Heart failure secondary to chronic pulmonary arterial hypertension: cardiac imaging and electrophysiologic characteristics

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Chapter 2

How valid are animal models to evaluate treatments for pulmonary hypertension?

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How valid are animal models to evaluate treatments for pulmonary hypertension?

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**List of abbreviations**

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ACE</td>
<td>angiotensin converting enzyme</td>
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<tr>
<td>BMPR2</td>
<td>one morphogenetic protein type II receptor gene</td>
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<tr>
<td>ET</td>
<td>endothelin</td>
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<tr>
<td>IPAH</td>
<td>idiopathic pulmonary arterial hypertension</td>
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<tr>
<td>5-HT</td>
<td>(5-hydroxytryptamine); serotonin</td>
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<tr>
<td>5-HTT</td>
<td>serotonin plasmatic membrane transporter</td>
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<tr>
<td>MCT</td>
<td>monocrotaline</td>
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<tr>
<td>PAP</td>
<td>pulmonary artery pressure</td>
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<tr>
<td>PASMCs</td>
<td>pulmonary artery smooth muscle cells</td>
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<tr>
<td>PDE5</td>
<td>phosphodiesterase 5</td>
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<tr>
<td>PH</td>
<td>pulmonary hypertension</td>
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<tr>
<td>RV</td>
<td>right ventricle</td>
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Abstract

Various animal models of pulmonary hypertension (PH) exist, among which injection of monocrotaline (MCT) and exposure to hypoxia are used most frequently. These animal models have not only been used to characterize the pathophysiology of PH but also to test novel therapeutic strategies. This manuscript summarizes the available treatment studies in animal models of PH and compares the findings to those obtained in patients with PH. The analysis shows that all approaches which have proven successful in patients, most notably prostacyclin and its analogs, and endothelin receptor antagonists, are also effective in various animal models. However, the opposite is not necessarily true. Therefore, promising results in animals have to be interpreted carefully until confirmed in clinical studies.

Key words: pulmonary hypertension, monocrotaline, hypoxia, prostacyclin, endothelin
Pulmonary hypertension (PH) is a serious illness with multiple potential causes that may progressively worsen and eventually prove fatal. PH was previously classified into two categories: primary pulmonary hypertension (PPH) and secondary pulmonary hypertension, depending on the absence or the presence of identifiable causes or risk factors. In 1998, during the Second World Symposium on Pulmonary Hypertension held in Evian, France, a clinical classification of PH was proposed. In 2003, the 3rd Symposium on Pulmonary Arterial Hypertension in Venice included several improvements over the 1998 Evian Classification. The current classification consists of five categories in which PH is grouped according to specific therapeutic interventions directed at dealing with the cause: 1) pulmonary arterial hypertension (this includes idiopathic pulmonary arterial hypertension, IPAH); 2) pulmonary hypertension associated with left heart disease; 3) PH associated with lung diseases and/or hypoxemia; 4) PH due to chronic thrombotic and/or embolic disease; 5) miscellaneous.

PH is characterized by progressive remodeling of the small pulmonary arteries, causing increased resistance to blood flow in the lung, which, in turn, raises the pulmonary artery pressure (PAP). As the pressure builds, the afterload on the right ventricle (RV) increases. Unrelieved PH, regardless of the underlying cause, leads to RV failure. PH is difficult to diagnose and a challenge to treat.

