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

Jinbao Huang, Liomar A. A. Neves, Peter Senese, Michael R. Gralinski

CorDynamics, Inc., Chicago, IL - www.cordynamics.com

Abstract # P516

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

Jinbao Huang, Liomar A. A. Neves, Peter Senese, Michael R. Gralinski

CorDynamics, Inc., Chicago, IL

Previous work from our labs has demonstrated development of increased terminal pulmonary artery pressures, hypertrophy of pulmonary arterial vascular smooth muscle, and proliferation of the endothelial vascular lumen in rats following extended exposure to hypoxia and VEGF-receptor antagonist. We have also reported the efficacy of bosentan and sildenafil in this preparation after three weeks of treatment. In this study we continue to describe this model by evaluating the time course of PAH development using telemetry catheters implanted in the pulmonary artery. Male Sprague-Dawley rats (250-275 gms) were surgically instrumented with telemetry catheters in the pulmonary artery, allowed to recover, then kept in their home cages inside a hypoxic room. Baseline atmospheric oxygen (21%, Chicago, IL--600 ft above sea level) was reduced to 11.5% (15,500 ft above sea level equivalency) using an oxygen scrubbing generator. Prior to placement inside the room, each rat received a single dose of semaxanib (200 mg/kg, s.c.). Rats were maintained in hypoxia for six weeks and pulmonary artery pressures were recorded daily via telemetry. During the course of six weeks hypoxia following semaxanib treatment, systolic pulmonary artery pressures (SPAP) increased on a daily basis. Baseline values for SPAP (pre-hypoxia) were 36 ± 2 mm Hg. At 14, 28, and 42 days into the study, SPAP was 77 ± 8 mm Hg, 114 ± 11 mm Hg, and 131 ± 6 mm Hg respectively. In summary, this data can support the timing selection to initiate intervention treatment in the context of hypoxia/semaxanib-induced pulmonary arterial hypertension in the rat.

Support: CorDynamics, Inc.

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 cytoproliferative process. The industry is focused in improving the available therapies to treat PAH however clinical relevant models are crucial for testing new articles.

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 overgrowth as occurs with PAH in humans. In 2001, Taraseviciene-Stewart et al demonstrated the additive effect of severe hypoxia + VEGF receptor antagonism on pulmonary artery hypertension in rats. The inclusion of VEGF receptor antagonist (semaxanib) in a hypoxic environment allows for pre-capillary arterial 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.

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.

Methods

Experimental Plan: Male SD rats (250-275 g, n=30) were instrumented with Data Sciences International (St. Paul, MN) C50-PXT, HDS-11, or HDS-21 implantable radiotelemetry devices by CorDynamics personnel. The pressure catheters were advanced through the right ventricle into the pulmonary artery and secured. Animals were allowed to recover from surgery at the vivarium for at least 7 days prior to initiation of semaxanib treatment and introduction of a low oxygen environment. The radiotelemetry devices were used to measure pulmonary artery pressure (PAP) and heart rate (HR); ECG leads were not implanted.

Following recovery, rats received a single dose of semaxanib (200 mg/kg, s.c.; Tocris Biosciences, Ellisville, MO) and were kept inside a commercially available hypoxic chamber (Hypoxico, New York, NY) for 6 weeks. The chamber was used to house up to 30 rats. Atmospheric oxygen was reduced to 11.5% using an oxygen scrubber. During this time, rats received intravenous doses of PlasmaLyte (3.33 mL/kg) once on Study Days 14, 21, and 28.

In a separate group of non-instrumented rats, vehicle (sesame oil, 10 ml/kg, n=10) or sildenafil (30 mg/kg twice daily, n=10) was administered by oral gavage during Study Days 1-28.

Hemodynamic Measurements (Anesthetized): On day 28, non-instrumented rats were removed from the chamber and anesthetized. A Millar 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 (Stinger et al., 1981 Hemodynamic values were automatically calculated by the physiological data acquisition system NOTOCORD-Hem Software 4.1 (NOTOCORD Inc., Croissy Sur Seine, France).

Hemodynamic Measurements (Telemetry): On a daily basis, 30 minutes epochs of telemetry data were recorded from each rat and used to construct comparisons of pulmonary artery pressure over the 42 days in this study.

Right Ventricular Hypertrophy Measurements: At the end of the study, rats were euthanized by pentobarbital overdose and hearts were isolated, flushed with saline and dissected to separate the right ventricle from the left ventricle+septum (LV+S). Dissected samples were weighed and the ratio of the RV to LV+septum weight [RV/LV+S] for each heart was calculated to obtain an index of RV hypertrophy.

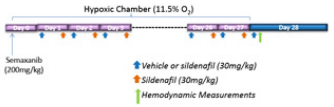

Figure 1. *Experimental design (anesthetized).*

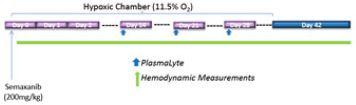

Figure 2. *Experimental design (telemetry).*

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

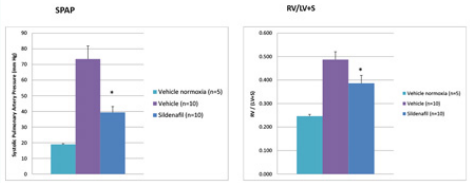

Figure 2. *Effect of sildenafil on hemodynamics and right ventricular hypertrophy in semaxanib and a low oxygen environment induced pulmonary arterial hypertension in rats. Systolic pulmonary arterial pressure (SPAP), and RV to LV + septum (RV/LV+S ratio) ratio in rats injected with semaxanib and submitted to low oxygen environment receiving vehicle (sesame oil, 10mL/kg once daily) or sildenafil (30mg/kg twice daily). Data are presented as mean ± S.E.M. (n=10). * = $p<0.05$ vs. vehicle determined by using a one-way ANOVA followed by post hoc Dunnett's test.*

Effects of Semaxanib and Low Oxygen Environment – Progression of Pulmonary Arterial Hypertension in Telemetered Rats

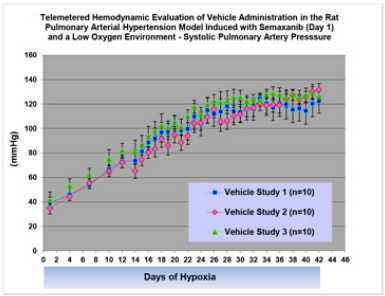

Figure 3. *Effect of semaxanib and a low oxygen environment on pulmonary arterial pressure in rats. Data are presented as mean ± S.E.M. (n=10 per study, 3 studies).*

To download this poster, go to **http://www.cordynamics.com/publications.php**

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 + S 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.