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*Review Article*

# Novel Approaches to Treat Experimental Pulmonary Arterial Hypertension: A Review

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*Background*. Pulmonary arterial hypertension (PAH) is a life-threatening disease characterized by an increase in pulmonary artery pressure leading to right ventricular (RV) hypertrophy, RV failure, and ultimately death. Current treatments can improve symptoms and reduce severity of the hemodynamic disorder but gradual deterioration in their condition often necessitates a lung transplant. *Methods and Results*. In experimental models of PAH, particularly the model of monocrotaline-induced pulmonary hypertension, efficacious treatment options tested so far include a spectrum of pharmacologic agents with actions such as anti-mitogenic, proendothelial function, proangiogenic, antiinflammatory and antioxidative. Emerging trends in PAH treatment are gene and cell therapy and their combination, like (progenitor) cells enriched with eNOS or VEGF gene. More animal data should be collected to investigate optimal cell type, in vitro cell transduction, route of administration, and number of cells to inject. Several recently discovered and experimentally tested interventions bear potential for therapeutic purposes in humans or have been shown already to be effective in PAH patients leading to improved life expectation and better quality of life. *Conclusion*. Since many patients remain symptomatic despite therapy, we should encourage research in animal models of PAH and implement promising treatments in homogeneous groups of PAH patients.

## 1. Background

*1.1. Pulmonary Arterial Hypertension.* Pulmonary arterial hypertension (PAH) is a progressive condition characterized by elevated pulmonary arterial pressures leading to RV failure. PAH is primarily a lung disorder associated with increased pulmonary vascular resistance, pulmonary vascular pathology, medial hypertrophy of arterioles, and lung inflammation. PAH may occur in association with underlying collagen vascular disease, congenital heart disease, HIV infection, or chronic thromboembolic disease. A minority of patients with PAH have idiopathic PAH. In familial PAH, 70% of the cases have heterozygous mutations of *BMPR2* encoding the bone morphogenetic protein receptor type 2 [1].

Current therapeutic strategies may improve symptoms and reduce the severity of the hemodynamic disorder, but in many cases deterioration of pulmonary and cardiac functions ultimately necessitates a lung transplant. Novel approaches in treating PAH include: (1) reversing the advanced occlusive structural changes in the pulmonary circulation that cause PAH and (2) regeneration of damaged pulmonary tissue using stem cells. These approaches have been tested using (i) pharmacotherapy, (ii) gene therapy, and (iii) cell therapy.

*1.2. Animal Models of Pulmonary Arterial Hypertension.* Various experimental models have been proposed for induction of PAH in animals including: (1) monocrotaline- (MCT-) induced PAH with or without aortocaval shunt; (2) chronic hypoxia-induced PAH; (3) chronic embolism-induced PAH; (4) ligation of ductus arteriosus; (5) overcirculation-induced PH; (6) genetically modified animal models, such as mice lacking the vasoactive intestinal peptide (*VIP*) gene. In the past 10 years most experimental studies on therapy of PAH have employed the MCT-induced PAH model.

## 2. Treatment Options for PAH

*2.1. Pharmacotherapy.* Several pharmacologic agents have been used either alone or in combination to treat PAH.

*2.1.1. Serine Elastase Inhibitors.* Progression of PAH is associated with increased serine elastase activity and deposition of extracellular matrix (ECM) protein tenascin-C in lung parenchyme. The recent studies using hypertrophied rat pulmonary arteries have shown that elastase inhibitors suppress tenascin-C and induce apoptosis of vascular smooth muscle cells (SMCs) [2, 3]. This therapy initiates complete regression of the hypertrophied vessel wall by a coordinated loss of cellularity and ECM. Elastase inhibitors were shown to reverse advanced MCT-induced pulmonary vascular disease. If the serine elastase inhibitors M249314 or ZD0892 were orally administered from 21 days after injection of MCT onwards, survival after 1 week was 92%, compared with only 39% survival in untreated or vehicle-treated rats [4].

*2.1.2. Platelet-Derived Growth Factor Inhibition.* Progression of PAH is associated with increased proliferation and migration of pulmonary vascular SMCs. Platelet-derived growth factor (PDGF) is a potent mitogen involved in this process. In rats with MCT-induced PAH daily administration of the PDGF receptor antagonist STI571 (imatinib) was started 28 days after disease induction, resulting in 100% survival in the first 2 weeks, compared with only 50% in shamtreated rats. PAH and RV hypertrophy were reversed to near-normal levels. Similar results were obtained in chronically hypoxic mice treated with STI571 after full establishment of PAH [5].

