myofibroblasts, and pericytes, play a role in pulmonary vascular remodeling [18, 19].

PH is a complex and diverse illness with various pathogenetic changes and various triggering stimuli (**Figure 2**). Environmental trigger factors like chronic hypoxia exposure, shear stress, smoke exposure [13]. Genetic susceptibility to different mutations like bone morphogenetic protein receptor 2 (BMPR2), activin receptor-like kinase 1 (ALK1) [20]. Parasites and infections such as schistosomiasis and HIV [2]. Systemic and circulating factors including hormones, iron availability, and endothelin. Cellular mechanisms in PAEC, PASMC, and fibroblasts include altered expression/function of ion channels and growth factor receptors, activation or deactivation of transcription factors [21, 22]. Activation or deactivation of transcription factors, as well as altered expression/function of ion channels and growth factor receptors, are cellular processes in pulmonary artery smooth muscle, endothelial cells and fibroblasts [4, 17]. In some ways, PH can be regarded as a pseudo-malignant condition with cancer-like characteristics such as apoptosis resistance, altered metabolism, and overexpression of growth factor receptors [23].

### 3-Models of induction of PH

The classic models of induction of PH include either exposure to hypoxia or injection of monocrotaline which are utilized expansively in the research of PH [24, 25]. However, the difficulty with these models is that they fail to induce some characteristics of the functional impairment and structural remodeling found in PH patients [26].

Because PH is so diverse, no single animal model is likely to be generally applicable. Investigating new animal models will aid in the search for the molecular targets by allowing a better understanding of the pathways regulating the switch from adaptive remodeling to right ventricular failure [17]. The unique experimental model has to show the important clinical and histological characteristics of human disease, including the elevation in mPAP or RVSP [27]. General histological characters such as small vessels muscularization and thickening of the medial and adventitial layers have to be occurred in the model of induction [17].

#### 4.1- MONOCROTALINE (MCT)

Monocrotaline is considered as the most commonly used model to investigate PH development, because of its low cost, widespread availability and simplicity. MCT, an alkaloid compound extracted from the natural plant *Crotalaria Spectabilis*, was discovered around 60 years ago to induce PH in rats [28]. However, the exact mechanism of PH induction by

monocrotaline is not clear yet. MCT pyrrole (MCTP), a hepatic metabolite of MCT, has been suggested to be the main participants in the pathophysiology of MCT induced PH [29].

One subcutaneous injection of MCT (60–80 mg/kg) is all that is required to cause the pathological characteristics of PH in rats. Pulmonary endothelial injury can be developed within hours of administration, and established after a week, with subsequent vascular inflammation. After 3 weeks of treatment with MCT, there has been significant vascular remodeling, as evident by the medial thickening of large arteries and the loss of peripheral vessels, simultaneously, RV hypertrophy appears and quickly advances to RV failure and death. The broad toxicity of MCT also results in a number of extrapulmonary aspects that may contribute to mortality [30].

Moreover, MCT can acts directly on the cardiac tissues causing the quick development to RV failure and death. Combination of MCT with surgical removal of one lung has led to severe model of PH including a plexiform-like lesions and advanced hypertrophy compared to single MCT administration. Similar pathological features are developed upon combining MCT with chronic hypoxia (CH) exposure [30, 31].

#### 4.2-CHRONIC HYPOXIA (CH)

Chronic hypoxia (CH) has become entrenched in high-altitude areas. It has been associated with the development of PH especially in individuals complaining from chronic lung diseases [32]. CH exposure is commonly used model for induction of PH due to its highly reproducibility, however the main limitation is that it is best suited to the evaluation of mild forms of PH only [33, 34].

The animals are housed in ventilated chambers (Biospherix, Ltd., Parish, USA). with a corresponding technical facility to which breathing gas is supplied, the composition of which corresponds to the room air, but with a reduced oxygen concentration (HOX; 10% O<sub>2</sub>). The hypoxic exposure should last from 21- 28 days [35]. Conditions were regulated by supplementing oxygen or nitrogen via an automated O<sub>2</sub>-controller, a soda lime container that removed CO<sub>2</sub> and a cooling system to drain off humidity from the system. During this period, the PH develops clearly and significantly. Elevated RVSP, RV hypertrophy, and ventricular remodeling have been developed in rats through 3–4 weeks of exposure to hypoxia [36]. Besides, small vessel muscularization and larger pulmonary arteries stiffening have been developed leading to PH [36]. Although rats tolerate CH well, they differently respond to hypoxia according to strain variations. The development of PH in Fawn-hooded rats and Sprague–Dawley (SD) rats is severe and show more PVR and higher RV mass than that developed in Wistar rats, while the Fischer strain is resistant to hypoxia-induced PH to a great extent [37].