Although the long-term prognosis for patients with PH is poor at present, there have been recent advances in our understanding of the pathophysiological mechanisms underlying the progression of PH. Accordingly, novel therapeutic approaches, which target various molecular pathways, hold promise for an improved prognosis. Most treatment studies have mainly targeted the vascular derangements (vasoconstriction, vascular remodeling) of PH. Fewer studies have addressed the RV sequelae of PH (RV hypertrophy and ultimately failure). In the following, we will briefly review the various animal models which have been used to investigate the pathophysiology of PH as well as to evaluate novel therapeutic approaches. The potential value of such models will be discussed in light of the available treatment data in humans. The most commonly used models of PH are the monocrotaline (MCT) model and the chronic hypoxia model.
ANIMAL MODELS

Monocrotaline injection

The MCT model was introduced more than 40 years ago. It is based upon a single injection of MCT (typically 60 mg/kg intraperitoneally or subcutaneously) which rapidly leads to severe pulmonary vascular disease in the absence of intrinsic heart and lung disease, thus suggesting its value as an animal model of IPAH. Despite its frequent use for many decades, the basic mechanism underlying PH induction by MCT remains to be fully resolved. It is accepted that MCT is not intrinsically toxic, but must be activated to the reactive MCT pyrrole, the initial dehydrogenation product of MCT, by hepatic cytochrome P450 3A4-6. The pulmonary vascular endothelium is thought to be an early target of MCT intoxication and also plays a central role in the development of human PH. Interestingly, only the combination of MCT-injection with one-sided pneumonectomy causes the neointima formation and vascular obliteration of small pulmonary arterioles that reproduces many of the pathological features of human IPAH.

Major differences exist between rat strains with regard to their MCT sensitivity, and even within a given strain the inter-individual differences in time of onset and extent of toxic effects can vary markedly. These differences in susceptibility may relate to the pharmacokinetics of MCT and possibly include differences in absorption, degradation, hepatic formation of the toxic MCT pyrrole or conjugation and excretion. Thus, MCT injection is an artificial model but mimics well the processes occurring secondary to dysfunction of the pulmonary arteries.

Chronic hypoxia

Reduction of the alveolar oxygen pressure to <70 mm Hg elicits strong pulmonary arterial vasoconstriction. Hypoxia-induced pulmonary vasoconstriction is common in mammals, but important species differences exist. Rabbits show almost no reaction to hypoxia, whereas cattle exhibit the strongest vasoconstriction; hypoxic...
vasoconstriction is weaker in humans than in rats. There is also a great variability among humans.

The pathophysiological mechanism of hypoxic pulmonary vasoconstriction is still under discussion. While a short exposure to hypoxia causes pulmonary vasoconstriction, prolonged hypoxia results in remodeling of the distal branches of pulmonary arteries. Experiments conducted in rats that were chronically exposed to hypoxia showed endothelial and myocyte hyperplasia in the walls of pulmonary arteries during the first days of continuous hypoxia.

Hypoxia applied in animal experiments has been much more severe than the hypoxia that generally develops in human disease states. This could result in a different severity of vasoconstriction and remodeling. It is also possible, that an episode of hypoxic pulmonary vasoconstriction in humans must last a certain time before it initiates reactions that ultimately lead to arterial wall remodeling. Another cause of discrepancy may be related to variations in the individual susceptibility to the hypoxic stimulus. In animal models, intermittent severe hypoxia leads to the development of PH, regardless of the duration of the hypoxia/normoxia intervals. Intermittent hypoxia in humans, however, seems to exert only a small, probably clinically unimportant, effect on pulmonary hemodynamics. Thus, hypoxia is a stimulus also occurring in some forms of human PH, but the duration and severity of hypoxia may differ between the animal models and humans.

**Ligation of ductus arteriosus**

Persistent PH of the newborn is a clinical syndrome characterized by elevated pulmonary vascular resistance, resulting in right-to-left shunting across the foramen ovale and ductus arteriosus with severe hypoxemia. Chronic intrauterine PH due to ligation of the ductus arteriosus in fetal lambs mimics this condition and causes marked elevation of intrauterine PAP, RV hypertrophy, hypertension-related structural changes in the lung, and failure to achieve the normal decline in pulmonary resistance at birth.
Ligation of the ductus arteriosus in late-gestation fetal lambs has provided an experimental model for studying mechanisms that contribute to structural and functional changes associated with perinatal PH. Studies of this experimental model of persistent PH of the newborn suggest that high pulmonary vascular resistance is partly due to vascular remodeling and an imbalance in production or responsiveness to vasodilator and vasoconstrictor stimuli\textsuperscript{20,21,23}.