*2.1.3. Prostacyclin Therapy.* Rats with flow-associated PAH due to an aortocaval shunt, in combination with MCT administration, were treated with low-dose aspirin (25 mg/kg/day) or iloprost (72 $\mu$g/kg/day), a prostacyclin analogue. 90% of the untreated rats with PAH developed dyspnea and pleural fluid; whereas this was seen in 50% and 10% of the aspirin and iloprost-treated rats, respectively. This could not be attributed to changes in pulmonary artery pressure, wall to lumen ratio of the pulmonary arterioles, or RV hypertrophy. However, both therapies restored the reduced RV capillary to cardiomyocyte ratio in rats with PAH and improved RV contractility [6]. Subcutaneous administration of a novel prostacyclin agonist (ONO-1301) markedly attenuated MCT-induced PAH and improved survival in rats. The beneficial effects of ONO-1301 occurred through its long-lasting stimulation of cAMP and inhibition of thromboxane synthase [7].

Although prostacyclin is recognized as a therapeutic breakthrough for PAH, it needs continuous infusion because of its short half-life in plasma. To overcome this problem, a new drug delivery system was developed by preparing ONO-1301MS, a novel sustained-release prostacyclin analogue polymerized with poly (D,L-lactic-co-glycolic acid) microspheres. A single injection of ONO-1301MS resulted in sustained activity for 3 weeks, and attenuated PAH, partly through its antiproliferative effect on vascular SMCs via inhibition of ERK phosphorylation [8]. Also the inhalation of iloprost has been shown to reverse PAH and vascular structural remodeling in MCT-treated rats [9].

*2.1.4. Combined Prostacyclin and Phosphodiesterase Inhibition.* Combination therapy with oral sildenafil, a phosphodiesterase (PDE)-5 inhibitor, and beraprost, an oral prostacyclin analogue, attenuated the development of MCT-induced PAH more than with either drug alone [10]. The combined administration of iloprost, a long-acting prostacyclin analogue, and a dual-selective PDE3/4 inhibitor, tolafentrine, reversed the development of PAH and cor pulmonale in response to MCT in rats [11].

*2.1.5. Rho-Kinase Inhibition.* Long-term treatment with a Rho-kinase inhibitor fasudil improved the mortality rate of MCT-induced PAH in rats [12], as well as ameliorated hypoxia-induced PAH in mice, partially by activation of endothelial nitric oxide synthase (eNOS) [13]. Fasudil exerts effective and selective vasodilatation of pulmonary arteries in rats with MCT-induced PAH, which explains its usefulness for the treatment of this fatal disorder [14]. In chronicly hypoxic rats, inhalation of Rho-kinase inhibitors nearly normalized PAH, but had no pulmonary vascular selectivity [15].

*2.1.6. Combined Rho-Kinase Inhibitor and Prostacyclin Therapy.* Combination therapy has been advocated based on potential for additive or synergistic effects. Long-term inhibition of Rho-kinase, an effector of the small GTPase Rho, ameliorated MCT-induced PAH in rats and hypoxia-induced PAH in mice. It was reported that prostacyclin and its oral analogue beraprost may lack direct inhibitory effect on Rho-kinase in vitro, suggesting that combination therapy with a Rho-kinase inhibitor and beraprost is effective for the treatment of PAH. Male rats were given a subcutaneous injection of MCT (60 mg/kg) and were subsequently treated without or with a Rho-kinase inhibitor (fasudil, 30 mg/kg/day), beraprost (200 mg/kg/day), or a combination of both for 3 weeks. The combination therapy, when compared with each monotherapy, showed significant improvement in PAH, RV hypertrophy, and pulmonary medial thickness without any adverse effects [16].

*2.1.7. Endothelin Receptor Antagonists.* Endothelin-1 (ET-1), a potent vasoactive and mitogenic peptide, has been implicated in the pathogenesis of several forms of PAH. A nonspecific endothelin-receptor antagonist, bosentan was shown to blunt MCT-induced PAH in rats [17].

The antagonism of the $ET_A$ receptor was shown to be essential for the protection from MCT-induced PAH, irrespective of the presence of the $ET_B$ receptors, although a protective role of $ET_B$ receptor-mediated action in the pathogenesis of this disease model could not be ruled out [18].

In another study from the same group, the functional roles of endothelin $ET_A$ and $ET_B$ receptors in the development of MCT-induced PAH were investigated using MCT-treated rats with or without daily administration of A-192621, a selective $ET_B$ receptor antagonist, ABT-627, a selective $ET_A$ receptor antagonist, or a combination of both. The results demonstrated that the $ET_A$ receptor-mediated action contributed exclusively to the pathogenesis of MCT-induced PAH [19].

*2.1.8. Combined Endothelin-A Receptor Antagonist and Prostacyclin Therapy.* Rats with MCT-induced PAH were treated with the oral $ET_A$ receptor antagonist TA-0201 and/or the oral prostacyclin analogue beraprost for 19 days. The PAH-associated RV systolic pressure elevation was significantly attenuated by TA-0201 and beraprost, and most strongly by their combination. The indexes of RV hypertrophy showed the same tendency as the increase in RV peak systolic pressure [20].

