The monocrotaline model of pulmonary hypertension in perspective

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Submitted 22 June 2011; accepted in final form 27 September 2011

Gomez-Arroyo JG, Farkas L, Alhussaini AA, Farkas D, Kraskauskas D, Voelkel NF, Bogaard HJ. The monocrotaline model of pulmonary hypertension in perspective. Am J Physiol Lung Cell Mol Physiol 302: L363–L369, 2012. First published September 30, 2011; doi:10.1152/ajplung.00212.2011.—Severe forms of pulmonary arterial hypertension (PAH) are characterized by various degrees of remodeling of the pulmonary arterial vessels, which increases the pulmonary vascular resistance and right ventricular afterload, thus contributing to the development of right ventricle dysfunction and failure. Recent years have seen advances in the understanding of the pathobiology of PAH; however, many important questions remain unanswered. Elucidating the pathobiology of PAH continues to be critical to design new effective therapeutic strategies, and appropriate animal models of PAH are necessary to achieve the task. Although the monocrotaline rat model of PAH has contributed to a better understanding of vascular remodeling in pulmonary hypertension, we question the validity of this model as a preclinically relevant model of severe plexogenic PAH. Here we review pertinent publications that either have been forgotten or ignored, and we reexamine the monocrotaline model in the context of human forms of PAH.

Investigations of the pathobiology of PAH have recently shifted from the early concepts of pressure and flow, to the state-of-the-art concepts of cell-to-cell interactions (45), apoptosis resistance (64), and quasi-malignancy (59). Yet, many questions remain unanswered, and, despite the development of new pharmacotherapies, treatments for PAH are still limited and PAH-related mortality remains unacceptably high (5). Elucidating the pathobiology of PAH continues to be critical for the design of new effective therapeutic strategies, and animal models are fundamental to achieve this objective. In this context, it is reasonable to interrogate experimental models presently in use to address which aspects of each model reproduce the salient features of the human disease. Although the MCT rat model has contributed to a better understanding of vascular remodeling in PH, we question whether this model remains productive as a preclinically relevant model of severe plexogenic PAH. Based on an extensive review of the literature and our own data, we wish to put this PH model in a greater perspective.

Monocrotaline Pyrrole Toxicity and the “MCT Syndrome”

MCT is an 11-membered macrocyclic pyrrolizidine alkaloid (PA) derived from the seeds of the Crotalaria spectabilis plant (Fig. 1, A and B). The MCT alkaloid is activated to the reactive pyrrole metabolite dehydromonocrotaline (MCTP) in the liver, a reaction that is highly dependent on cytochrome P-450 (CYP3A4) (61, 84). Specific metabolic inducers of this cytochrome increase the MCTP production by the rat liver, whereas specific anti-CYP3A4 antibodies inhibit it (31, 61). When ingested, MCT induces a syndrome (Table 1) characterized, among other manifestations, by PH, pulmonary mononuclear vasculitis (acute necrotizing pulmonary arteritis in about one-third of the animals), and RV hypertrophy (32, 36).

Although it has been reported that MCT injures pulmonary endothelial cells (32, 63), the exact toxicological mechanisms by which MCT initiates lung toxicity remain unclear. Lee et al. (38) have shown that pulmonary arterial endothelial cells (PAEC) exposed to MCT develop megalocytosis characterized by an enlarged Golgi apparatus, displacement of endothelial nitric oxide synthase, and decreased cell-surface/caveolar nitric oxide. MCT-treated endothelial cells demonstrate marked disruptions of intracellular membrane trafficking that affect several cell membrane proteins (68). Huang et al. (30) have reported that MCT-induced loss of membrane proteins results in the activation of proliferative and antiapoptotic factors, and deregulation of nitric oxide signaling, leading to lung vascular changes. The initial MCT-induced endothelial cell damage has also been linked to bone morphogenetic protein receptor II (BMPR II) dysfunction and BMP signaling disruption, as well as increased expression of intracellular elements involved in the sequestration and inhibition of the BMPR II activity (60).
Nakayama et al. (46) demonstrated that, in human PAEC, the monocrotaline pyrrole significantly induced the Nrf2-mediated stress response pathway and increased caspase-3 activation. Paradoxically, although there is vast evidence to suggest that MCT elicits PAEC dysfunction on multiple levels, the MCT PAH model is characterized predominantly by pulmonary arterial medial hypertrophy (Fig. 1D) but not by endothelial cell-mediated angioobliteration. In addition to the vascular changes, monocrotaline-treated rats exhibit marked perivascular edema (F, blue line), alveolar septal thickening (G, long arrow; arrowhead marks a normal septa), and megalocytosis of type I pneumocytes (H, long arrows; arrowhead marks a normal type I pneumocyte nucleus).