**Chronic embolic PH**

Repeated microembolizations with Sephadex microspheres generate moderate chronic PH in dogs\textsuperscript{24}. Based upon the size of the injected microspheres, the vascular lesions can be targeted at smaller or larger vessels. Primary vascular mechanical obstruction and vasoconstriction\textsuperscript{25} are the mechanisms of the high pulmonary vascular resistance\textsuperscript{24}. Weimann et al.\textsuperscript{26} established a model of sustained PH in pigs using three repeated embolizations with polydextrane microspheres, which lead to a sustained elevation in PAP. In this model, PAP was increased for at least 1 week. Models of acute pulmonary embolism using various different materials\textsuperscript{27,28} or autologous blood clots\textsuperscript{29} have been used to study the pathophysiological mechanisms or drug effects within the first hour following the embolization.

**Genetically modified animals**

Genetic screening has identified a number of potentially important gene variants that may contribute to the development of PH. The discovery a heterozygous mutation of the \textit{BMPR2} gene, which encodes for the bone morphogenetic protein receptor–II (BMPR-II), in a substantial proportion of patients with IPAH\textsuperscript{30,31} represents a major advance towards an understanding of the molecular mechanisms underlying PH. However, heterozygous \textit{BMPR2}-deficient mice generally exhibit only a mild phenotype with slight increases in PAP and evidence of reduced arterial remodeling after chronic
exposure to hypoxia, indicating that this mouse model may not appropriately reflect human PH.

A role of serotonin (5-hydroxytryptamine, 5-HT) and its plasmatic membrane transporter (5-HTT) was recently reported in the pathogenesis of PH in human and experimental models. Specifically, genetically engineered mice lacking the 5-HTT exhibit attenuated hypoxia-induced PH, whereas 5-HHT overexpression can induce PH in mice.

TREATMENT TARGETS IN PH

Various drug classes are currently used or under investigation for the treatment of PH, both in animal models and in patients. They include prostanoids, endothelin receptor antagonists, phosphodiesterase 5 inhibitors, serotonin transporter inhibitors, NO (for a review, see Badesch et al.), other vasodilators, and statins.

Prostanoids

Prostacyclin and several of its analogs have been tested in animal models and patients with PH with good success. Their conceptual role in the treatment of PH has been confirmed using MCT-treated rats which were rescued by gene therapy with prostacyclin synthase. Repeated inhalation of iloprost also reversed both hemodynamic derangements and structural changes of the small pulmonary arteries in the MCT rat model of PH. Beraprost sodium, a stable and orally active prostacyclin congener, exhibited protective effects on the development of PH in the MCT rat model. Interestingly, a combination of the phosphodiesterase 5 (PDE5) inhibitor sildenafil plus beraprost was significantly more effective than either drug alone in MCT-induced PH. Recently, subcutaneous administration of the prostacyclin agonist ONO-1301 markedly attenuated PH and improved survival in MCT-treated rats. Potential benefits of ONO-1301 as compared to prostacyclin may include its long plasma half-life, which results in
long lasting increases in plasma cAMP levels and attenuation of increases in plasma thromboxane levels. Thus, various prostacyclin receptor agonists have shown beneficial effects in the rat MCT model. Differences among the compounds may largely relate to differential pharmacokinetic properties.