*2.1.9. Serotonin Transporter Inhibition.* Progression of PAH is associated with increased pulmonary expression of the serotonin transporter (5-HTT), which leads to hyperplasia of the pulmonary artery SMCs (PA-SMCs). Given the fact that overexpression of the 5-HTT gene in PA-SMCs leads to PAH [21], it was investigated whether the highly selective 5-HTT inhibitor fluoxetine prevented and/or reversed PAH in MCT-treated rats. Selective antagonists to the $5\text{-}HT_{1B/1D}$ receptor, $5\text{-}HT_{2A}$ receptor, and $5\text{-}HT_{2B}$ receptor were used for comparative testing. MCT injection (60 mg/kg s.c.) was followed by an early peak in lung 5-HTT expression on day 1, which preceded the onset of PAH. Established PAH on day 15 was associated with a sustained 5-HTT increase. Continued fluoxetine treatment completely prevented PA-SMC proliferation and PAH development, and also suppressed the late 5-HTT increase, without affecting the early peak. The 5-HT receptor antagonists did not affect PAH. Oral fluoxetine (10 mg/kg/day) started 3 weeks after MCT injection completely reversed established PAH, normalizing pulmonary artery pressure and structure [22].

Furthermore, a selective serotonin reuptake inhibitor, sertraline, protects against MCT-induced PAH by decreasing pulmonary artery pressure, RV hypertrophy index, and pulmonary artery remodeling, probably related to a reduction in serotonin transporter mRNA [23].

A serotonin receptor antagonist, MCI-9042, attenuated the development of MCT-induced PAH, suggesting a pivotal role of serotonin in the development of PAH induced by MCT [24].

Specific $5\text{-}HT_{2A}$ receptor blockade with sarpogrelate, a $5\text{-}HT_{2A}$ receptor antagonist, immediately after MCT administration, inhibited PAH and prolonged survival in rats. These effects were accompanied by anti-inflammatory and antiproliferative effects in the lung tissue and marked improvement of pulmonary vascular endothelial dysfunction and activation [25].

*2.1.10. Phosphodiesterase Inhibition.* PDE inhibitors for treatment of PAH have been studied extensively. Oral sildenafil, a PDE5 inhibitor, prevented (in a study in which sildenafil was given 1 day after MCT) and reversed (in a study in which sildenafil was given 3 weeks after MCT) the development of PAH in MCT-treated rats, associated with a reduction in the $ET_A$-receptor density in SMCs of pulmonary small arteries (diameter $< 100\,\mu$m) [26]. The effects of oral pumafentrine, a mixed-selective PDE3/4 inhibitor, have been investigated in rats with MCT-induced PAH. When chronically administered in weeks 4 to 6 after a single injection of MCT (60 mg/kg), pumafentrine (10 mg/kg daily) partially reversed PAH and RV hypertrophy in rats [27]. In addition, small pulmonary arterial muscularization, medial hypertrophy, and decrease in lumen area were largely reversed. Inhibition of PA-SMC proliferation by pumafentrine was demonstrated in vivo [27].

Four weeks after a single injection of MCT in rats, the animals displayed nearly threefold elevated pulmonary artery pressure and vascular resistance values, with a concomitant decline in central venous oxygen saturation and arterial oxygenation. Marked RV hypertrophy was evident, and massive thickening of the arteriolar SMC layer was histologically apparent. Sildenafil, administered from day 14 to day 28, significantly increased plasma cGMP and inhibited the development of PAH and RV hypertrophy, with improvement of central venous oxygen saturation and arterial oxygenation. A corresponding efficacy profile was also noted for treatment with sildenafil started at day 28 till day 42. Moreover, the death rate significantly decreased in those animals treated with sildenafil [28].

Inhalation of tolafentrine, a combined PDE3/4 inhibitor, reversed PAH that occurred in response to MCT in rats. This "reverse remodeling" effect included structural changes in the lung vascular wall and key molecular pathways of matrix regulation, concomitant with 60% normalization of hemodynamics [29].

*2.1.11. Combination of Phosphodiesterase and Endothelin Receptor Inhibition.* In a study with 5 groups of rats, a first group consisted of control rats without MCT injection. Four other groups of rats received MCT subcutaneously and were assigned to receive no treatment, 300 mg/kg/day bosentan in the food, 100 mg/kg/day sildenafil in drinking water, or their combination for 4 weeks. The doses of bosentan and sildenafil were the maximally effective doses based on a dose-rangefinding study. Mortality was 0%, 53%, 11%, 11%, and 0%, respectively, in the five different groups. Bosentan and sildenafil significantly attenuated the increase in mean pulmonary arterial pressure, and the combination had an additional effect [30].