Table 1. The monocrotaline syndrome

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Dose Range, mg/kg</th>
<th>Ref. No.</th>
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<tbody>
<tr>
<td>Acute lung injury</td>
<td>60–100</td>
<td>20, 33, 62, 66, 74</td>
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<tr>
<td>Interstitial pulmonary fibrosis</td>
<td>2.4–100</td>
<td>29, 43, 44</td>
</tr>
<tr>
<td>Necrotizing pulmonary arteritis</td>
<td>Not quantified</td>
<td>32, 36, 84</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>45–60</td>
<td>8, 32, 49, 56, 65, 66, 86, 87</td>
</tr>
<tr>
<td>RV hypertension</td>
<td>45–60</td>
<td>8, 10, 25, 32, 49, 56, 65, 66, 86, 87</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>50–60</td>
<td>1, 10, 25</td>
</tr>
<tr>
<td>Hepatic venooclusive disease</td>
<td>60–300</td>
<td>13–17, 19, 21, 39, 40, 54, 83, 84</td>
</tr>
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RV, right ventricle.
Hematocyte megakaryocytosis (Fig. 1), significant alveolar septal thickening (Fig. 1), and occlusion of pulmonary veins (20, 37). MCT-induced interstitial pulmonary fibrosis has also been described in mice (with variable doses and time points) (20, 29). Electron micrographs of MCT-treated animals revealed degeneration of both lung endothelial and type I epithelial cells, as well as marked interstitial hypercellularity and fibrosis (43, 44). Hayashi et al. (29) reported that a single dose of 100 mg/kg is sufficient to induce severe pulmonary fibrosis and/or interstitial pneumonia in mice, whereas other investigators have reported that pulmonary fibrosis seems to be only a late manifestation of MCT exposure (20).

The monocrotaline pyrrole has also been described as an anti-mitotic agent (16, 55). In astrocytes, the monocrotaline pyrrole can induce DNA damage by generating DHP-derived DNA adducts, which induce DNA cross links, DNA-celullar protein conjugates, and apoptosis (46, 71, 81). MCT-induced DNA damage is reflected in persistent cell cycle arrest and is responsible for the cyto- and karyomegaly (megalocytosis) described in pneumocytes, human pulmonary endothelial cells, glial cells, and hepatocytes of MCT-treated animals (38, 71, 83, 84). Particularly, type II pneumocytes seem to be highly affected by MCT-induced mitotic inhibition (85), which has been shown to induce significant portal hypertension in dogs (13, 14, 19). The monocrotaline pyrrole has also been described as an anti-mitotic agent (16, 55).

MCT-induced liver toxicity in rats appears to be triggered only by large doses of the monocrotaline pyrrole, in MCT PH studies, the potential contribution of liver disease, and perhaps portal hypertension, to the development of the pulmonary vascular disease has not been considered.

MCT-Induced Liver Toxicity: A Model for Venoocclusive Hepatic Disease

It is well known that PA can induce liver toxicity, and this has been a serious problem in third world countries (13, 14, 19). The most frequent outcome of PA toxicity, in either humans or animals, is hepatic injury (84). MCT induces damage of sinusoidal endothelial cells, central venular endothelial cells, and hepatic parenchymal cells (17, 39). These initial lesions give way to a subacute phase of fibrotic occlusion of central and sublobular veins and sinusoidal fibrosis (Fig. 2E), making MCT a suitable model for hepatic venoocclusive disease (15). In dogs, MCT induces hepatic venoocclusive disease, which is accompanied by an increase in splenic pressure (54), and a single dose of 60 mg/kg has been shown to induce significant portal hypertension in dogs (21). Whereas the MCT liver toxicity in rats appears to be triggered only by large doses of the monocrotaline pyrrole, in MCT PH studies, the potential contribution of liver disease, and perhaps portal hypertension, to the development of the pulmonary vascular disease has not been considered.

MCT-Induced Myocarditis

MCT-treated rats develop significant PH and marked RV hypertrophy (Fig. 2A, also see Refs. 8, 32, 56, 65, and 75). Tradi-
tional concepts suggest that the RV dysfunction of MCT-treated rats is a direct consequence of pressure overload. Interestingly, despite having lower pulmonary artery pressure and a similar degree of RV dysfunction compared with the SU5416/hypoxia model of PH (Fig. 2, B and C), MCT-treated rats exhibit a significantly higher mortality rate compared with the SU5416/hypoxia model (D). Rats treated with a dose of 160 mg/kg or higher develop liver alterations consistent with hepatic venoocclusive disease [E, reproduced with permission of Frank Snow (19)]. Histological analysis of MCT-treated right ventricles demonstrated a severe inflammatory infiltrate in the RV (F and G). A similar inflammatory infiltrate was present in the left ventricle of MCT-treated rats (H, arrows) and was associated with medial hypertrophy of coronary arterioles (H, arrowhead) and marked perivascular fibrosis (I). Immunohistochemistry reveals that the majority of the inflammatory infiltrates are prominently positive for the B cell marker CD20 (K), negative for CD68+/H11001 (J), and identifies few CD8+ cells (L). These results are consistent with an MCT-induced lymphocytic myocarditis. SuHx, SU5416/hypoxia-exposed rats; mPAP, mean pulmonary arterial pressure; RVID, right ventricular internal diameter.