In line with the consistent efficacy of prostacyclin and its analogs in the MCT model, such compounds have also been studied extensively in the treatment of human PH. Based upon such studies, prostacyclin has become part of the standard treatment for PH patients (for reviews see McLaughlin & Rich; Wanstall & Jeffery). The vasoprotective effects of prostacyclin include vasodilatation and inhibition of platelet aggregation and pulmonary artery smooth muscle cells (PASMC) proliferation. While prostacyclin can be administered intravenously or by inhalation, its pharmacokinetic properties, particularly its very short half-life, are clearly not optimal for chronic treatment. Therefore, several prostacyclin analogs have been tested. The prostacyclin analog iloprost has vasodilatory and antithrombotic properties and exhibits long-term beneficial effects in PH patients upon daily inhalations. Inhalation of aerosolized iloprost promoted selective pulmonary vasodilatation in severe PH of both primary and secondary origin. On the other hand, the effectiveness of the prostacyclin analog beraprost sodium was limited in patients with primary and secondary PH. Intravenous epoprostenol improves exercise capacity and survival in patients with PH (Archer and Michelakis, 2006). Moreover, continuous intravenous epoprostenol treatment prior to pulmonary endarterectomy in patients with chronic thromboembolic PH produced beneficial hemodynamic and clinical effects. In a direct comparative cross-over study treprostinil had even better overall therapeutic efficacy than epoprostenol after intravenous administration in PH patients. Of interest, according to that study treprostinil also exhibited bioequivalence whether administered subcutaneously or intravenously, and it has a longer half-life than epoprostenol.

In conclusion, prostacyclin and its analogs have largely been tested in the MCT rat model, and the animal data, with the possible exception of beraprost, are largely in
good agreement with those found in clinical studies. Therefore, the MCT rat model appears useful for studies on prostacyclin analogs. Whether beneficial long-term effects of prostanoids are due to the sustained pulmonary dilatatory effects, or whether they indicate reverse remodeling of the pulmonary vasculature, is still unclear.

**Endothelin antagonists**

Endothelin (ET) levels are elevated in PH, providing the rationale for the use of endothelin receptor antagonists. BQ-123, a selective antagonist of the ETₐ subtype of ET receptors was the first ET receptor antagonist to exhibit beneficial effects in animal models of PH. BQ-123 is a peptide and hence not orally active. It has a short duration of action, requiring continuous intravenous infusion. Medial wall thickness and neomuscularisation were successfully inhibited using continuous infusion of BQ-123 in a rat hypoxia model, the MCT rat model, and in newborn sheep in which the ductus arteriosus was ligated. It was demonstrated that inhibition of pulmonary vascular remodeling by BQ-123 is associated with concomitant reductions in PAP, but the rise in PAP was prevented completely only with the highest dose of BQ-123 (9.6 mg/day).

Other ETₐ receptor antagonists, which are orally active and can be administered once daily, e.g. Ambrisentan, LU135252, and sitaxsentan, have also been examined in various animal models of PH, where they consistently attenuated or even prevented medial thickening of pulmonary arteries.

Bosentan, an antagonist of both ETₐ and ETₜ receptors, has also been examined in various animal models of PH. Similar to ETₐ-selective receptor antagonists, bosentan had beneficial effects on medial thickening and neomuscularisation of distal pulmonary arteries and reduced PAP in a rat hypoxia model and rat MCT model. In a canine model of chronic thromboembolic hypertension treatment with bosentan not only reduced medial thickening of pulmonary arteries, but also reduced adventitial thickening and prevented intima fibrosis and peripheral neomuscularisation. In hypoxic models of PH, bosentan administration after PH had developed not only attenuated the remodeling
of the pulmonary vessels but actually reversed it towards values seen in normoxic animals; additionally, any further rise in PAP was either prevented or reversed\(^61,62,66\). Of note, inhibition of the remodeling in the rat MCT model required higher doses of bosentan and sitaxsentan than in the rat hypoxia model\(^61,66-68\). Another mixed ET\(_{A/B}\) antagonist, BSF420627, was effective in a hypoxia model of PH\(^63\). Thus, ET receptor antagonists appear to readily inhibit remodeling associated with hypoxic exposure. The combined therapy of an oral ET receptor antagonist and the prostacyclin analogue beraprost was superior to the single use of each drug alone in PH induced by MCT injection in rats, even if started after the onset of PH\(^70\).