#### 4.3-SUGEN + HYPOXIA (SuHx)

Administration of Sugden, VEGF receptor-2 antagonist, in combination with chronic hypoxia exposure could develop the severe form of PH with the pathological features of human PAH [38]. Sugden given to chronically hypoxic rats causes severe and progressive pulmonary vascular remodeling that is irreversible, even with recovery to normoxia, whereas VEGF inhibition alone causes only mild PH in normoxic rats [38]. One subcutaneous Sugden injection (20 mg/kg) is given as part of a common protocol for the Sugden + hypoxia (SuHx) mouse, which is then exposed to hypoxia for three to four weeks (10 percent oxygen) [39].

Therefore, the loss of responsiveness to vasodilators like iloprost and NO in SuHx rats and PAH patients could be explained due to the incidence of intimal thickening in both forms [40]. Induction of SuHx in rats leads in producing vascular lesions similar to PAH's plexiform lesions, followed by RV maladaptive remodeling after 6 weeks of treatment, concomitant with increased diastolic diameter of the RV, RVH and a decrease in cardiac output (CO) and the tricuspid annular plane systolic excursion (TAPSE) compared to control rats [39, 40].

#### 4.4-CIGARETTE SMOKE EXPOSURE

Pulmonary hypertension related to chronic lung diseases (group III PH), including COPD, is mostly developed via the chronic cigarette smoke exposure (daily cigarette smoke exposure for 6 months) [41, 42], which considered gold standard model for COPD induction, because it shares most of the pathogenic pathways as human, including inflammation, emphysema, airway remodeling and subsequent PH [43].

Cigarette smoke exposure to animals has been increased RVSP and induced medial hypertrophy, mostly after 3 months. Three months of smoke exposure is also sufficient to induce elevated RVSP, medial hypertrophy [44]. However, 6 months of exposure might lead to vascular remodeling [45].

It was demonstrated that, smoke exposure in comparison with chronic hypoxia exposure, did not result in hypoxemia or alveolar hypoxia, moreover, it did not induce apoptosis like that happened in CH- induced PH [43]. This discrepancy may be the result of profound changes in how genes are regulated in the tiny pulmonary vessels, while exposure to smoke increases the expression of proapoptotic genes, exposure to CH increases the expression of genes associated mostly with oxidative stress [46].

#### 4.5-PULMONARY ARTERY BANDING (PAB)

The PAB paradigm is appropriate for testing prospective cardioprotective compounds as a supplement to standard therapy because it devoid the toxicity of MCT, hypoxia and VEGF suppression on the cardiac function [47]. PAB was first utilized surgical palliation of newborns with heart abnormalities characterized by pulmonary over-circulation and severe left-to-right vascular shunting [48]. In PAB, the pulmonary trunk is mechanically constricted using a suture tie or metal clip (**Figure 3**) [49].

The abrupt elevation of afterload is considered the main disadvantage of this model because it does not identify the progressive increase in PVR found in PH, and this can be mitigated by using juvenile animals which have gradual increase in afterload [49]. As a result, PAB necessitates a high level of technical expertise, as the placement of the pulmonary trunk constriction must be meticulously reproduced to assure reproducibility [50].

However, the main advantage of this model is that the severity of the disease- induced by PAB model can be changed to any desired level by varying the width of constriction, ranging from adaptive RV hypertrophy to decompensated RV failure, in addition to the fixed elevated afterload created by PAB allows for studying RV remodeling [51].

#### 4.6 SCHISTOSOMIASIS

Schistosomiasis is a significant cause of PAH in countries where Schistosomiasis is commonly takes place [52]. When *Schistosoma* eggs obstruct the portal system and vessels entering the liver, portosystemic vascular shunts occur which, in turn, raise shear stress on the pulmonary vessels and allow egg deposition into the lung [53].

The incidence of *Schistosoma*-associated PAH (Sch-PAH) in mice is higher than in rats, the vulnerability of mice to schistosomiasis development is greater than rats [54]. This have substantially aided knowledge of the participating mechanisms, especially the involvement of systemic type 2 inflammation [55]. The common pathogenic features of the human disease have been developed after 12 weeks, and involving pronouncer PVR, increased levels of inflammatory and cytokines mediators, and formation of the plexiform-like lesions [56].

#### 4.7-BLEOMYCIN

The inflammatory and profibrotic effects of bleomycin, an anticancer agent, have restricted its use as model for induction of PH [57]. Intratracheal bleomycin instillation in mice is frequently utilized as a model for induction of pulmonary fibrosis [58]. Besides, it is also a leading cause for secondary development of PH. The bleomycin- induced PH model shares similar levels of increased RVS and RVH to that developed during exposure to chronic hypoxia [59]. However, the usefulness of this model may be limited due to the early death or gradual remission of PH depending on the amount of bleomycin employed [59].