*2.1.12. Caveolin-1 Peptide.* Caveolins, the principal structural proteins of caveolar microdomains, have been implicated in the development of PAH. Mice with homozygous deletion of the caveolin-1 (*Cav-1*) gene develop PAH and RV hypertrophy. In several animal models of PAH and in patients with severe PAH, reductions in pulmonary Cav-1 expression were apparent.

To study whether in vivo modulation of Cav-1 expression could affect the development of PAH and RV hypertrophy, rats were injected with MCT, followed by a daily injection of (1) saline, (2) a peptide corresponding to the homeodomain of the Drosophila transcription factor antennapedia (AP; 2.5 mg/kg/day), or (3) a peptide consisting of the Cav-1–scaffolding domain coupled to AP (AP-Cav; 2.5 mg/kg/day) for 2 weeks. MCT and MCT+AP rats developed PAH. Administration of AP-Cav to MCT rats significantly reduced the pulmonary artery pressure. MCT and MCT+AP rats also developed pulmonary artery medial hypertrophy and RV hypertrophy, which was normalized by administration of AP-Cav [31].

*2.1.13. Estradiol.* Daily supplementation of genistein, a phytoestrogen, potently attenuated MCT-induced PAH, RV hypertrophy, and pulmonary vascular remodeling in rats [32]. Also 2-methoxyestradiol (2ME), a nonestrogenic estradiol metabolite, prevented the development and retarded the progression of MCT-induced PAH [33]. 2ME significantly attenuated RV hypertrophy and pulmonary arterial medial hypertrophy and reduced proliferative and inflammatory responses in the lungs. Furthermore, 2ME given from day 14 to 28 to diseased animals significantly decreased RV pressure and RV hypertrophy and reduced mortality [33, 34].

Recently, it was shown that 2-ethoxyestradiol strongly inhibits vascular remodeling in PAH which suggests that antiproliferative agents, including synthetic analogues of estradiol metabolites, may be protective in PAH [35].

*2.1.14. Statins.* Various statins have been shown to be effective in treating experimental PAH. Simvastatin attenuated MCT-induced pulmonary vascular remodeling, PAH, and RV hypertrophy in rats [36]. Pravastatin reduced the development of MCT-induced PAH and improved endothelium-dependent pulmonary artery relaxation through reduced apoptosis and a restored eNOS expression of endothelial cells [37]. Rosuvastatin provided protection against the development of PAH and RV hypertrophy [38].

*2.1.15. Miscellaneous Therapeutic Agents.* (1) Amlodipine, a third-generation calcium channel blocker, inhibited the development of PAH and improved survival in rats independent of its effect on lowering blood pressure. These effects were associated with marked inhibition of the downregulation of eNOS and improvement of pulmonary vascular endothelial activation, as well as anti-inflammatory, antiproliferative, and antifibrotic effects in the lung tissue. However, amlodipine failed to reverse established PAH [39].

(2) *Nicorandil,* an ATP-sensitive potassium channel opener with a nitrate-like action, inhibited development of MCT-induced PAH but failed to reverse it. These effects were associated with marked upregulation of diminished lung eNOS concentration along with improvement of pulmonary vascular endothelial activation and antiinflammatory and anti-proliferative effects in the lung tissue [40].

(3) *Infusion of C-type natriuretic peptide*, the third member of the natriuretic peptide family, attenuated MCT-induced PAH and improved survival. The beneficial effects were mediated by regeneration of pulmonary endothelium, inhibition of endothelial cell apoptosis, prevention of monocyte/macrophage infiltration, and restoration of fibrinolytic activity [41].

(4) *Repeated inhalation of adrenomedullin*, a potent vasodilator peptide that was originally isolated from human pheochromocytoma, has been shown to inhibit MCT-induced PAH without systemic hypotension, and thereby improving survival in MCT rats [42].

(5) Heme oxygenase-1 was shown to be critical for the antiproliferative and vascular protective effects of *rapamycin*, an immunosuppressive agent with antiproliferative activity not only against lymphocytes but also against vascular cells, in vitro and in vivo in MCT-induced PAH [43].

(6) *Granulocyte colony-stimulating factor* inhibited the progression of PAH in a rat model, possibly by stimulating pulmonary endothelial cells to proliferate at sites of impaired lung vasculature [44].

(7) The NF-$\kappa$B nuclear localization and vascular cell adhesion molecule (VCAM-1) expression are temporally and spatially associated with the development of MCT-induced PAH in rats. Administration of a *$NF_{-\kappa}B$ inhibitor*, pyrrolidine dithiocarbamate, reversed the MCT-induced development of PAH in rats [45].

(8) In rats with MCT-induced PAH treatment with an *interleukin-1 receptor antagonist* started at the same time as MCT administration and continued for the first 2 weeks prevented the development of PAH and RV hypertrophy as assessed 3 weeks after MCT injection [46].

