Bosentan and Sildenafil Reduce Pulmonary Arterial Hypertension in Rats Induced by Semaxanib and a Low Oxygen Environment

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Previous work has demonstrated the development of increased pulmonary artery pressures, hypertrophy of the pulmonary arterial vascular smooth muscle, and proliferation of the endothelial vascular lumen in rats following extended exposure to hypoxia and VEGF-receptor antagonists. We validated this model of pulmonary arterial hypertension (PAH) by evaluating its utility as a clinically relevant paradigm to determine the efficacy of test articles against PAH. Male SD rats (240-370 g) were kept inside a hypoxic tent. Atmospheric oxygen was reduced to 13.5% using an oxygen scrubber. Rats received a single dose of semaxanib (200 mg/kg, s.c.) and were maintained inside the tent for 3 weeks. Rats received oral doses of vehicle (20% cyclodextrin, 10mL/kg once daily), bosentan (BS- 300mg/kg once daily) or sildenafil (SL- 30mg/kg twice daily). At day 21, pulmonary and systemic arterial pressures and thoracic organ weights were measured. BS and SL-treated rats exhibited systolic, diastolic and mean pulmonary arterial pressures (21 and 19; 16 and 15; 18 and 17 mmHg, respectively) that were significantly lower compared to the vehicle (30, 22 and 26 mmHg, respectively). There was a trend toward decreased right ventricle to left ventricle weight ratio when comparing BS and SL with the vehicle. There were no significant differences in mean arterial pressure among rats treated with BS and SL as compared to vehicle. In summary, oral administration of BS and SL reduces PAH induced semaxanib and a low oxygen environment in a clinically relevant model.

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 validated this model of pulmonary arterial hypertension (PAH) by evaluating its utility as a clinically relevant paradigm to determine the efficacy of test articles against PAH.

Objectives

Validated the PAH rat model following extended exposure to hypoxia and VEGF-receptor antagonists by evaluating its utility as a clinically relevant paradigm to determine the efficacy of test articles against PAH.

Methods

Experimental Plan: Male SD rats (240-370 g) received a single dose of semaxanib (200 mg/kg, s.c.; Tocris Biosciences, Ellisville, MO) and were kept inside a commercially available hypoxic tent (Hypoxico, New York, NY) for 3 weeks. The tent was used to house up to 30 rats. Atmospheric oxygen was reduced to 13.5% using an oxygen scrubber. During this time, rats received oral doses of vehicle (20% cyclodextrin, 10 mL/kg once daily), bosentan (300 mg/kg once daily) or sildenafil (30 mg/kg twice daily).

Hemodynamic Measurements: On day 21, rats were removed from the tent and anesthetized by intra-muscular injection of ketamine/ xylazine (80/10 mg/kg) and placed on a heating pad to maintain body temperature at 37°C. 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). Briefly, a 3.5 fr umbilical vessel catheter (Utah Medical Products LTD, Midvale, UT), angled to 90° over the distal 1 cm and curved slightly at the tip, was introduced into the right external jugular vein. With the angle directed interiorly, the catheter was inserted proximally, which placed the catheter in the right atrium. The catheter was rotated 90° counterclockwise and inserted further, which placed the catheter in the right ventricle, and then advanced approximately 1.5 cm, into the pulmonary artery. Placement at each stage was confirmed by monitoring the respective pressure contours. Hemodynamic values were automatically calculated by the physiological data acquisition system NOTOCORD-Hem Software 4.1 (NOTOCORD Inc., Croissy Sur Seine, France).

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.

Plasma Concentration of Bosentan and Sildenafil: Approximately two hours post-dose, before the anaesthetized rats were euthanized, approximately 1 mL of blood was collected in presence of K_2EDTA. Plasma was separated, frozen on dry ice, and stored at -80°C until determination of Bosentan and Sildenafil concentration.

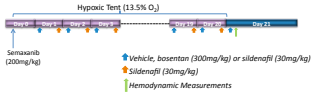

Figure 1. Experimental design.

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

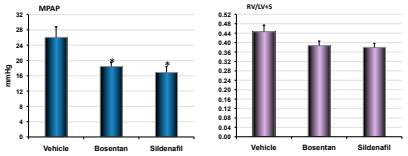

*Figure 2. Effect of bosentan and sildenafil on hemodynamics in semaxanib and a low oxygen environment induced pulmonary arterial hypertension in rats. Mean pulmonary arterial pressure (PAP), and RV to LV + septum (RV/LV+S ratio) ratio in rats injected with semaxanib and submitted to low oxygen environment receiving vehicle (20% cyclodextrin, 10mL/kg once daily), bosentan (300mg/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 Bosentan and Sildenafil on Mean Arterial Pressure and Heart Rate in Anesthetized Rats with Semaxanib and Low Oxygen Environment-Induced Pulmonary Arterial Hypertension

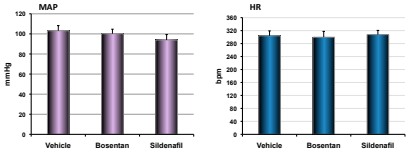

Figure 3. Effect of bosentan and sildenafil on hemodynamics in semaxanib and a low oxygen environment induced pulmonary hypertension in rats. Mean arterial pressure (MAP), and heart rate in rats injected with semaxanib and submitted to low oxygen environment receiving vehicle (20% cyclodextrin, 10mL/kg once daily), bosentan (300mg/kg once daily) or sildenafil (30mg/kg twice daily). Data are presented as mean ± S.E.M. (n=10).

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Plasma Concentration of Bosentan and Sildenafil 2hrs post-Dose in Male Rats Injected with Semaxanib and Submitted to Low Oxygen Environment

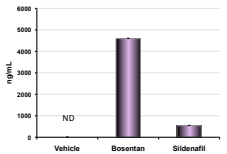

Figure 4. Plasma concentration of bosentan (300mg/kg once daily) and sildenafil(30mg/kg twice daily) in male rats injected with semaxanib and submitted to low oxygen environment. Plasma was obtained on the 21th day of consecutive oral dosing, 2hrs post-dose. Values are expressed as mean±SEM (n=10). ND- non-detectable.

Summary

- Bosentan and sildenafil treated rats exhibited systolic, diastolic and mean pulmonary arterial pressures that were significantly lower (29-36% decrease) as compared to the vehicle.
- There was a trend toward decreased right ventricular hypertrophy (as measured by RV/LV + S ratio) in the bosentan and sildenafil treated rats, but these effects did not reach statistical significance when compared to the vehicle arm.
- No significant differences in heart rate or systemic arterial pressure (or its components; systolic, diastolic pressures) were noted for rats treated with bosentan and sildenafil as compared to vehicle.

Conclusion

Oral administration of bosentan and sildenafil reduces PAH induced by semaxanib and a low oxygen environment demonstrating that this is a clinically relevant model to evaluate efficacy of test articles.