Oxidative Stress in Pulmonary Artery Hypertension

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Vinu Wilson and Subir Kumar Maulik

Abstract

Pulmonary artery hypertension (PAH) is a progressive disorder characterized by pulmonary vascular remodeling ultimately leading to right ventricular failure and death. The last few decades have seen considerable progress in PAH therapy based on drugs targeting three major mechanistic pathways, viz., prostacyclin, endothelin and nitric oxide pathways. A growing body of research has documented that "oxidative stress" is intricately associated with development of PAH. Experimental studies have shown that markers of oxidative tissue damage are present in different genetic and chemical models of PAH. Animal studies have also shown the preventive and therapeutic potential of endogenous antioxidants and/or drugs with antioxidant activity in experimental PAH. Though the evidence implicating oxidative stress in PAH has also been generated in human PAH studies, the clinical trials of antioxidants have not yet yielded encouraging results. Further studies are warranted to unravel the reason(s) underlying this paradox in order to develop potential curative drugs for this morbid disorder.

Keywords

Pulmonary hypertension · Oxidative stress · Reactive oxygen species · Antioxidants

V. Wilson
Department of Pharmacology, Govt. T. D. Medical College, Alappuzha, Alappuzha, Kerala, India

S. K. Maulik (✉)
Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, India

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16.1 Introduction

Pulmonary arterial hypertension (PAH) can be idiopathic or associated with several heritable as well as acquired systemic disorders. PAH forms the first category of the current WHO clinical classification of pulmonary hypertension adopted in 2013 [1]. It is characterized by a resting mean pulmonary artery pressure ≥25 mm of Hg with elevated pulmonary vascular resistance (>3 Wood units) and a normal left atrial pressure (≤15 mm of Hg) [1]. PAH is a progressive disorder leading to right ventricular hypertrophy and failure reducing the median survival in affected patients to 2.8 years without treatment [2].

Despite the advent of several therapeutic agents such as prostaglandin analogues, phosphodiesterase 5 inhibitors, and endothelin receptor antagonists in the last few decades, PAH remains incurable, steadily progressive, and eventually fatal [3]. The symptoms of PAH are nonspecific making diagnosis difficult. Further, low awareness of PAH among primary caregivers as well as socioeconomic constraints of patients lead to a very low percentage of PAH patients actually being referred to the few tertiary centers equipped to perform definitive diagnoses [4].

16.2 Pathophysiological Mechanisms in PAH

The pathophysiology of PAH has been significantly unraveled in the past several decades as involving dynamic pulmonary artery vasoconstriction, thrombosis, and remodeling of small pulmonary arteries characterized by hypertrophy of pulmonary vascular smooth muscle cells and hyperplasia of endothelial and connective tissue cells resulting in plexiform lesions [5]. These pathologic processes are targeted with empirical treatment modalities such as oxygen therapy, oral anticoagulants, diuretics, digoxin, and vasodilators especially calcium channel blockers [6]. Calcium channel blockers are recommended only in patients showing a positive acute vasoreactivity to them during a right heart catheterization study. Unfortunately, none of these therapeutic measures have shown any long-term survival benefit in the limited number of uncontrolled studies done with them [6]. Over the past two decades, significant progress in therapy has been achieved by targeting three mechanistic pathways discovered to be dysregulated in PAH, viz., the prostacyclin pathway, the endothelin pathway, and the nitric oxide pathway [6].

16.2.1 Prostacyclin Pathway

Prostacyclin (PGI_2) and thromboxane A_2 (TxA_2) are the major derivatives of arachidonic acid metabolism in vascular cells. PGI_2 is a potent vasodilator, inhibits platelet activation, and has antiproliferative properties, while TxA_2 is a potent vasoconstrictor and platelet agonist. In PAH, the imbalance between these two

molecules is found to be shifted toward TxA_2. In the urine of patients with pulmonary hypertension, the levels of 6-keto-prostacyclin $F_{2\alpha}$ (a metabolite of PGI_2) are decreased, whereas the levels of thromboxane B_2 (a metabolite of TxA_2) are increased [7]. Furthermore, the production of prostacyclin synthase is decreased in the small- and medium-sized pulmonary arteries of patients with pulmonary hypertension, particularly those with idiopathic PAH [8].