#### 4.8-GENETIC MODELS

Pulmonary hypertension can be developed in case of down-regulation or overexpression of certain genes which have been studied via genetic manipulation in animals [20]. In spite of the usefulness of this approach in targeting certain molecular and cellular pathways, the single gene modification might not achieve the complex pathology of the disease. That is why, stimulation by other factors like hypoxia might be needed to allow a robust phenotype [60, 61].

##### 4.8.1-BONE MORPHOGENETIC PROTEIN RECEPTOR TYPE II (BMPR-II)

The Bone morphogenetic protein receptor type II (BMPR-II), is a serine/threonine receptor kinase [62]. The transcription growth factor (TGF)-beta superfamily ligands are bound together via the BMPR-II. Decreased BMPR-II levels have been found in various non-genetic animal studies of PH [63].

It was found that, in endothelial cells, conditional removal of BMPR-II can result in higher RVSP and elevated number and thickness of muscularized precapillary arteries [63, 64]. Genetic deletion of BMPR-II mutant alleles developed mice with partially increased RVSP, but did not induce RV hypertrophy [62].

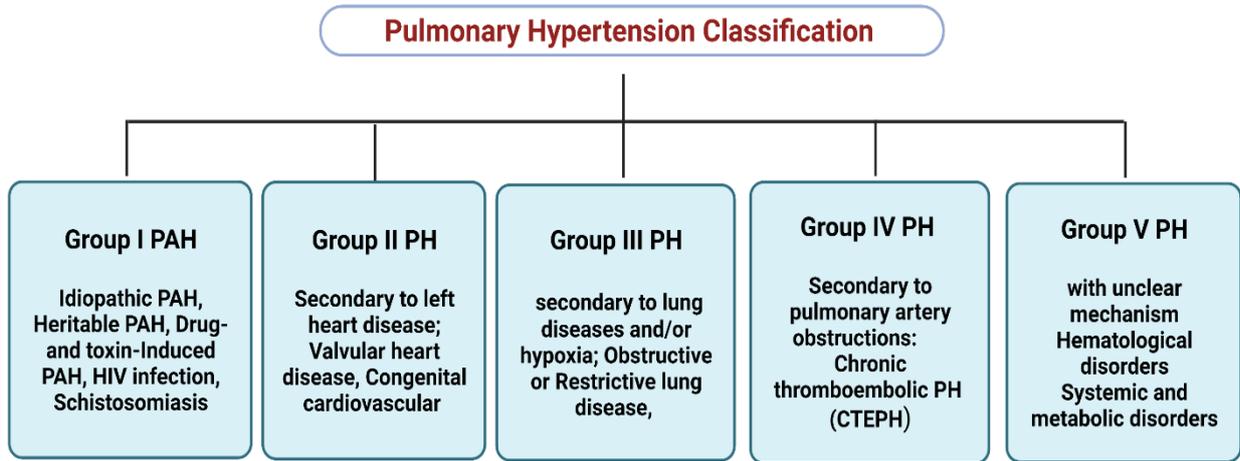


Figure 1: Clinical categorization of Pulmonary hypertension according to the underlying causes.

