Animal Models of Human Severe PAH

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Based on differences in clinical presentation, diagnostic findings, and response to treatment, human pulmonary hypertension (PH) has been subdivided into 5 categories. These include[1]:

- Pulmonary arterial hypertension (PAH)
- PH with left-sided heart disease
- PH associated with respiratory disorders and/or hypoxemia
- PH caused by chronic thrombotic and/or embolic disease
- PH caused by miscellaneous other disorders affecting the pulmonary vasculature.

The PAH category encompasses the idiopathic and familial forms of PH as well as those that occurr secondarily to several other diseases or conditions, including connective tissue disease, congenital systemic-to-pulmonary shunts, HIV infection, portal hypertension, hemoglobinopathies, and ingestion of drugs and toxins. This group also includes pulmonary veno-occlusive disease, pulmonary capillary hemangiomatosis, and persistent pulmonary hypertension of the newborn.

Characteristics of PAH

While any form of PH can contribute to patient debilitation and mortality, PAH is a particularly severe and progressive form that frequently leads to right heart failure and premature death.[2-4] The pathogenesis of the increased precapillary pulmonary vascular resistance (PVR) is generally ascribed to combined effects of vasoconstriction, arterial wall remodeling, and in situ thrombosis.[5-8] What appears to distinguish PAH from other forms of PH is the severity of the arteriopathy. Whereas the early phase of PAH has been described as histologically nonspecific, showing medial hypertrophy and mild intimal thickening of muscular pulmonary arteries, the later more progressive stage involves formation of complex cellular and fibrotic neointimal and plexiform lesions that obstruct and obliterate medium and small pulmonary arteries and arterioles.[9-13] This cellular and fibrotic luminal obliteration presumably accounts for the poor responsiveness of most adult PAH patients to acute administration of conventional pulmonary vasodilators, and the irreversibility of the hypertension following corrective surgery in some PAH patients with congenital heart diseases.[12-16]

Current Treatment of PAH

The goals for the treatment of PAH are to reduce PVR and pulmonary arterial pressure, and thereby to reverse the pressure overload of the right ventricle to prevent failure and death.[2,3,17] In addition to adjunctive therapy with anticoagulants, diuretics, inotropes, and supplemental oxygen, patients with PAH who are not candidates for calcium channel blockers are currently treated with prostacyclin analogs, endothelin-1 receptor blockers, and/or phosphodiesterase type 5 inhibitors. This treatment improves symptoms and quality of life, but a recent meta-analysis of several clinical trials of these agents in patients with severe PAH showed only moderate reductions in PVR and pulmonary arterial pressure.[18,19] There was no statistically significant decrease in mortality. These disappointing results do not duplicate those found in animal studies, which show that these classes of drugs, and numerous others, largely prevent and in some cases reverse chronic hypoxia- and monocrotaline-induced PH in rats.[20-23]

Classical Animal Models of PH

The limitations of using chronically hypoxic and monocrotaline-injected rats as models of human severe PAH have been previously discussed.[22,24-28] The PH in these models is due largely to sustained vasoconstriction.[29-31] Notably, there is no formation of obstructive intimal lesions in the peripheral pulmonary arteries. Whether there is loss, rarefaction, of pulmonary microvessels, or simply impaired filling of these vessels with indicator due to spasm of upstream hypertensive arteries, is controversial.[31-33] In any case, it is apparent that preventing or reversing the sustained constriction and increased muscularization and adventitial thickening of pulmonary arteries in these 2 rodent models is not equivalent to "dissolving" the obliterative neointimal and other complex vascular lesions, and/or reversing unconventional mechanisms of vasoconstriction, that seemingly account for the high pulmonary vascular resistance (PVR) in human severe PAH. The same limitations apply to chronically hypoxic and monocrotaline pyrrole-injected mice, which typically show even less pulmonary artery

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Table. Animal Models That Develop Obstructive, Neointimal Lesion-Associated Pulmonary Hypertension

Animal	Model	Cells of Obstructive Lesions
Rat	Left pneumonectomy + MCT[53]	SMCs
	Left pneumonectomy + MCT + MCT in younger animals[63]	ECs in perivascular lesions
	ET_B receptor deficient +MCT[45]	ECs and SMCs
	Sugen 5416 + chronic hypoxia[57]	ECs
	Athymic and Sugen 5416[58]	ECs and B-lymphocytes with perivascular inflammatory cells
Mouse	S100A4/Mts1 over expression[43]	SMCs with perivascular inflammatory cells
	S100A4/Mts1 over expression exacerbated by infection with M1γherpesvirus 68[54]	SMCs with perivascular inflammatory cells
	Lung-specific IL-6 over expression exacerbated by chronic hypoxia[55]	ECs and T-lymphocytes with perivascular inflammatory cells
	Repeated inhalation of *Stachybotrys chartarum* spores[51]	?
Beagle	Dehydromonocrotaline[64]	?
Macaque	SHIV-nef infection[65]	ECs and SMCs with lymphatic infiltration
Calf	Aorta-pulmonary artery anastamosis[66]	?
Piglet	Aorta-pulmonary artery anastamosis[67]	?