Based on these findings, intravenous epoprostenol (PGI_2 analogue) was first used in idiopathic PAH in the 1980s. Several randomized clinical trials have shown improvement in resting hemodynamics and clinical and functional status of NYHA class III and IV PAH patients given intravenous epoprostenol [6]. Epoprostenol is the only drug to have shown survival benefit in PAH patients. To obviate the need for cumbersome continuous intravenous administration of epoprostenol through central veins and associated complications, several longer acting prostacyclin analogues which could be given by intravenous (iloprost, treprostinil), subcutaneous (treprostinil), oral (beraprost, iloprost, treprostinil), or inhalational (iloprost, treprostinil) routes were developed and tested in clinical trials. An oral, non-prostanoid, selective prostacyclin receptor agonist, selexipag, was recently approved for PAH therapy [9]. Although these drugs reproduce the beneficial effects of prostacyclin, they are still far from being ideal treatments for PAH owing to their adverse effects, short half-lives necessitating frequent dosing, and high cost to the patients [6].

16.2.2 Endothelin Pathway

Endothelin-1 (ET-1), a potent vasoconstrictor chiefly produced by endothelial cells, stimulates the proliferation of pulmonary artery smooth-muscle cells. The plasma levels of ET-1 are found to be increased and inversely proportional to the magnitude of the pulmonary blood flow and cardiac output in PAH [5]. ET-1 can induce fibrosis and is a pro-inflammatory mediator by virtue of its capacity to enhance the expression of cellular adhesion molecules. The effects of ET-1 are mediated through the ET_A and ET_B endothelin receptors. Activation of ET_A receptors causes sustained vasoconstriction and proliferation of vascular smooth-muscle cells, whereas ET_B receptors mediate pulmonary endothelin clearance and induce the production of nitric oxide and PGI_2 by endothelial cells leading to vasodilatation [8]. Bosentan is an orally active dual (ET_A and ET_B) endothelin-receptor antagonist (ETRA) found to be beneficial in clinical trials of NYHA class III–IV PAH patients. Selective ET_A receptor antagonists (ambrisentan and sitaxsentan) have the theoretical advantage of sparing ET_B receptor mediated ET-1 clearance and vasodilatation and showed lesser perturbation of hepatic transaminase levels in clinical trials [6]. Macitentan, a tissue-targeting oral dual ET-1 receptor antagonist, was recently approved by the Food and Drug Administration (FDA) for PAH patients [10]. The use of ETRAs is, however, limited by their dose-limiting hepatotoxicity, teratogenic potential, and high cost [6].

16.2.3 Nitric Oxide Pathway

Nitric oxide (NO) is a potent endogenous, endothelium-derived vasodilator that directly relaxes the underlying vascular smooth muscle through stimulation of soluble guanylate cyclase (sGC) and increased production of intracellular cyclic guanosine monophosphate (cGMP). A number of experimental and clinical studies have documented that PAH is associated with a defect in NO availability and thereby decreased NO-induced vasodilatation [8]. Therapeutic trials showed that short-term NO administration improves pulmonary hemodynamics in PAH. However, long-term NO inhalation therapy is cumbersome to administer and associated with rebound deterioration in pulmonary hemodynamics on withdrawal [3].

An indirect strategy employed to increase the biological activity of endogenous NO in PAH is through inhibition of phosphodiesterase type 5 (PDE5), the predominant enzyme metabolizing cGMP in pulmonary vascular smooth muscle cells. PDE5 inhibitors (sildenafil, tadalafil) have shown improvement in pulmonary hemodynamics and functional status of patients when used as adjunctive treatments with prostacyclin analogues in New York Heart Association (NYHA) class III–IV PAH patients [6]. A direct sGC stimulator, Riociguat, which produces cGMP even in the absence of NO, is undergoing clinical trials in PAH [11]. However, all these drugs are expensive and associated with adverse effects including visual disturbances, dyspepsia, flushing, headache, and limb pain [3].