Fig. 2. Both monocrotaline (MCT) and SU5416/hypoxia animals develop pulmonary hypertension, however, MCT-treated rats present with a lower degree of pulmonary hypertension compared with the SU5416/hypoxia model (A). Both models develop a similar degree of right ventricular (RV) dysfunction assessed by increased RV internal diameter (B) and decreased tricuspid annular planar systolic excursion (TAPSE, C), two heart rate-independent variables to evaluate RV function by echocardiogram. Although the pulmonary artery pressure is lower in MCT-treated rats, and RV dysfunction is similar in both models, MCT-treated rats exhibit a higher mortality rate compared with the SU5416/hypoxia model (D). Rats treated with a dose of 160 mg/kg or higher develop liver alterations consistent with hepatic venoocclusive disease [E, reproduced with permission of Frank Snow (19)]. Histological analysis of MCT-treated right ventricles demonstrated a severe inflammatory infiltrate in the RV (F and G). A similar inflammatory infiltrate was present in the left ventricle of MCT-treated rats (H, arrows) and was associated with medial hypertrophy of coronary arterioles (H, arrowhead) and marked perivascular fibrosis (I). Immunohistochemistry reveals that the majority of the inflammatory infiltrates are prominently positive for the B cell marker CD20 (K), negative for CD68+/H11001 (J), and identifies few CD8+ cells (L). These results are consistent with an MCT-induced lymphocytic myocarditis. SuHx, SU5416/hypoxia-exposed rats; mPAP, mean pulmonary arterial pressure; RVID, right ventricular internal diameter.
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tistic) hypothesis is that mice metabolize MCT differently 
of severe PAH has never been described. One (perhaps sim-
enced in patients with idiopathic PAH (IPAH) and PAH associated 
pared with RV samples from IPAH patients. Whereas these 
histological findings make a case for a worse prognosis ob-
served in patients with SScPAH, and could plausibly argue in favor of the MCT model, the number of CD45+ cells is far greater in the MCT-treated rats. Moreover, the authors investi-
gating RV samples from PAH patients reported a significantly higher number of CD68+ cells while our results indicate that CD68+ cells are absent in the MCT-treated RV.

Of Mice and MCT

Because mice provide the opportunity of a vast spectrum of genetic manipulations, it is peculiar that a MCT mouse model of severe PAH has never been described. One (perhaps sim-
pristic) hypothesis is that mice metabolize MCT differently from other species. Of the four members of the CYP3A family, CYP3A4 is of greatest importance in drug metabolism (18). Mice express >100 putative CYP genes with functional impact on the CYP3A cluster (47), which make the metabolism of a drug by this cytochrome highly unpredictable (27). The question if CYP isoforms influence or modify the development of the MCT syndrome (Table 1) in mice has never been addressed mechanistically. However, researchers have tried to circum-
vent the problem of MCT metabolism by injecting ex vivo synthesized MCTP (20). Surprisingly, even MCTP (active MCT) was insufficient to reproduce a rat MCT syndrome in mice. In contrast, mice developed multiple signs of ALI in the first 7–10 days post-MCT injection, followed by resorption of lung edema by day 14–21, finally developing foci of lung fibrosis by day 28 (20). A possible explanation for this se-
quence of events in mice treated with MCTP is the fact that this compound is extremely unstable.

In conclusion, the MCT rat model of PH remains a model favored by many investigators. Here we reviewed pertinent publications that have either been forgotten, or ignored, and reexamined the MCT model in the pathobiological context of human forms of PAH. The MCT rat model continues to impact preclinical PAH research (73), and a significant amount of time and funding continues to be invested in testing new drugs in this model (78). We suggest that the MCT model may be informative in the context of inflammation and the role of lung injury and chronic inflammation in pulmonary vascular dis-
ases. Pulmonary vasoconstriction seems to be an important mechanistic component of the MCT model (11, 41, 50, 67), and, while vasoconstriction occurs in a certain subpopulation of patients with PAH (those documenting a large vasoconstric-
tion component), pulmonary interstitial edema, myocarditis, and hepatic venoocclusive disease [not to mention renal alterations (34)], which are part of the MCT syndrome, are certainly not associated with the human forms of severe PAH [renal insufficiency is, however, a late manifestation (69)]. It is true that (almost) all animal models are imperfect and that it matters which aspect, mechanism, or manifestation of disease a particular model can reproduce or investigate. Whereas the “two hits” model of MCT/pneumonectomy can reproduce the pertinent pulmonary vascular pathology of human PAH, the other mentioned components of the MCT syndrome may be con-
founding. In contrast to the SU5416/hypoxia model (75), a very large number of drugs and compounds have either pre-
vented or improved PH in the MCT-alone (single-hit) model. Paradoxically, the animals, when untreated, die from undeter-
mined causes. Hence the question: do MCT-treated rats die with PH or from PH? As a model of inflammation-associated PAH, the MCT-alone model may be informative but limited as a model of severe angioproliferative PAH, since the full syndrome associated with the development of PH (after a single injection of MCT) and the successful treatment of the MCT syndrome with practically any drug investigated (73) may have little in common with human forms of angioprolif-
erative PAH.

DISCLOSURES

No conflicts of interest are declared by the authors.

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