Clinical data with selective ET\(_A\) receptor antagonists have not been communicated, and hence it remains unclear whether selective inhibition of this subtype is sufficient to yield effective treatment in PH patients. Bosentan has shown therapeutic efficacy in several studies with patients with PH (for a review see Dingemanse and van Giersbergen 2004\(^71\)). Completed randomized, placebo controlled pilot trials of bosentan showed significant improvement in exercise capacity, functional class, and pulmonary hemodynamics in patients with PH\(^72,73\). In patients with chronic thromboembolic PH, bosentan provided an alternative medical therapy to improve six-minute walking distance, functional class, cardiac index, total pulmonary resistance, and one-year survival\(^74\). Retrospective studies in children with PH (IPAH or associated with congenital heart or connective tissue diseases), suggested that bosentan, with or without concomitant prostanoid therapy, is efficient and safe and may improve survival\(^75,76\). Taken together, these data show that hypoxia-related PH models were more sensitive towards inhibition of ET receptors than other models; however, it remains unclear whether this translates in a differential sensitivity of PH patients based upon the underlying cause of their condition.
Ca\textsuperscript{2+}-channel blockers

In both hypoxic and MCT-treated rats, dihydropyridine Ca\textsuperscript{2+}-entry blockers such as nifedipine, nitrendipine, and amlodipine had beneficial effects on pulmonary vascular remodeling\textsuperscript{77-79}. Although Ca\textsuperscript{2+}-entry blockers are widely used in the treatment of human PH, the evidence base for such use is insufficient. As compared to other uses of this drug class, relatively high doses are needed in PH patients, possibly due to both impaired drug absorption and lower sensitivity of the pulmonary vasculature\textsuperscript{80}. It had been proposed that acute responses to such drugs may predict long-term responses, as improved survival in acute responders as compared to non-responders has been reported\textsuperscript{80}. More recent analyses, however, found that <10% of the IPAH evaluated patients exhibited long-term benefit upon Ca\textsuperscript{2+}-entry blocker treatment\textsuperscript{81}. These authors strongly advised not to consider Ca\textsuperscript{2+}-entry blockers as a routine first-line treatment for PH. While a comprehensive discussion of the benefits and risks of Ca\textsuperscript{2+}-entry blockers in the treatment of PH is beyond the scope of this manuscript, these data highlight the possibility that positive data in animal models, even if shown in multiple models, do not necessarily predict clinical efficacy in PH, particularly with regard to end-points such as survival.

PDE5 inhibitors

The pulmonary arterial vasodilating effects of PDE5 inhibitors, which were originally developed for the management of erectile dysfunction, were discovered incidentally. Most available studies have been performed with sildenafil, but limited data with other compounds such as T-1032\textsuperscript{82} suggest that the sildenafil findings may represent class effects. Chronic oral treatment of MCT-treated rats with sildenafil significantly attenuated PH despite delayed administration, i.e. commencement of treatment after PH had already develope\textsuperscript{83}. Sildenafil was also found to be effective in PH of newborn rats.
exposed to hypoxia\textsuperscript{84}. In vitro studies suggest that this may involve beneficial effects on endothelial capillary network formation\textsuperscript{84}, on calcium signaling in pulmonary artery smooth muscle cells from hypoxia-exposed rats leading to a reduced vascular reactivity\textsuperscript{85} and on smooth muscle cell proliferation\textsuperscript{86}. Moreover, sildenafil also blunted the acute hypoxia-induced vasoconstriction response in isolated lungs obtained from both wild-type mice and those genetically engineered to lack the endothelial NO synthase\textsuperscript{87}. Interestingly, a combination of the prostanoid beraprost and sildenafil was significantly more effective than either drug alone in MCT-induced PH; plasma levels of cAMP and cGMP also increased substantially more and remained elevated for a longer period of time in animals treated with the combination\textsuperscript{88}.