16.3 Oxidative Stress

As has already been discussed elsewhere in this book, oxidative stress is implicated in the pathophysiology of varied cardiovascular disorders. A considerable amount of literature generated over the last few decades supports its involvement in pulmonary vascular remodeling in PAH as well [12]. “Oxidative stress” is the abnormal cellular state of redox imbalance characterized by enhanced production of reactive oxygen species (ROS) and/or subdued antioxidant defenses. ROS contain at least one reactive oxygen atom and include relatively stable molecules such as NO and hydrogen peroxide (H_2O_2) as well as highly reactive ones such as superoxide ($O_2^{-\cdot}$) and hydroxyl ($OH^{\cdot}$) radicals. NO can react with superoxide to form the highly damaging peroxynitrite ($ONOO^-$) anion. While a low level of ROS is involved in cellular signaling, their excess production is shown to not only damage cellular macromolecules in a runaway “chain reaction” but also stimulate pathological cellular proliferation [12, 13]. Under physiological conditions, ROS overactivity is kept in check by endogenous enzymatic (catalase, superoxide dismutase, glutathione peroxidase) and nonenzymatic (glutathione, urate) antioxidant defenses. Pathological oxidative stress occurs when ROS production overwhelms the antioxidant defenses.

16.3.1 Sources of ROS in PAH

The multiple enzymatic and metabolic processes known to generate ROS within cells of the pulmonary vascular wall are similar to those found elsewhere in the body and are most abundant in the mitochondrion [14]. They include the nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Nox) [15], the mitochondrial electron transport chain complexes, xanthine oxidase (XO) [16], and uncoupled nitric oxide synthase (NOS) [17]. It is widely accepted that NADPH oxidases are not only the principal generator of ROS in the vasculature [18], but their activities regulate the activities of other ROS-generating oxidases such as XO [19]. Among the members of Nox enzyme family, Nox4 was selectively increased in the pulmonary vasculature and lungs of hypoxia-exposed mice and in pulmonary vascular tissue from patients with pulmonary arterial hypertension [20]. Hypoxia also upregulated Nox4 in pulmonary artery adventitial fibroblasts in vitro and in adventitial fibroblasts from patients with idiopathic pulmonary arterial hypertension [21]. Recently, Nox1, Nox2 (gp91phox), and Nox4 expression was found to be upregulated in monocrotaline (MCT)-induced model of PAH in rats which was shown to be attenuated by treatment with resveratrol [22].

16.3.2 Oxidative Stress in PAH

16.3.2.1 Experimental Studies

16.3.2.1.1 *Elevated ROS and/or Suppressed Antioxidant Defenses in Experimental PAH*

Monocrotaline (MCT)-induced PAH is one of the most commonly employed experimental models of PAH in rats. Oxidative stress has been documented in MCT-induced model in both lungs and the failing right ventricle. Elevated levels of lung malondialdehyde and inducible NOS (iNOS) expression and reduced levels of catalase, glutathione, and superoxide dismutase have been documented in MCT-treated rats [23]. In the right ventricle of MCT-treated rats, an initial rise and later decline in antioxidant enzyme (catalase, superoxide dismutase, and glutathione peroxidase) activity and increased lipid peroxidation have been shown [24].

In the mouse model of chronic hypoxia-induced pulmonary hypertension (CH-PH), intrapulmonary artery superoxide levels have been shown to be elevated [25]. Initially, it was thought that hypoxia would attenuate the generation of ROS due to the lack of molecular oxygen to generate superoxide radical. However, it was later recognized that hypoxia enhanced ROS generation in relative rather than absolute amounts [26].

The recently introduced caveolin-1 knockdown model of PAH also shows elevated ROS levels primarily derived from an uncoupled endothelial NOS (eNOS) [27]. Caveolin-1, a protein expressed in vascular smooth muscle caveolae, acts as a scaffold maintaining eNOS in an inactive form. Knockdown of caveolin-1 leads to widespread eNOS uncoupling and excess NO generation and resultant peroxynitrite

anion formation. Experimental studies have shown that eNOS uncoupling also contributes to the persistent pulmonary hypertension of newborn [28].

16.3.2.1.2 *Genetic Loss/Gain of Function Studies*

It has been shown that Nox2 knockout mice fail to develop CH-PH which suggests a critical role for superoxide generated by Nox2 containing NADPH oxidases in this model [29]. Caveolin null mice have been shown to develop PAH due to elevated NO-mediated ROS production mediated by uncoupling of eNOS besides bone morphogenetic protein (BMP) receptor activation [30]. This observation was further strengthened by study which showed that rats with double knockout of caveolin and eNOS genes do not develop PAH due to lack of formation of peroxynitrite anion [27].