(9) In rats with MCT-induced PAH, treatment with *antibodies to monocyte chemotactic and activating factor/monocyte chemoattractant protein-1* was started at the same time as MCT administration or at day 3.5, 7, or 14 after MCT administration. Antibody therapy started together with MCT administration was the most effective in alleviating RV hypertrophy, as compared to later starts [47]. Antibody therapy started together with MCT administration did not prevent development of pulmonary hypertension, but RV peak systolic pressure was significantly lower than observed in rats treated with MCT only.

(10) One day after MCT administration (60 mg/kg), rats were treated without or with a specific *inhibitor of p38 mitogen-activated protein kinase,* FR167653, for 27 days. Four weeks after MCT administration pulmonary artery pressure and RV weight had hardly increased, compared to rats that received only MCT. The beneficial effects of FR167653 were ascribed to attenuated expression of inflammatory cytokines, thereby preventing the progression of PAH [48].

(11) PAH is associated with endothelial injury [49] and with NO-dependent endothelial dysfunction [50]. Rats treated with daily i.p. doses of *L-arginine* (500 mg/kg), started 3 days before MCT administration, and continuing till sacrifice of the animals at day 17 after MCT injection, prevented the development of PAH, RV hypertrophy, and pulmonary vascular disease [51].

(12) Rats were, 2 days before MCT administration (60 mg/kg), treated without or with an *inhibitor of lipoxygenase pathways*, diethylcarbamazine, for 23 days. Three weeks after MCT administration, therapy with diethylcarbamazine

had blocked the development of PAH and RV hypertrophy, associated with inhibition of influx of polymorphonuclear cells and alveolar macrophages into the alveoli and the activation of these cells by lipoxygenase-related products [52].

(13) Therapy of rats with MCT-induced PAH was started 3 weeks later using daily i.p. injections of the *activin receptor-like kinase-5 signaling inhibitor* IN-1233 for an additional 2 weeks. At that time RV peak systolic pressure and RV hypertrophy index were significantly lower in the treated rats than in the untreated rats with PAH [53].

### 2.2. Gene Therapy

*2.2.1. Extracellular Superoxide Dismutase Gene.* There is ample evidence that oxidative stress contributes to the pathogenesis and/or development of PAH. MCT-injected rats were intratracheally administered (1) vehicle (MCT group), (2) an adenovirus encoding $\beta$-galactosidase (Ad$\beta$gal group), or (3) an adenovirus encoding *human extracellular superoxide dismutase (EC-SOD)* (AdEC-SOD group). After intratracheal gene transfer, EC-SOD was successfully expressed in lung tissue, bronchoalveolar lavage fluid, and plasma. Twenty-eight days after MCT injection, RV systolic pressure and the weight ratio of the RV to the LV plus septum were significantly lower in the AdEC-SOD group than in the MCT group and the Ad$\beta$gal group. Moreover, vascular remodeling and proliferation of vascular SMCs in pulmonary arteries were markedly suppressed in the AdEC-SOD group [54].

*2.2.2. Plasmid Inhibiting MCP-1.* Monocyte/macrophage chemoattractant protein-1 (MCP-1) is a potent chemoattractant chemokine and an activator for mononuclear cells. Intramuscular injection of a plasmid encoding a *7-terminal deleted dominant negative inhibitor of MCP-1* in rats simultaneously injected with MCT inhibited the progression of MCT-induced PAH, RV hypertrophy, medial hypertrophy of pulmonary arterioles, and mononuclear cell infiltration into the lungs [55].

*2.2.3. Prostacyclin Synthase Gene.* Prostacyclin is a potent vasodilator that also inhibits platelet adhesion and cell growth. Intratracheal transfer of the *human prostacyclin synthase (PGIS)* gene to rats with MCT-induced PAH augmented pulmonary prostacyclin synthesis, ameliorated MCT-induced PAH, and improved survival in MCT rats [56]. An intramuscular injection of adeno-associated virus (AAV) vector harboring the PGIS gene (AAV-PGIS) also prevented MCT-induced PAH in rats [57].

*2.2.4. Angiopoietin-1 Gene.* Cell-based gene transfer with *angiopoietin-1 (Ang-1)*, a newly discovered ligand of the endothelial-specific tyrosine kinase receptor Tie-2, has been shown to improve survival and pulmonary hemodynamics in experimental PAH by a mechanism involving the inhibition of apoptosis and protection of the pulmonary microvasculature [58].

*2.2.5. Interleukin-10 Gene.* *Interleukin (IL)-10* is a pleiotropic anti-inflammatory cytokine with vasculoprotective properties. After rats were injected intramuscularly with an AAV serotype 1 vector expressing IL-10, followed by MCT injection, it was demonstrated that IL-10 expression prevented MCT-induced PAH in rats [59].

