Rodent models of PAH: are we there yet?

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A recent meta-analysis of 16 clinical trials of prostacyclin analogs, endothelin receptor antagonists, and phosphodiesterase type 5 inhibitors in severe pulmonary arterial hypertension (PAH) from 1990 to 2005 shows that although the pharmacological agents moderately improve symptoms and hemodynamic parameters, none significantly reduces mortality (13). These disappointing results are not predicted by animal model studies, which show these classes of drugs largely prevent, and in some cases reverse, chronic hypoxia- and monocrotaline-induced pulmonary hypertension in rats (e.g., 6, 25, 29, 30).

The limitations of using chronically hypoxic or monocrotaline-injected rats as models of human PAH have been previously noted (9, 28, 39, 42). It is apparent that preventing or reversing sustained constriction and neomuscularization of pulmonary arteries in these rodent models (15, 31) is not equivalent to “dissolving” obstructive neointimal and other complex vascular lesions that seemingly account for the high pulmonary vascular resistance (PVR) in PAH (26, 37). Investigators evaluating new therapies for PAH should consider using more recent rodent models of neointimal lesion-associated pulmonary hypertension rather than the classic chronically hypoxic and monocrotaline-injected models. As an anonymous critic once quipped, “Almost everything inhibits monocrotaline-induced pulmonary hypertension.”

Six different rodent models of distal pulmonary artery neointimal lesion formation have been described and studied. These include: left pneumonectomized + monocrotaline-injected rats (7, 18–21, 23, 24, 38), VEGF receptor blocker (Sugen 5416)-injected + chronic hypoxia-exposed rats (1, 22, 32, 35, 36), Sugen 5416-injected athymic nude rats (34), chronically hypoxic athymic nude rats (33), monocrotaline-injected ETβ receptor-deficient rats (10), and S100A4/Mts1 protein overexpressing mice (8, 16). These models develop pulmonary hypertension accompanied by formation of occlusive cellular lesions in the lumen of small pulmonary arteries and arterioles, in addition to neomuscularization of pulmonary arteries. The proliferative neointimal lesions are variously reported to comprise phenotypically abnormal smooth muscle cells, endothelial cells, or cells expressing both endothelial and smooth muscle cell markers. Some of the lesions are considered to resemble the plexogenic arteriopathy of human PAH. The question of whether or not these rodent models do, in fact, develop the human PAH form of plexiform lesion brings us to the focus of this editorial.

In this issue of AJP-Lung, White and colleagues (40) have now modified the usual left pneumonectomized + monocrotaline-injected rat model by using younger rats, reasoning that the pulmonary vasculature of young rats would be more “re-active.” As expected (24), pneumonectomy alone did not cause pulmonary hypertension, and pneumonectomized + monocrotaline rats developed more severe hypertension and right ventricular hypertrophy than did monocrotaline-alone rats. It is implied these younger rats developed more severe PAH than older rats, but White et al. did not directly compare younger and older groups. Also, the effects of anesthesia, lack of measurement of cardiac output, and an unconventional index of right ventricular hypertrophy (RV+S/LV instead of RV/LV+S) prevent comparison of the hemodynamic parameters with those in previous studies of older rats.

Regardless, the intriguing finding of White et al. (40) is that in addition to the previously observed pulmonary artery medial thickening and formation of lumen-occluding neointimal lesions, these younger rats also developed perivascular, proliferative lesions described as remarkably similar to the plexiform lesions of human PAH (40). These rat plexiform-like lesions, which comprised a diffuse population of cells that expressed VEGF receptor-2 and/or smooth muscle α-actin, appeared to form outside the arterial lumen and be permeated by disorganized vascular channels lined with von Willebrand factor-positive endothelial cells. Fluorescence microangiography indicated they were connected to the pulmonary circulation. They were occasionally observed within 1 wk of monocrotaline injection, before severe hypertension had developed, and became more numerous over time. Whether or not these perivascular lesions contributed directly to the increased PVR is unknown. Acute vasodilator studies were not done in this initial study, and the relative contributions of reversible vasoconstriction and fixed structural remodeling to the increased resistance remain to be determined. Similarly, because the hypertensive lungs were not maximally vasodilated during perfusion of the vasculature with microsphere-containing agarose, future studies will be needed to assess whether the apparent vascular pruning was due to loss of vessels, in situ thrombosis, or incomplete filling due to vasospasm (14, 31).