In humans, sildenafil can attenuate the acute pulmonary vascular response to hypoxia\textsuperscript{87}. Hence, sildenafil was shown to be effective in treating PH in humans. This applies to high altitude-induced transient PH in healthy volunteers\textsuperscript{89} as well as to patients suffering from PH\textsuperscript{90,91}. Moreover, sildenafil treatment of PH patients has been associated with reduction in RV mass\textsuperscript{91}, suggesting that PDE-5 inhibitors may have a role in the prevention or reversal of remodeling of the RV secondary to PH. Similar to the animal studies, the combination of sildenafil and beraprost has also yielded beneficial effects in PH patients\textsuperscript{92}. Nevertheless, the long-term benefits of sildenafil are not clear and the risk for adverse effects should be considered\textsuperscript{93}. Interestingly, sildenafil has been effective in various pathophysiologically different models of PH, raising the possibility that it may be suitable for the treatment of PH patients irrespective of the underlying cause. It is also interesting to note that sildenafil exhibited its beneficial clinical effects upon once daily dosing despite its relatively short half-life.

**NO and L-arginine**

Within PASMC, NO and PDE5 are part of the same signaling cascade, as inhibition of the latter potentiates effects of the former, i.e. reduced breakdown of cGMP enhances cGMP-dependent effects of NO. A possible role of NO in the management of
PH has been studied using inhaled NO gas, NO donor drugs, L-arginine (the precursor of NO), and gene transfer of NO synthase. Animal and human studies have demonstrated that NO causes selective pulmonary vasodilatation, lowering PAP and pulmonary vascular resistance\textsuperscript{94,95}.

In animal studies, the effectiveness of NO seems to vary depending on the experimental model. In adult rats injected with MCT, inhalation of NO had no effect on remodeling\textsuperscript{96,97} but this approach was effective in newborn rats treated with MCT\textsuperscript{98}. Treatment of both hypoxia-induced and MCT-induced PH in rats with L-arginine inhibited medial thickening and neomuscularisation, and this was associated with a reduction in PAP\textsuperscript{99}. The beneficial effect of L-arginine in the rat MCT model was observed in studies aimed at reversing rather than preventing PH and its sequelae. Neomuscularisation was reduced, but there was no effect on medial thickening or PAP. The beneficial effect of L-arginine in the MCT model is surprising in light of the lack of effect on NO gas in adult rats under similar conditions. A recent experimental approach for increasing NO in the pulmonary vasculature is via gene transfer of NO synthase, the enzyme responsible for the production of NO from L-arginine. Using the hypoxia model in rats, the transfer by aerosol of an adenoviral vector containing the gene for inducible NO synthase was found to decrease neomuscularisation of small pulmonary arteries and to reduce both pulmonary vascular resistance and PAP\textsuperscript{100}.

Until now, elevation of NO tone has been tested clinically only on a limited basis. In critically ill adults, NO is used as an option in the short term management of PH, because it reduces PAP and improves oxygenation by increasing the fraction of blood flow to lung regions with a normal ventilation-perfusion ratio in acute respiratory syndrome \textsuperscript{101}. A possible role for NO donors or L-arginine in the chronic treatment of PH remains to be established. This also makes it difficult to compare clinical and experimental data based upon an NO approach.
Statins

Based upon their anti-proliferative and anti-inflammatory cardiovascular benefits in addition to cholesterol-lowering effects\textsuperscript{102,103}, statins have also been tested in animal models and patients with PH. Most of these studies have used simvastatin. Originally, it was reported that simvastatin was effective in the rat MCT model when administered at the time of induction of vascular injury, but had only smaller effects when given 2 weeks after the induction of PH\textsuperscript{104}. In a later study from the same group using the more severe MCT/pneumonectomy rat model of fatal PH, simvastatin attenuated and reversed both PH and neointimal formation and conferred a 100\% survival; this was accompanied by a reversed vascular occlusion through reduced intima proliferation and increased apoptosis of pathological smooth muscle cells in pulmonary arteries\textsuperscript{105}. Similar results were noted in a rat model of hypoxic pulmonary hypertension\textsuperscript{106}. A study in this issue of the journal\textsuperscript{107} extends findings on the use of statins in PH to pravastatin by demonstrating beneficial effects when administered at the time of PH induction by MCT. This is interesting because pravastatin differs from simvastatin and other statins due to its open lactone ring chemical structure\textsuperscript{108}. While it is too early to determine the utility of statins in the treatment of PH, it is encouraging that simvastatin was shown to improve exercise capacity in an observational study with PH patients\textsuperscript{109}.