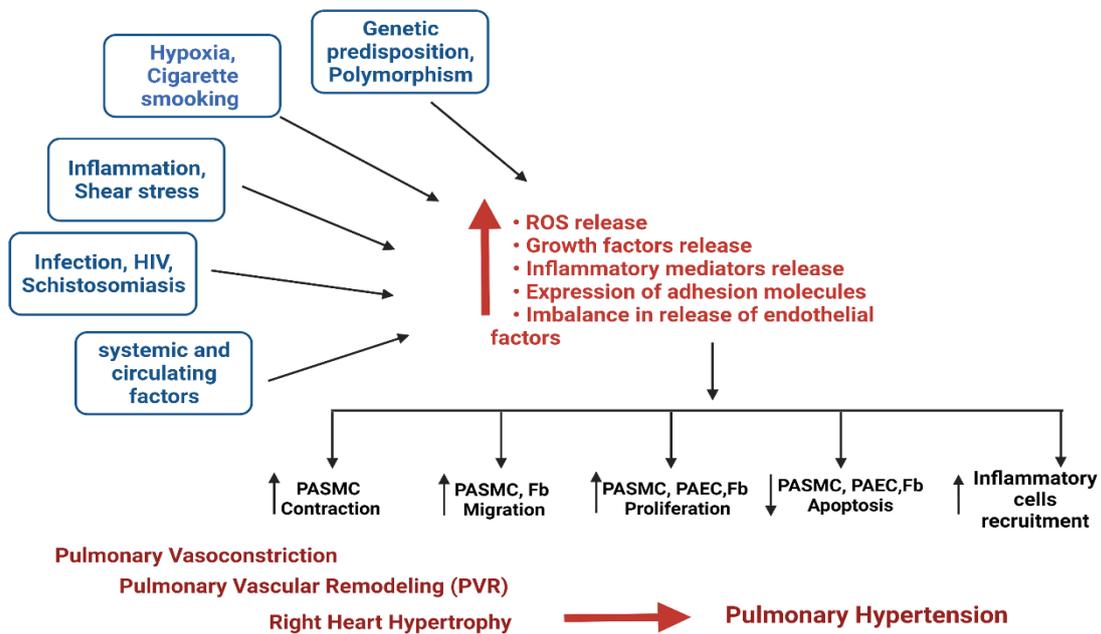


Figure 2: An overview of the pathological mechanisms underlying development of pulmonary hypertension. Fb: fibroblasts, HIV: human immunodeficiency virus, PAEC: pulmonary artery endothelial cell, PASM: pulmonary artery smooth muscle cells, ROS: reactive oxygen species

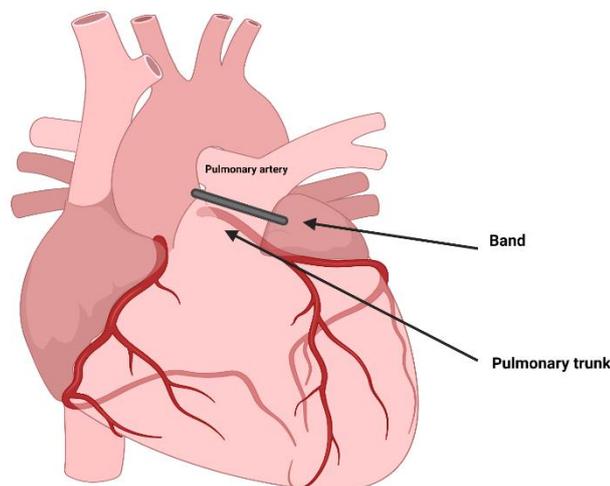


Figure 3: Illustrating diagram for pulmonary artery banding showing the position of the pulmonary trunk ligation.

#### 4.8.2-The Hypoxia-Inducible Factor/ Prolyl Hydroxylase Domain Enzymes HIF/PHD2

The hypoxia-inducible factors (HIF), master regulators of oxygen homeostasis, are the main determinants of the adaptive mechanisms of vascular cells to chronic hypoxia [65]. HIF heterodimers comprise of oxygen-dependent  $\alpha$  subunit and  $\beta$  subunit which is oxygen-independent subunit [66]. HIF stabilization is regulated by the availability of oxygen through prolyl hydroxylases (PHDs) that utilize oxygen for degradation of HIF $\alpha$  [67].

PHDs use molecular oxygen ( $O_2$ ) as a co-substrate with  $Fe^{2+}$  cation and 2-oxoglutarate to hydroxylate HIF $\alpha$ , which is then ubiquitinated via the ligase complex enzyme von Hippel–Lindau (VHL), a well-known tumor suppressor protein [68]. Thus, the PHDs, along with HIF, work as an efficient cellular oxygen sensor that is switched off in hypoxic states and dictates HIF $\alpha$  stabilization and its dimerization with HIF $\beta$ , or its proteasomal degradation under normoxia [69].

Evidence from previous study of HIF1 $\alpha$  and HIF2 $\alpha$  knockout mice strengthened the importance of the HIF system in developing PH. Heterozygous mice with partial loss of HIF1 $\alpha$  or HIF2 $\alpha$  showed normal embryonic development, and upon exposure to chronic hypoxia (10%  $O_2$ ), these animals exhibited little increase in PAP and right ventricular hypertrophy relative to respective littermates, animals with inherited deficiency of HIF1 $\alpha$  and HIF1 $\beta$  did not survive because of heart and vascular defects, indicating the importance of the HIF system in cardiovascular development [70].

However, animals with functional mutations in HIF 2 $\alpha$ , showed increased RVSP, RV wall thickness, as well as increased vascular remodeling which are characteristics of PH. Thus, this remarkable scenario may be the greatest portrayal of clinical PAH in a mouse to date [71]. Despite the fact that complete PHD2 deficiency is fatal during development. Reduced expression of PHD2 in endothelial cells of pulmonary arteries is seen in patients with PAH [67].

#### 5-Conclusion

There are various models for induction of PH, each with a certain pattern for adaptation to the highly increased pulmonary vascular resistance and persistent afterload. However, the whole range of pathological alterations seen in the human lung acquired from PH patients is not replicated by any animal model. Therefore, interested investigators could utilize more than one model to investigate the subsequent cardiac insults such as inflammation, neurohormonal signaling, metabolic remodeling, fibrosis and oxidative stress.

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