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RP5063, a novel, multimodal, serotonin receptor modulator, prevents Sugen 5416-hypoxia–induced pulmonary arterial hypertension in rats

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ABSTRACT

RP5063, a multimodal dopamine (DA) and serotonin (5-HT) modulator with high affinity for $DA_{2/3/4}$ and 5-$HT_{2A/2B/7}$ receptors and moderate affinity for SERT, is a novel therapeutic of special interest in the treatment of pulmonary arterial hypertension (PAH). Evidence indicates that therapeutics targeting the 5-$HT_{2A/2B}$ receptors can influence the pathogenesis of PAH. However, the therapeutic effect of RP5063 in humans has yet to be investigated. A Sugen 5416-hypoxia (SuHx)–induced PAH model was used to evaluate twice-daily (b.i.d.) RP5063 at 10 mg/kg (RP-10) and 20 mg/kg (RP-20), as compared with positive (sildenafil 50 mg/kg b.i.d.; Sil-50) and negative controls (SuHx+vehicle; SuHx+veh), in 24 adult male Wistar-Kyoto rats. RP5063 showed significantly lower systolic pulmonary arterial (both doses) and systolic right ventricular (RP-10) pressures, and improvement in oxygen saturation (RP-20). It significantly reduced small-vessel wall thickness (RP-20), lowered the percentage of muscular vessels (both doses). Both doses limited arterial obliteration due to endothelial cell proliferation, prevented plexiform lesion formation, and stemmed the release of leukotriene B4. Sildenafil showed statistically greater effects on vessel structure than that seen in both RP5063 groups and improved oxygen saturation. Additionally, Sildenafil did not demonstrate any significant effect on arterial obliteration, plexiform lesion development, or pulmonary arterial or right ventricular pressure. As PAH gains in severity, the impact of RP5063 inhibition of $5HT_{2B}$ increases, preventing arterial constriction and improving pulmonary hemodynamics. Due to its functional, structural, and chemokine effects, RP5063 represents a promising candidate for investigation in late-phase PAH.

1. Introduction

Pulmonary arterial hypertension (PAH) is a progressive condition defined by increased pulmonary artery pressure due to pulmonary arterial constriction/remodeling (Bogaard et al., 2009; Frumkin, 2012). These changes cause right ventricle (RV) pressure overload, hypertrophy, and failure, leading to death (Kamar et al., 2016; Voelkel et al., 2006). PAH affects 15–50 cases per 1,000,000 (Peacock et al., 2007). Survival rates at 5 and 7 years following diagnosis are 57% and 49%, respectively (Benza et al., 2012).

Treatment involves use of the following strategies: 1— decreasing phosphodiesterase 5 (PDE-5) expression; 2— blocking endothelin; and 3— supplementing prostacyclins (Christman et al., 1992; Corbin et al., 2005; Galie et al., 2009; Humbert et al., 2004a, 2004b; McLaughlin et al., 2009). However, these approaches fail to ameliorate the underlying cytoproliferation that influences the pulmonary vascular structure (Zopf et al., 2011). Thus, new therapies are needed to stem these cytoproliferative processes that influence PAH development and progression.

Modulation of serotonin (5-HT) is of great interest. This mediator exerts its effects through the 5-$HT_{1B/2A/2B}$ receptors and the 5-HT transporter (SERT); all are enhanced in pulmonary artery smooth muscle and endothelial cells in the setting of PAH (Baliga et al., 2011; Dumitrascu et al., 2011; Esteve et al., 2007; Farber and Loscalzo, 2004; Humbert et al., 2004a, 2004b; MacLean and Dempsie, 2010). These 5-HT-receptor and SERT interactions stimulate pulmonary artery smooth muscle cell and fibroblast proliferation, leading to medial layer thickening and pulmonary artery narrowing and remodeling (Welsh et al., 2004).