The factors causing development of neointimal, and now plexiform-like, lesions in left pneumonectomized + monocrotaline-injected rats are uncertain. Botney et al. (4, 24) originally suggested the combined effects of increased blood flow (shear stress) to the remaining right lung and endothelial cell injury by monocrotaline were responsible for the neointimal lesions. However, a subsequent study by Nishimura et al. (18) found that similar increases in lung blood flow via an aorto-caval fistula in monocrotaline-injected rats did not mimic the effects of pneumonectomy, and, in fact, the increased flow actually attenuated the severity of hypertension and formation of neointimal lesions in pneumonectomized + monocrotaline-injected rats. White et al. (40) did not directly address how pneumonectomy augments the hypertensive effects of monocrotaline and leads to formation of neointimal and plexiform-like lesions in young rats, but they implied it might have more to do with the compensatory lung growth and effects of various...
growth factors than with increased blood flow per se. This will be an interesting lead to follow up on in future studies, particularly if the hyperplastic lung growth is more vigorous in younger rats.

One substance White et al. (40) have implicated in the pulmonary arteriopathy of the young pneumonectomized + monocrotaline-injected rats is the prothrombotic and proinflammatory mediator, tissue factor (TF). They observed that while TF-antigen staining was low in normotensive pulmonary arteries, it was increased early and intensified with severity of hypertension in both thickened arteries and plexiform-like lesions. Similarly, they observed in lung tissue from PAH patients that TF staining was increased in remodeled pulmonary arteries and was particularly intense and diffuse in plexiform lesions. They therefore suggested TF might be playing an important role in the thrombotic diathesis, endothelial dysfunction, and plexiform lesion formation in PAH. In the rats, pneumonectomy alone did not increase TF staining, but it is unclear whether or not its expression was increased with monocrotaline alone. A simple increase in TF expression is apparently not sufficient to induce formation of neointimal and plexiform lesions, because it is also increased, at least in the early stages of exposure, in the pulmonary arteries of hypoxic mice (41), which typically show minimal hypertensive vascular remodeling.

The exact roles TF may play in the pulmonary arteriopathy of either young pneumonectomized + monocrotaline-injected rats or in patients with PAH will clearly require more investigation. However, the increase in its expression prompts the consideration of microparticles (MPs). MPs are ∼0.2- to 1-μm-diameter vesicles released from cell plasma and/or endosomal membranes directly into surrounding tissue and fluid spaces (12, 27). Essentially, all cells can generate MPs, including platelets, endothelial cells, smooth muscle cells, erythrocytes, monocytes, and granulocytes. MPs convey a variety of proteins, lipids, and mRNA molecules, the nature of which depends on the cell of origin and whether they are generated during cell activation or apoptosis. The levels of circulating MPs are increased in numerous cardiovascular diseases, and it is postulated they may act as biomarkers, and perhaps even as mediators, of vascular injury (5, 12, 17). For example, circulating TF-positive MPs may be recruited to sites of vascular injury and contribute to thrombus formation (11). Thus, we wonder whether MPs bearing TF, and/or other prothrombotic and proinflammatory signals, might be contributing to the pathogenesis of PAH. Two recent preliminary reports of increased circulating MPs, including TF-positive MPs, in patients with PAH (2, 3) indicate that others are also interested in this possibility.

Although the various rat models of neointimal, and perhaps plexogenic, pulmonary arteriopathy address Heath’s (9) major criticism that the rat is a poor animal model for study of PAH because it lacks intimal proliferation, it is not yet clear how closely any of the models mimic the cellular and molecular pathobiology of human PAH. However, it stands to reason that study of these models will provide insights into pathological molecular signaling pathways and potentially efficacious therapies that would not be revealed or rigorously tested in the classic chronically hypoxic and monocrotaline-injected models. We might not be there yet, but with the work of White et al. (40) and others (8, 10, 21, 23–35), we seem to be getting closer.

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REFERENCES

Editorial Focus