### 2.3. Cell Therapy

*2.3.1. Intratracheal Mesenchymal Stem Cell Therapy.* Rat bone marrow-derived mesenchymal stem cells (rMSCs) transfected with the lacZ gene were administered intratracheally to rats 2 weeks after administration of MCT. Intratracheal cell therapy attenuated the rise in pulmonary arterial pressure and pulmonary vascular resistance and restored pulmonary responses to acetylcholine toward values measured in control rats. Treatment with rMSCs decreased RV hypertrophy induced by PAH. Immunohistochemical studies showed widespread distribution of lacZ-labeled rMSCs in lung parenchyme surrounding airways in MCT-treated rats. These rMSCs retained the expression of von Willebrand factor and $\alpha$-smooth muscle-actin, being markers specific for endothelial cell and SMC phenotypes, respectively. However, lacZ expressing rMSCs were not detected in the wall of pulmonary vessels [60].

*2.3.2. Intravenous Administration of Pulmonary Artery Smooth Muscle Cells.* Primary cultures of rat PA-SMCs were transfected with either the full-length coding sequence of the eNOS gene or with the control vector. Transfected PA-SMCs were delivered to syngeneic recipient rats by injection into the jugular vein, simultaneously with MCT. At 28 d after injection, rats injected with the eNOS-transfected SMCs had significantly lower RV systolic pressure than rats injected with the null-transfected SMCs [61]. Cultured PA-SMCs that were in vitro transfected with the gene encoding vascular endothelial growth factor (VEGF)-A were administered i.v. to rats simultaneously with MCT injection. Four weeks later, PAH, RV hypertrophy, and medial hypertrophy of pulmonary arterioles were significantly less in the VEGF-treated animals compared to MCT-treated animals that did not receive cell therapy. If cell-based gene transfer using VEGF-expressing PA-SMCs was delayed till PAH had developed, also a significant decrease in the progression of PAH and RV hypertrophy was documented [62]. Thus, a therapeutic role for angiogenic factors in the therapy of PAH is very likely.

*2.3.3. Intravenous Administration of Fibroblasts.* Twenty one days after MCT injection, rats received i.v. (1) fibroblasts transfected with the eNOS gene, (2) fibroblasts transfected with the VEGF-A gene, and (3) null-transfected fibroblasts. Fourteen days later it appeared that eNOS-transfected fibroblasts reduced pulmonary artery pressure; whereas VEGF-transfected fibroblasts prevented further increases of pulmonary artery pressure but did not reverse established PAH [63].

*2.3.4. Intravenous Administration of Endothelial Progenitor Cells.* Rat bone marrow-derived endothelial progenitor cells (EPCs), cultured for 7 to 10 days in endothelial growth medium and injected i.v. in syngeneic MCT-treated rats, engrafted at the level of the distal pulmonary arterioles and incorporated into the endothelial lining of the MCT-injured lung. The administration of EPCs 3 days after MCT administration nearly completely prevented the increase in RV pressure observed at 3 weeks with MCT alone. Delayed administration of EPCs 3 weeks after MCT prevented the further progression of PAH 2 weeks later; whereas animals receiving EPCs transfected with the human eNOS gene exhibited significant regression of established disease at day 35 compared with day 21. Moreover, the delivery of EPCs to rats with established PAH resulted in marked improvement in survival, which was the greatest in the group receiving eNOS-transduced EPCs [64]. In a study by Yip and coworkers EPCs were harvested from bone marrow 7 days after MCT injection (75 mg/kg) [65]. The EPCs were grown in vitro for 3 weeks and then administered, after labeling, intravenously at day 28 in the rat from which the EPCs were isolated. The final analysis was done at day 90. Labeled EPCs were recovered in the endothelial lining of distal pulmonary arterioles, where they may have differentiated into mature endothelial cells and restored the integrity of vascular endothelial function. The i.v. administration of autologous EPCs led to reduced (i) RV hypertrophy, (ii) RV systolic pressure, and (iii) wall thickening of pulmonary arterioles, associated with increased pulmonary arteriolar-capillary density, although most values remained different from those of healthy rats.

*2.3.5. Intravenous Administration of Bone Marrow-Derived Cells.* The recent evidence suggests that bone marrow-derived cells may differentiate into vascular cells that participate in arterial repair and/or lesion formation [66]. However, it remains uncertain whether bone marrow-derived cells can also participate in vascular remodeling associated with PAH. The bone marrow of Sprague-Dawley rats was reconstituted with that of green fluorescent protein- (GFP-) transgenic rats. The bone marrow-chimeric rats were injected intraperitoneally with 60 mg/kg MCT after unilateral pneumonectomy. After 28 days, they had RV peak systolic pressures more than 2 times the pressure in control animals. The pulmonary arterioles were markedly thickened, with an infiltration of GFP-positive macrophages into the perivascular areas. The endothelium of pulmonary arterioles contained only a few GFP-positive cells, and hardly any GFP–positive cells were detected in the media of thickened pulmonary arterioles [67]. Thus, unfractionated bone marrow-derived cells do not contribute substantially to pulmonary arterial remodeling associated with MCT-induced PAH in pneumonectomized rats.

