Inhaled PLGA Particles of Rosiglitazone as a Promising Targeted Therapy for PAH
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Purpose
Peroxisome proliferator-activated receptor-γ (PPAR-γ) is a ligand activated nuclear transcription factor (TF) that is known to regulate various cellular and physiological processes including cell differentiation, lipid metabolism, inflammation and tumorigenesis. A series of recent studies point to the involvement of PPAR-γ in the development of pulmonary arterial hypertension (PAH), a debilitating condition that encompasses a group of diseases characterized by a mean pulmonary arterial pressure (mPAP) of ≥25 mmHg at rest and a normal mean pulmonary capillary wedge pressure (≤ 15 mmHg). Pulmonary arterial endothelial (PAECs) and smooth muscle cells (PASMCs) and fibroblast of the pulmonary arteries/arterioles are known to be intricately intertwined in the development and progression of the disease. Although PPAR-γ is profusely expressed in healthy PAECs and PASMCs, its expression in PAECs and PASMCs, collected from PAH afflicted patients and animal models, declines dramatically. Indeed, the expression of PPAR-γ drops significantly in the plexiform lesions of human subjects with PAH. Further, the erasure of PPAR-γ is a therapeutic target for PAH. The thiazolidinediones are a class of ligands that have high affinities for and a potent agonist of PPAR-γ. A series of recent studies point to the involvement of PPAR-γ in PAH pathogenesis, rosiglitazone, a PPAR-γ agonist, appears to have beneficial effects in PAH. Published studies suggest that rosiglitazone mediated activation of PPAR-γ slows PASMC proliferation by modulating cell growth and apoptosis. In this study, we investigated the underlying mechanisms by which rosiglitazone may act on PAECs and PASMCs and assessed the feasibility of PLGA inhalable particulate formulations of rosiglitazone in the treatment of PAH and pulmonary arterial remodeling.

Methods
PASMCs and PAECs treated with 100 μM and 10 μM of rosiglitazone under both normoxic and hypoxic condition for 24 and 48 hr, the expression of eNOS, ET-1, expression of PPAR-γ, NOX-4 expression using western blot analysis. Cell proliferation study, performed using CCK-8 cell counting kit after 24 and 48 hr of rosiglitazone treatment. Pulmonary arterial pressure in a SUGEN-5416 plus hypoxia induced PAH rats. We recorded mPAP and mSAP for 3-6 hours using a fluid filled catheter and PowerLab Data Acquisition System and calculated the percentage reduction in mPAP and mSAP using the initial baseline pressure as 100%. Rats divided into four groups received (i) intra-tracheal saline (100μL), (ii) plain rosiglitazone oral (0.3 mg/kg), (ii) plain rosiglitazone intra-tracheal (0.3 mg/kg), and (iv) optimized rosiglitazone particles via the intratracheal route (equivalent to 0.3 mg/kg rosiglitazone).

Results
When we treated diseased PASMCs and PAECs with 100 μM and 10 μM of rosiglitazone under both normoxic and hypoxic condition for 24 and 48 hr, the expression of eNOS increased but that of ET-1 decreased. The same treatment increased the expression of PPAR-γ in both PAECs and PASMCs but decreased NOX-4 expression only in PASMCs. We assayed all markers using western blot analysis. Moreover, cell proliferation study, performed using CCK-8 cell counting kit after 24 and 48 hr of rosiglitazone treatment, showed inhibition of PASMC proliferation. Thus, mechanistic studies on human derived PAH cells shows that rosiglitazone slows PAH progression by altering the expression of key signaling proteins responsible for arterial remodeling. We have studied the efficacy of the optimized rosiglitazone particles in reducing pulmonary arterial pressure in a SUGEN-5416 plus hypoxia induced PAH rats. Rats divided into four groups received (i) intra-tracheal saline (100μL), (ii) plain rosiglitazone oral (0.3 mg/kg), (ii) plain rosiglitazone intra-tracheal (0.3 mg/kg), and (iv) optimized rosiglitazone particles via the intratracheal route (equivalent to 0.3 mg/kg rosiglitazone). We recorded mPAP and mSAP for 3-6 hours using a fluid filled catheter and PowerLab Data Acquisition System and calculated the percentage reduction in mPAP and mSAP using the initial baseline pressure as 100%. Optimized formulation, as administered via the pulmonary route, produced pulmonary selective vasodilation in PAH animals over an extended period and minimize the systemic exposure of the drug. We observed 30% reduction in PAH after giving plain rosiglitazone and formulation by intra-tracheal route compared with vehicle treated group and orally treated group. When PAH rats were treated with plain rosiglitazone, the reduction in mPAP lasted only for ~30 minutes suggesting the selective vasodilatory effect of pulmonary vasculature and then it returned to its normal baseline. The inhalable PLGA-mircoparticles of rosiglitazone extended the effect of lowering mPAP over a period of ~4.5 hours (Fig. 1).

Conclusion
Using both cellular and intact animal models of PAH, in this study, we showed that rosiglitazone, an oral antidiabetic, can potentially be repurposed into an inhalated formulation for the treatment of PAH.