remodeling than rats.[34-35] Although chronically hypoxic bovine calves and fawn-hooded rats develop severe PH with marked medial and adventitial thickening of pulmonary arteries, there are no reports of obliterative neointimal lesions in the resistance arteries of these models.[36-40]

Animal Models of PAH

Studies of the classic chronically hypoxic and monocrotaline-injected models of PH have produced an abundance of important information on cellular and molecular mechanisms of pulmonary vasoconstriction and medial and adventitial remodeling. However, investigators who evaluate new therapeutic strategies for severe PAH should consider using more recent animal models of obstructive, neointimal lesion-associated PH. At least 10 different rodent models of peripheral pulmonary artery neointimal lesion formation have now been described and studied (**Table**).[41-67]

These models develop PH accompanied by formation of obstructive cellular lesions in the lumen of small pulmonary arteries and arterioles, in addition to increased medial muscularization of proximal and distal pulmonary arteries. The proliferative neointimal lesions are variously reported to comprise phenotypically abnormal smooth muscle cells, endothelial cells, cells that express both endothelial and smooth muscle cell markers, and inflammatory cells. This is similar to the cellular heterogeneity reported in the lesions of human forms of PAH.[9-11,13] The lesions in some of these models are considered to resemble the plexiform lesions of human PAH.[43,54,55,57,63]

In addition to the rodent models, PAH arteriopathy has also been observed in young beagles who have been exposed to dehydromonocrotaline (an endothelial cell-toxic metabolite of monocrotaline), macaques infected with SHIV-*nef* (a chimeric viral construct containing the HIV *nef* gene in a simian immunodeficiency virus backbone), and calves and piglets with anastamosis of the left lower lobe pulmonary artery to the aorta.[64-67] Not all studies of aortopulmonary shunts in young pigs, however, have found formation of peripheral neointimal lesions.[68]

Treatment of Animal Models

With regard to using the rodent models of neointimal PAH to identify more effective therapeutic drugs, the 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitor simvastatin has been found to attenuate the development of PAH and, in a more clinically relevant experiment, to reverse the established disease and promote survival in left pneumonectomized plus monocrotaline-injected rats.[48,50] Similar results, albeit not with complete reversal of hypertension and neointimal lesions, were seen with triptolide treatment, an agent that has antitumor, antiangiogenic, and antiproliferative effects; rapamycin, an immunosuppressant and antiproliferative agent; and the naturally occurring steroid hormone dehydroepiandrosterone (DHEA).[42,44,49,61] Although DHEA treatment did not completely reverse the PAH, it was associated with 100% survival as compared to 30% in DHEA-untreated rats.

In the Sugen 5416 (vascular endothelial growth factor [VEGF] receptor blocker) -injected plus chronic hypoxia-exposed rat model, treatment with the bradykinin antagonist B9430, the caspase inhibitor Z-Asp-2,6-dichlorobenzoyloxymethylketone, or the anti-cancer drug sorafenib prevented development of the PAH.[47,56,57] With respect to reversal studies, treatment with the bradykinin receptor agonist B9972 or simvastatin arrested progression of the established PAH but did not reverse the hypertension or neointimal lesions.[59,60] Several other drugs with a variety of actions, including the anticancer drugs cyclophosphamide and paclitaxel, the angiotensin-converting enzyme inhibitor lisinopril, the angiotensin II type 1 receptor blocker irbesartan, the bradykinin antagonist B9430, the antiangiogenic agent thalidomide, the peroxisome proliferator-actived receptor-γ agonist PGJ2, and the calcium channel blocker nifedipine, failed to arrest progression of the PAH. No reversal experiment with sorafenib has been reported. Even so, clinical trials with both simvastatin and sorafenib in PAH are currently under way.

Conclusion

Although it is not clear how closely any of the neointimal animal models mimic the multifactorial pathobiology of human PAH, it is probable that they will provide insights into pathological cellular and molecular signaling pathways and potentially effective therapies that would not be revealed or rigorously tested in the classic chronically hypoxic and monocrotaline-injected models. Another point is that while prevention studies may provide useful information, the more clinically relevant experiment is to determine if the treatment reverses the neointimal arteriopathy and hypertension once they are well established. Finally, it needs to be noted that even if a novel drug or therapeutic strategy is found to effectively reverse PAH, and/or prevent right ventricular failure and death, in one or more of the animal models, that doesn't necessarily mean it will work in the human forms of PAH. The cellular and molecular pathogenesis of obstructive vascular lesions, and the mechanisms of right ventricular dysfunction, that develop over a few weeks in the animal models may not duplicate that which occurs over months or years in human PAH. Careful and rigorous clinical trials will be required to establish the safety and efficacy of any new therapy in patients.[69]

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