In another study the effect of bone marrow-derived cells on PAH induced by either MCT or exposure to chronic hypoxia in mice was investigated. I.v. administration of the active MCT metabolite (monocrotaline pyrrole, MCTp) to mice induced PAH within 15 days, due to remodeling of small pulmonary arterioles. Three days after MCTp injection, the mice were injected with bone marrow-derived cells of donor mice treated with 5-fluorouracil (3.5 mg i.p./animal) to deplete mature cells and to allow proliferation of progenitor cells. Bone marrow-derived cells significantly attenuated PAH as assessed by reductions in RV pressure, RV weight, and percentage of muscularized pulmonary arterioles, compared to irradiated bone marrow-derived cells administered to MCTp-treated animals. In contrast, chronically hypoxic mice subjected to the same procedure failed to show improvement in PAH [68].

*2.3.6. Intravenous Administration of Mesenchymal Stem Cells Overexpressing Endothelial Nitric Oxide Synthase (eNOS)* . Bone marrow-derived MSCs transfected by the eNOS gene (MSCs/eNOS) were studied in rats with MCT-induced PAH. One week after MCT administration, the rats received 3 different treatments: MSCs (MSC group), MSCs/eNOS, (MSC/eNOS group), or no treatment (PAH group). As the negative control, rats received saline instead of MCT (control group). RV pressures in the MSC and MSC/eNOS groups were significantly lower than in the PAH group, and RV pressure in the MSC/eNOS group was significantly lower than in the MSC group. Similar results were obtained with regard to RV hypertrophy in the 3 groups. The survival time of rats receiving MSCs/eNOS was significantly longer than survival time of PAH rats without treatment [69].

*2.3.7. Transplantation of Endothelial Progenitor Cells into the Lung.* The effects of EPC transplantation into the lungs were studied in dogs with dehydroMCT-induced PAH. The lung parenchyme was injected with ex vivo-expanded, autologous EPCs using a bronchoscope. EPC transplantation resulted in significant improvements in pulmonary artery pressure, pulmonary vascular resistance and cardiac output. Histological evaluation revealed improvement in the medial thickness of the small pulmonary arteries and neovascularization of lung tissue [70].

*2.3.8. Intravenous Administration of MSCs from Donor Rats Suffering with PAH.* Recently, we completed a study in which rats with MCT-induced PAH were, 14 days after MCT injection, treated with (1) a single i.v. MSC injection ($10^6$ cells/rat) obtained from bone marrow of donor rats with MCT-induced PAH, mimicking autologous stem cell therapy, or (2) a single injection with saline. Two weeks later RV pressures were measured, the rats were sacrificed, and heart and lungs were dissected. The saline-treated MCT rats developed PAH but in the MSC-treated MCT rats the RV pressures were significantly lower. Accordingly, RV hypertrophy in the MSC-treated MCT rats was significantly lower compared to the PBS-treated MCT rats. In this study we have demonstrated that bone marrow-derived MSCs obtained from donor rats suffering from PAH when administered to recipient rats with PAH reduceing RV pressure overload and RV hypertrophy [71].

Table 1: Classification of published therapies of experimental pulmonary hypertension.

| | (Ref.) |
|---|---|
| Antimitogenic (particularly towards PA-SMCs) | |
| PDGF inhibitors | [4] |
| Endothelin receptor blockers | [16–19, 29] |
| Prostacyclin (analogue) | [5–10, 15, 19] |
| Serotonin transporter inhibitors | [21] |
| Serotonin receptor blockers | [23, 24] |
| Serotonic re-uptake inhibitor | [22] |
| PDE 4/5 and 3/4 inhibitors | [9, 10, 25–29] |
| Caveolin-1 peptide | [30] |
| Estradiol (derivatives) | [31–34] |
| Amlodipine | [38] |
| Rapamycin | [42] |
| Cell therapy using differentiated cells transduced with eNOS gene | [60, 62, 63, 68] |
| Prostacyclin synthase gene | [55, 56] |
| NF-$\kappa$B inhibitor | [44] |
| Serine-elastase inhibitors | [3] |
| Proendothelial function, vasodilatation, and proangiogenesis | |
| Rho-kinase inhibitors | [11–15] |
| Statins | [35–37] |
| Nicorandil | [39] |
| Granulocyte-colony stimulating factor | [43] |
| L-arginine | [50] |
| Prostacyclin (analogue) | [5–10, 15, 19] |
| Endothelin receptor blockers | [16–19, 29] |
| PDE 4/5 and 3/4 inhibitors | [9, 10, 25–29] |
| Adrenomedullin | [41] |
| Prostacyclin synthase gene | [55, 56] |
| Angiopoietin-1 gene | [57] |
| Cell therapy using EPCs and MSCs | [59, 63–65, 68–71] |
| Cell therapy using differentiated cells transduced with eNOS gene | [60, 62, 63, 68] |
| Cell therapy using differentiated cells transduced with VEGF gene | [61, 62] |
| Anti-inflammatory and antioxidative | |
| Serotonin receptor blockers | [23, 24] |
| Amlodipine | [38] |
| Nicorandil | [39] |
| Statins | [35–37] |
| C-type natriuretic factor | [40] |
| Interleukin-1 receptor antagonist | [45] |
| Antibodies to monocyte chemotactic and activating factor/MCP1 | [46] |
| Inhibitor of p38 mitogen-activated protein kinase | [47] |
| Inhibitor of lipoxygenase pathways | [51] |
| Antimonocyte chemoattractant protein-1 gene | [54] |
| Interleukin-10 gene | [58] |
| Extracellular superoxide dismutase gene | [53] |