5-HTT inhibitors

Perivascular inflammation, i.e. infiltration with macrophages and lymphocytes in the region of occlusive lesions, is a histopathological feature of PH\textsuperscript{110}. It may involve endothelial dysfunction with deregulated expression of vasoactive, mitogenic and pro-inflammatory mediators\textsuperscript{111}. These findings are the rationale to test anti-inflammatory approaches, such as immunosuppressant and cytokine antagonists, in the treatment of PH. Indeed the immunosuppressants rapamycin and triptolide were shown to significantly
reduce PAP in rats that had undergone pneumectomy and subsequent MCT injections\textsuperscript{112,113}. Interleukin-1 is excessively produced in the lungs of MCT-treated rats, but not in hypoxia-induced PH; accordingly, repeated injections of a recombinant interleukin-1 receptor antagonist reduced PH and RV hypertrophy in the MCT model, but not in the chronic hypoxia model\textsuperscript{114}. The possible implications of such findings for the treatment of PH patients are difficult to evaluate in the absence of clinical data.

**Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers**

The effects on PH or its sequelae of drugs that prevent either the production or the action of the potent mitogen/growth factor angiotensin II have been examined in both hypoxic and MCT rat models. In chronically hypoxic rats, various ACE inhibitors have been shown to inhibit pulmonary vascular remodeling associated with the development of PH. When rats were studied for the entire hypoxic period\textsuperscript{115,116}, medial thickening and muscularisation of the pulmonary arteries were attenuated. In one study with the ACE inhibitors cilaprazil, medial thickening of these vessels was even totally prevented\textsuperscript{117}. The beneficial effects of ACE inhibitors were even found when treatment started 7 or 12 days after commencement of hypoxic exposure\textsuperscript{118}. On the other hand, the effects of ACE inhibitors in MCT-treated rats are not fully clear. While treatment with captopril (12 mg/kg/day for 4 weeks) had no effects on medial thickness, neomuscularisation or on PAP\textsuperscript{116}, a longer treatment with a higher dose (60 mg/kg/day for 6 weeks) reduced the degree of neuromuscularisation of peripheral pulmonary arteries \textsuperscript{119}. The role of angiotensin II in PH is further confirmed by findings with angiotensin receptor antagonists. Thus, losartan, inhibited medial hypertrophy and neuromuscularisation in hypoxic rats\textsuperscript{120} as well as in the MCT-plus-pneumonectomy rats\textsuperscript{121}. Similarly, olmesartan medoxomil inhibited RV hypertrophy and also inhibited increases in mRNA levels of various markers of RV dysfunction such as atrial and brain natriuretic peptides in a
chronic hypoxia model of PH. The possible implications of such findings for the treatment of PH patients are difficult to evaluate in the absence of clinical data.

Conclusion

In conclusion, a variety of therapeutic strategies have been tested in various animal models of PH, most often hypoxia- or MCT-based models. Several of these approaches were also shown to be effective in PH patients, and all clinically proven treatments also work in the animal models. However, some models may be more sensitive to certain approaches than others (e.g. the greater potency of ET receptor antagonists in hypoxia than in other models). Moreover, the clinical role of Ca\(^{2+}\)-entry blockers remains unclear despite their consistent beneficial effects in animal models. Therefore, promising animal data e.g. 5-HTT inhibitors, inhibitors of the rennin-angiotensin-system, statins and anti-inflammatory drugs need to be interpreted with caution until confirmed in clinical studies.
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