Intratracheal delivery of adenovirus transfected with gene for extracellular superoxide dismutase (EC-SOD) was shown to reverse pathological remodeling of pulmonary vascular cells as well as the right ventricle in MCT-treated rats [31]. Recombinant human SOD was shown to restore eNOS function, reduce oxidative stress, and reduce pulmonary vascular resistance while breathing 100% oxygen in a lamb model of persistent pulmonary hypertension of the newborn [32].

16.3.2.1.3 *Drug/Antioxidant Intervention Studies*

Several interventional studies employing drugs or herbal products with antioxidant properties have shown to attenuate the development of MCT-induced PAH and right ventricular hypertrophy. For instance, intratracheal delivery of adenovirus containing the gene for human extracellular SOD ameliorated development of MCT-PAH [31]. More recently, it was reported that the antioxidant resveratrol decreased pulmonary artery smooth muscle cell proliferation, NADPH oxidase-induced oxidative stress and prevented the development of MCT-PAH [22].

Our group has shown the preventive potential of the peroxisome proliferator-activated receptor α (PPARα) agonist, fenofibrate, and two herbal drugs, viz., *Ocimum sanctum* (Linn.) and *Terminalia arjuna* (Roxb.), against development of MCT-induced PAH in rats [33–35]. The antioxidant effect of these drugs is thought to be involved in their beneficial effect because all of them attenuated markers of oxidative stress and/or enhanced antioxidant defenses.

The pathological changes in experimental PAH associated with exposure to chronic hypoxia are abolished by administration of the antioxidant, N-acetylcysteine, or the XO inhibitor, allopurinol [36]. Excess iron has been implicated in accelerating the conversion of hydrogen peroxide to highly reactive superoxide and hydroxyl radicals by Fenton chemistry. Iron chelation therapy with deferoxamine has been shown to reverse chronic hypoxia-induced PAH in rats [37].

16.3.2.2 Clinical Studies

16.3.2.2.1 *Elevated ROS and/or Reduced Antioxidant Levels*

A large body of evidence attests to the involvement of oxidative stress in the lungs of patients with PAH. Oxidative stress has been shown to be associated with elevated pulmonary artery systolic pressure and with survival in PAH patients [38, 39].

Recently, it was shown that patients with idiopathic PAH have elevated XO activity compared to control patients and that XO-mediated oxidative stress could be reversed by treatment with XO inhibitors [40]. Lung biopsy samples of patients with idiopathic PAH have shown depletion of SOD and catalase and elevation of 3-nitrotyrosine, a widely used biomarker of oxidative protein damage caused by reaction of peroxynitrite with tyrosine residues [41]. 8-Hydroxyguanosine staining is present within the plexiform lesions from patients with PAH and is absent in the pulmonary vascular endothelium of control patients [42]. 8-Hydroxyguanosine is a biomarker of oxidative nuclear damage caused by reaction of superoxide with guanine. In the lungs of the same PAH patients, the amount and activity of SOD were lower, indicating decreased capacity to scavenge superoxide [42]. Genetic polymorphisms of antioxidant enzymes such as catalase and superoxide dismutase have been implicated in some cases of persistent primary hypertension of the newborn [43]. The valvular fibrosis caused by anti-obesity drugs such as fenfluramine and sibutramine has been shown to be due to excess serotonin-mediated monoamine oxidase-dependent superoxide generation [44]. The evidence from these studies suggests that the lungs of patients with PAH are under chronic oxidative stress.

16.3.2.2.2 *Effects of Drug/Antioxidant therapy*

The clinical trials of the currently approved drugs in PAH have shown beneficial effects in PAH patients by evaluating hemodynamic and functional endpoints [6]. However, studies exploring the effect of such drugs on markers of oxidative stress in PAH patients have been few and far between [12]. For instance, sildenafil has been shown to reduce serum 4-hydroxynonenal levels and improve heart rate variability in PAH patients [45]. Vardenafil administration in treatment-naïve PAH patients has been shown to reduce 8-iso-prostaglandin-F2α and 3-nitrotyrosine blood levels while significantly increasing NO levels [46]. Another study showed that the beneficial hemodynamic response to inhaled iloprost was attenuated in association with endothelial dysfunction and oxidative stress in PAH patients [47].