Abbreviations: PA-SMCs: pulmonary artery-derived smooth muscle cells; PDGF: platelet-derived growth factor; PDE: phosphodiesterase; NO: nitric oxide; EPC: endothelial progenitor cells; MSC: mesenchymal stem cells; eNOS: endothelial NO-synthase; VEGF: vascular endothelial growth factor; MCP1: monocyte chemoattractant protein-1.

## 3. Cell Therapy for PAH, from Experimental Models to Clinical Disease

Cell therapy is a promising novel therapeutic option, and several cell types have been tested in experimental models of PAH. EPCs have recently been explored as a potential source for neovascularization of the diseased pulmonary circulation in patients with PAH. A first randomized trial has indicated that i.v. infusion of autologous EPCs to patients with idiopathic PAH appears to be feasible and safe and has beneficial effects on exercise capacity and pulmonary hemodynamics [72].

Also MSC is a cell type that can improve the pulmonary pathology of PAH by either differentiating into other cell types leading to regeneration of the diseased vasculature or secreting an array of substances, the "prosurvival factors" including various growth factors and cytokines, leading to improvement in lung pathology by paracrine mechanisms. Autologous bone marrow-derived MSC therapy has a practical advantage over other types of cell therapies, as the mode of administration is rather simple and has proven to be safe.

## 4. Conclusions

In conclusion, a wealth of therapeutic modalities including pharmacotherapy, gene therapy, and cell therapy has been tested in several animal models of PAH. In these therapeutic modalities we distinguish three approaches: (i) antiproliferative and proapoptotic therapies towards PA-SMCs, (ii) therapies that improve endothelial function, promote angiogenesis, and induce vasodilatation, and (iii) antiinflammatory and antioxidative therapies (seeTable 1 ). Although many of these therapies have demonstrated to be effective in monocrotaline-induced PAH in experimental animals, any therapy to be planned for use in human PAH should be tested before in other animal models of PAH. As many patients remain symptomatic despite therapy, new therapeutic modalities are being developed. As such, cell therapy is a novel treatment option, and stem cell therapy is most promising. Although many questions about optimal cell type, in vitro cell transduction, route of cell administration, and number of cells to inject are waiting for answers, we expect that autologous MSC therapy is a safe and efficacious option to treat patients with PAH.

### *List of Abbreviations*

AAV: Adeno-associated virus
Ang-1: Angiopoietin-1
AP: Drosophila transcription factor atennapedia
ATP: Adenosine triphosphate
cAMP: Cyclic adenosine monophosphate
Cav-1: Caveolin-1
CNP: C-type natriuretic peptide
ECM: Extracellular matrix
EC-SOD: Extracellular superoxide dismutase
eNOS: Endothelial nitric oxide synthase
EPC: Endothelial progenitor cell
ET: Endothelin
G-CSF: Granulocyte colony stimulating factor
AAV: Adeno-associated virus
GFP: Green fluorescent protein
cGMP: Cyclic guanosine monophosphate
GTPase: Guanosine triphosphatase
5-HT: 5-hydroxy tryptamine
5-HTT: 5-hydroxy tryptamine transporter
IL-10: Interleukin-10
MCT: Monocrotaline
MCTp: Monocrotaline pyrrole
2-ME: 2-Methoxyestradiol
MSC: Mesenchymal stem cells
NF-$\kappa$B: Nuclear factor-$\kappa$B
PA-SMC: Pulmonary artery smooth muscle cell
PAH: Pulmonary arterial hypertension
PDE: Phosphodiesterase
PDGF: Platelet derived growth factor
PGIS: Prostacyclin synthase
RV: Right ventricle
SMC: Smooth muscle cell
VCAM-1: Vascular cell adhesion molecule-1
VEGF: Vascular endothelial growth factor.

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