Pulmonary Arterial Hypertension in Rats Induced by Combination of Semaxanib and a Low Oxygen Environment: Time Course of Pulmonary Artery Pressure Increases Measured by Telemetry

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Introduction
Pulmonary arterial hypertension (PAH) is a chronic disease characterized by sustained elevation of pulmonary arterial pressure that leads to right ventricle failure and death. Pulmonary arterioles in PAH undergo progressive narrowing and/or occlusion. Currently approved therapies for PAH are directed primarily at relief of symptoms by interfering with vasoconstrictive signals, but do not halt the microvascular cytopathological process. The industry is focused on improving the available therapies to treat PAH however clinical relevant models are crucial for testing new treatments. Animal models of PAH usually focus on monocrotaline-induced injury, hypoxia challenge (both acute and chronic), or serotonin overload. In isolation, these models induce hypertrophy and muscularization in pulmonary arterioles resulting in increased pulmonary arterial pressures. However, they do not induce epithelial growth as occurs with PAH in humans. In 2001, Tarsa-Marek et al demonstrated the additive effect of severe hypoxia + VEGF receptor antagonism on pulmonary artery hypertension in rats. The inclusion of VEGF receptor antagonist bovine FGF-1 in a hypoxic environment allows for proangiogenic capillary occlusion by proliferating endothelial cells – an environment more closely resembling the clinical condition.

In this study, we conducted additional validation of this model by evaluating the daily progressive increase in pulmonary artery pressures using telemetry.

Methods

Experimental Plan: Male Sprague-Dawley rats (200-250 g, n=10) were instrumented with Data Science International (DSI, St. Paul, MN, USA) pressure transducers (CP-15, P23XDC, P23XDP) before being placed in a hypobaric chamber (Precision Hypobaric Chambers Inc., New York, NY) for 6 weeks. The chamber was used to house animals up to 20 min. Average oxygen tensions were reduced to 11.3% using oxygen and nitrogen mixtures. During this time, rats received intravenous doses of Phentolamine (0.3 mg/kg) once per day (at 4 PM) for 6 weeks. In a separate group of non-instrumented rats, 10 mg/kg ibuprofen or sildenafil (20 mg/kg twice daily, n=10) was administered by oral gavage during study Days 1-4 and 15.

Telemetric Measurements (Anesthetized): On Day 28, non-instrumented rats were removed from the chamber and anesthetized. A Miller catheter (1.4 French, Millar Instruments, Houston, TX) was inserted into the femoral artery to measure arterial blood pressure. Additionally, the pulmonary artery pressure was measured as described previously (Dong et al., 2011). Pulmonary arterial pressures were automatically recorded by the physiological data acquisition system (DMS, Millar Instruments, Houston, TX) and the data were recorded by a computer.

Telemetric Measurements (Telemetry): On a daily basis, 20 minute epochs of telemetry data were recorded from each rat and used to construct a comparison of pulmonary arterial pressures over the 28 days in this study.

Right Ventricular Hypothyroid Hypertrophy: At the end of the study, rats were euthanized by pentobarbital overdose and hearts were isolated, fixed in saline and dissected to separate the right ventricle from the left ventricle/crown sinus (LV+PV). Dissected samples were weighed and the ratio of the RV to LV+crown sinus weight (RV/LV+crown) for each heart was calculated to obtain an index of RV hypertrophy.

Results

Figure 1: Effects of sildenafil on Systolic Pulmonary Artery Pressure and Right Ventricle to Left Ventricle Ratio in Anesthetized Rats with Semaxanib and Low Oxygen Environment-Induced Pulmonary Arterial Hypertension

Figure 2: Effects of sildenafil on systolic pulmonary arterial pressure in semaxanib and low oxygen environment-induced pulmonary arterial hypertension in rats. (A) Systolic pulmonary arterial pressure (SPP, mmHg) and RV/LV+crown ratio (right ventricle/left ventricle+crown sinus) at baseline and after treatment with sildenafil (20 mg/kg, twice daily) for 6 weeks. *p<0.05 vs. vehicle treated rats. (B) Effect of sildenafil on systolic pulmonary arterial pressure after 6 weeks of semaxanib and low oxygen environment treatment. *p<0.05 vs. vehicle treated rats.

Figure 3: Effects of Semaxanib and a Low Oxygen Environment on Pulmonary Artery Pressure in Telemetered Rats

Figure 4: Effects of Semaxanib and Low Oxygen Environment-Induced Pulmonary Arterial Hypertension

Figure 5: Effect of semaxanib and a low oxygen environment on pulmonary arterial pressure in rats. Data are presented as mean ± S.E.M. (n=10 per group, 6 studies).

Summary
Sildenafil treated rats exhibited systolic pulmonary arterial pressures that were significantly lower (~46% decrease) compared to the vehicle.

There was a decreased right ventricular hypertrophy (as measured by RV/LV+crown ratio) in the sildenafil treated rats compared to the vehicle arm.

The pulmonary artery pressure increase in the model is linear between Weeks 1 and 4. Following this period, the increased PAP begins to plateau. There is limited benefit to continue assessment beyond Week 4 if hemodynamic endpoints are the sole arbiters of efficacy. The optimal time to initiate reversal treatment appears to be at Weeks 2 to 3.

Conclusion
Oral administration of sildenafil reduces PAH induced by semaxanib and a low oxygen environment. The increase in pulmonary artery pressure reaches a plateau after approximately 4 weeks. Putative reversal therapy should be started between two and three weeks after initiation of PAH.

Objectives
Continuing validation of the PAH rat model following extended exposure to hypoxia and VEGF receptor antagonists by evaluating progressive increases in pulmonary artery pressure and examining optimal time window for reversal mode intervention.