On the other hand, studies exploring the utility of antioxidants or of drugs with antioxidant properties in PAH patients have yielded disappointing results [6]. A variety of antioxidants showing beneficial effect in animal models of PAH failed to demonstrate similar effect in clinical studies. For instance, supplementation with coenzyme Q, a mitochondrial constituent, improved red blood cell redox status in PAH patients but not 6-min walk distance or BNP levels [48]. Similarly, in spite of promising experimental studies, neither atorvastatin nor simvastatin improved functional status of PAH patients in terms of the distance covered in the 6-min walking test [49, 50]. This was further endorsed by a recent meta-analysis of trials of statins in PAH patients [51].

16.4 Quasi-Cancerous Phenotype

A growing body of research has shown that PAH develops a quasi-cancerous phenotype over time characterized by pulmonary artery endothelial cell precursors and smooth muscle cells developing several hallmarks of cancerous cells [52]. These characteristics include self-sufficiency in several growth factors, resistance to apoptosis, and a metabolic switch to glycolysis instead of oxidative phosphorylation known as Warburg effect [52, 53]. Activation of several intracellular signaling pathways such as Rho kinase (ROCK) and mitogen-activated protein kinase (MAPK) have been implicated in conferring these properties [52]. Drugs targeting various mediators in these pathways such as imatinib (tyrosine kinase inhibitor), sorafenib (multikinase inhibitor), fasudil (Rho-kinase inhibitor), and dichloroacetate (mitochondrial pyruvate dehydrogenase inhibitor which inhibits glycolysis) have been tested in animal as well as clinical studies but have shown only modest benefit against risk of significant adverse effects [6].

16.5 Summary and Conclusion

The last few decades have seen considerable progress in the understanding of the molecular pathophysiology and drug therapy of PAH. Oxidative stress has been shown to be intricately involved in the underlying pathobiology of PAH. However, the exact pathways and mechanisms leading to dysregulated effects of oxidative stress signaling remain to be unraveled. In spite of the promising results shown by antioxidants and drugs with antioxidant properties in experimental studies, their clinical trials in PAH patients have yielded indeterminate results at best. What this points to is our incomplete knowledge of ROS kinetics, their subcellular compartmentalization, or the inability of drugs to reach appropriate subcellular targets. Whatever may be the reason(s), the answers to these questions will determine the fate of millions of patients suffering from this currently incurable disorder.

References

1. Simonneau G, Gatzoulis MA, Adatia I et al (2013) Updated clinical classification of pulmonary hypertension. J Am Coll Cardiol 62:D34–D41
2. D'Alonzo GE, Barst RJ, Ayres SM et al (1991) Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. Ann Intern Med 115:343–349
3. Humbert M, Sitbon O, Simonneau G (2004) Treatment of pulmonary arterial hypertension. N Engl J Med 351:1425–1436
4. Menon S (2009) Pulmonary hypertension in the south east Asia region: an analysis of indexed publication profile. PVRI Rev 1:167
5. Humbert M, Morrell NW, Archer SL et al (2004) Cellular and molecular pathobiology of pulmonary arterial hypertension. J Am Coll Cardiol 43:13S–24S
6. Badlam JB, Bull TM (2017) Steps forward in the treatment of pulmonary arterial hypertension: latest developments and clinical opportunities. Ther Adv Chronic Dis 8:47–64
7. Christman BW, McPherson CD, Newman JH et al (1992) An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. N Engl J Med 327:70–75

8. Farber HW, Loscalzo J (2004) Pulmonary arterial hypertension. N Engl J Med 351:1655–1665
9. Bruderer S, Hurst N, Remenova T, Dingemanse J (2017) Clinical pharmacology, efficacy, and safety of selexipag for the treatment of pulmonary arterial hypertension. Expert Opin Drug Saf 16:743–751
10. Sidharta PN, Treiber A, Dingemanse J (2015) Clinical pharmacokinetics and pharmacodynamics of the endothelin receptor antagonist macitentan. Clin Pharmacokinet 54:457–471
11. Ghofrani H-A, Galiè N, Grimminger F et al (2013) Riociguat for the treatment of pulmonary arterial hypertension. N Engl J Med 369:330–340
12. Wong C-M, Bansal G, Pavlickova L et al (2013) Reactive oxygen species and antioxidants in pulmonary hypertension. Antioxid Redox Signal 18:1789–1796
13. Pagel J-I, Deindl E (2012) Disease progression mediated by egr-1 associated signaling in response to oxidative stress. Int J Mol Sci 13:13104–13117
14. Wolin MS, Ahmad M, Gupte SA (2005) The sources of oxidative stress in the vessel wall. Kidney Int 67:1659–1661
15. Mohazzab KM, Wolin MS (1994) Sites of superoxide anion production detected by lucigenin in calf pulmonary artery smooth muscle. Am J Physiol 267:L815–L822
16. Terada LS, Guidot DM, Leff JA et al (1992) Hypoxia injures endothelial cells by increasing endogenous xanthine oxidase activity. Proc Natl Acad Sci USA 89:3362–3366
17. Grobe AC, Wells SM, Benavidez E et al (2006) Increased oxidative stress in lambs with increased pulmonary blood flow and pulmonary hypertension: role of NADPH oxidase and endothelial NO synthase. Am J Physiol Lung Cell Mol Physiol 290:L1069–L1077
18. Cai H, Griendling KK, Harrison DG (2003) The vascular NAD(P)H oxidases as therapeutic targets in cardiovascular diseases. Trends Pharmacol Sci 24:471–478
19. McNally JS, Davis ME, Giddens DP et al (2003) Role of xanthine oxidoreductase and NAD(P) H oxidase in endothelial superoxide production in response to oscillatory shear stress. Am J Physiol Heart Circ Physiol 285:H2290–H2297
20. Mittal M, Roth M, König P et al (2007) Hypoxia-dependent regulation of nonphagocytic NADPH oxidase subunit NOX4 in the pulmonary vasculature. Circ Res 101:258–267
21. Li S, Tabar SS, Malec V et al (2008) NOX4 regulates ROS levels under normoxic and hypoxic conditions, triggers proliferation, and inhibits apoptosis in pulmonary artery adventitial fibroblasts. Antioxid Redox Signal 10:1687–1698
22. Csiszar A, Labinskyy N, Olson S et al (2009) Resveratrol prevents monocrotaline-induced pulmonary hypertension in rats. Hypertension 54:668–675
23. Wang X, Yang Y, Yang D et al (2016) Tetrandrine prevents monocrotaline-induced pulmonary arterial hypertension in rats through regulation of the protein expression of inducible nitric oxide synthase and cyclic guanosine monophosphate-dependent protein kinase type 1. J Vasc Surg 64:1468–1477
24. Farahmand F, Hill MF, Singal PK (2004) Antioxidant and oxidative stress changes in experimental cor pulmonale. Mol Cell Biochem 260:21–29
25. Nisbet RE, Graves AS, Kleinhenz DJ et al (2009) The role of NADPH oxidase in chronic intermittent hypoxia-induced pulmonary hypertension in mice. Am J Respir Cell Mol Biol 40:601–609
26. Araneda OF, Tuesta M (2012) Lung oxidative damage by hypoxia. Oxid Med Cell Longev 2012:856918
27. Zhao Y-Y, Zhao YD, Mirza MK et al (2009) Persistent eNOS activation secondary to caveolin-1 deficiency induces pulmonary hypertension in mice and humans through PKG nitration. J Clin Invest 119:2009–2018
28. Konduri GG, Bakhutashvili I, Eis A, Pritchard K (2007) Oxidant stress from uncoupled nitric oxide synthase impairs vasodilation in fetal lambs with persistent pulmonary hypertension. Am J Physiol Heart Circ Physiol 292:H1812–H1820
29. Liu JQ, Zelko IN, Erbynn EM et al (2006) Hypoxic pulmonary hypertension: role of superoxide and NADPH oxidase (gp91phox). Am J Physiol Lung Cell Mol Physiol 290:L2–L10
30. Maniatis NA, Shinin V, Schraufnagel DE et al (2008) Increased pulmonary vascular resistance and defective pulmonary artery filling in caveolin-1-/- mice. Am J Physiol Lung Cell Mol Physiol 294:L865–L873

31. Kamezaki F, Tasaki H, Yamashita K et al (2008) Gene transfer of extracellular superoxide dismutase ameliorates pulmonary hypertension in rats. Am J Respir Crit Care Med 177:219–226
32. Farrow KN, Lakshminrusimha S, Reda WJ et al (2008) Superoxide dismutase restores eNOS expression and function in resistance pulmonary arteries from neonatal lambs with persistent pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 295:L979–L987
33. Galhotra P, Prabhakar P, Meghwani H et al (2018) Beneficial effects of fenofibrate in pulmonary hypertension in rats. Mol Cell Biochem. https://doi.org/10.1007/s11010-018-3355-3
34. Meghwani H, Prabhakar P, Mohammed SA et al (2018) Beneficial effect of ocimum sanctum (Linn) against monocrotaline-induced pulmonary hypertension in rats. Med Basel Switz 5. https://doi.org/10.3390/medicines5020034
35. Meghwani H, Prabhakar P, Mohammed SA et al (2017) Beneficial effects of aqueous extract of stem bark of Terminalia arjuna (Roxb.), An ayurvedic drug in experimental pulmonary hypertension. J Ethnopharmacol 197:184–194
36. Jankov RP, Kantores C, Pan J, Belik J (2008) Contribution of xanthine oxidase-derived superoxide to chronic hypoxic pulmonary hypertension in neonatal rats. Am J Physiol Lung Cell Mol Physiol 294:L233–L245
37. Wong C-M, Preston IR, Hill NS, Suzuki YJ (2012) Iron chelation inhibits the development of pulmonary vascular remodeling. Free Radic Biol Med 53:1738–1747
38. Ghasemzadeh N, Patel RS, Eapen DJ et al (2014) Oxidative stress is associated with increased pulmonary artery systolic pressure in humans. Hypertens Dallas Tex 63:1270–1275
39. Cracowski J-L, Degano B, Chabot F et al (2012) Independent association of urinary F2-isoprostanes with survival in pulmonary arterial hypertension. Chest 142:869–876
40. Spiekermann S, Schenk K, Hoeper MM (2009) Increased xanthine oxidase activity in idiopathic pulmonary arterial hypertension. Eur Respir J 34:276
41. Masri FA, Comhair SAA, Dostanic-Larson I et al (2008) Deficiency of lung antioxidants in idiopathic pulmonary arterial hypertension. Clin Transl Sci 1:99–106
42. Bowers R, Cool C, Murphy RC et al (2004) Oxidative stress in severe pulmonary hypertension. Am J Respir Crit Care Med 169:764–769
43. Dani C, Poggi C (2014) The role of genetic polymorphisms in antioxidant enzymes and potential antioxidant therapies in neonatal lung disease. Antioxid Redox Signal 21:1863–1880
44. Peña-Silva RA, Miller JD, Chu Y, Heistad DD (2009) Serotonin produces monoamine oxidase-dependent oxidative stress in human heart valves. Am J Physiol Heart Circ Physiol 297:H1354–H1360
45. Semen K, Yelisyeyeva O, Jarocka-Karpowicz I et al (2015) Sildenafil reduces signs of oxidative stress in pulmonary arterial hypertension: Evaluation by fatty acid composition, level of hydroxynonenal and heart rate variability. Redox Biol 7:48–57
46. Fan Y-F, Zhang R, Jiang X et al (2013) The phosphodiesterase-5 inhibitor vardenafil reduces oxidative stress while reversing pulmonary arterial hypertension. Cardiovasc Res 99:395–403
47. Gabrielli LA, Castro PF, Godoy I et al (2011) Systemic oxidative stress and endothelial dysfunction is associated with an attenuated acute vascular response to inhaled prostanoid in pulmonary artery hypertension patients. J Card Fail 17:1012–1017
48. Sharp J, Farha S, Park MM et al (2014) Coenzyme Q supplementation in pulmonary arterial hypertension. Redox Biol 2:884–891
49. Zeng W-J, Xiong C-M, Zhao L et al (2012) Atorvastatin in Pulmonary Arterial Hypertension (APATH) study. Eur Respir J 40:67–74
50. Kawut SM, Bagiella E, Lederer DJ et al (2011) Randomized clinical trial of aspirin and simvastatin for pulmonary arterial hypertension. Circulation 123:2985–2993
51. Rysz-Górzynska M, Gluba-Brzózka A, Sahebkar A et al (2016) Efficacy of statin therapy in pulmonary arterial hypertension: a systematic review and meta-analysis. Sci Rep 6:30060
52. Rai PR, Cool CD, King JAC et al (2008) The cancer paradigm of severe pulmonary arterial hypertension. Am J Respir Crit Care Med 178:558–564
53. Xu W, Koeck T, Lara AR et al (2007) Alterations of cellular bioenergetics in pulmonary artery endothelial cells. Proc Natl Acad Sci USA 104:1342–1347