Evidence suggests that 5-HT receptor targeting can influence the pathogenesis of PAH (Dumitrascu et al., 2011; Liu et al., 2012; Porvasnik et al., 2010; Welsh et al., 2004). SB204741, a 5-HT_{2B}

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antagonist, was found to reduce inflammatory cell recruitment to the lungs, pulmonary artery smooth muscle muscularization, and vascular stiffness by limiting 5-HT effects on Src tyrosine kinase and downstream activity (West et al., 2016). Terguride, a potent 5-$HT_{2A/2B}$ antagonist, was reported to suppress pulmonary vessel smooth muscle inflammatory histopathologic changes and proliferation, reduce inflammatory cytokines, and inhibit pulmonary vasoconstriction (Dumitrascu et al., 2011). PRX08066, a 5-HT_{2B} antagonist, was shown to reduce vascular remodeling, pulmonary arterial pressure (PAP), and RV hypertrophy (Porvasnik et al., 2010; Shacham et al., 2006). Rosiglitazone, a peroxisome proliferator-activated receptor γ agonist, was demonstrated to reverse pulmonary artery vasoconstriction and thickening due to 5-HT_{2B} induction (Liu et al., 2012).

RP5063, a novel, multimodal dopamine (DA) and 5-HT modulator, shows high affinity for $DA_{2/3/4}$ and 5-$HTR_{2A/2B//7}$ receptors and moderate affinity for SERT (Cantillon et al., 2017). Targeting multiple 5-HT receptors may provide an attractive option for PAH, while potentially reducing comorbid mental disorders that can exist in up to 35% of PAH patients (Verma et al., 2016). RP5063 brings an established efficacy, safety, and pharmacokinetic profile based on data from a phase 1 evaluation and phase 2 studies of acute schizophrenia and schizoaffective disorders (Cantillon et al., 2017).

This study was undertaken to evaluate RP5063 efficacy in the mitigation of the symptoms of PAH induced by Sugen 5416 and hypoxia (SuHx) in rats.

2. Materials and methods

2.1. Experimental design

In this parallel-design study, 38 male Wistar–Kyoto rats (weights: 200–250 g; ages: 10–12 weeks; Charles River Laboratories, St Constant, Quebec, Canada) were randomized according to their body weight into five experimental groups. The study schedule was such that an equal number of animals per treatment group were monitored and processed on each terminal surgery day (when possible).

The institutional animal ethics committee of IPS Therapeutique (IPST) approved the study in accordance with the principles of the Canadian Council on Animal Care (CCAC). IPST identified, housed, and cared for the animals per the CCAC guidelines.

On Day 0, dimethyl sulfoxide (DMSO; Sigma-Aldrich Canada Ltd.) solution (0.5 ml) was injected subcutaneously into five healthy animals (Group 1), and Sugen solution (SU5416, 10 mg/ml diluted in DMSO, Sigma-Aldrich Canada Ltd.) dosed at 20 mg/kg was administered subcutaneously to 33 animals (Groups 2–5). Animals in Groups 2–5 were kept under hypoxic conditions (FiO_2 of 10%) for 3 weeks (Days 0–21), then in ambient oxygen levels (FiO_2 of 21%) for 2 weeks (Days 22–35). From Day 14–35, these animals were gavaged b.i.d. as follows: 1— Group 2 (vehicle, SuHx+veh; n=8); 2— Group 3 (RP5063 10 mg/kg (RP-10); n=10); 3— Group 4 (RP5063 20 mg/kg (RP-20); n=10); and 4— Group 5 (sildenafil 50 mg/kg (Sil-50); n=5).

The 10 and 20 mg/kg b.i.d. doses of RP5063 were prepared by dissolving 300 or 600 mg of active drug into 400 ml of sterile 5% glucose solution, resulting in solutions of 0.75 mg/ml or 1.5 mg/ml, respectively. For sildenafil, 1895 mg of active drug was dissolved in 500 ml of sterile 5% glucose solution to obtain a solution of 3.79 mg/ml. To deliver the vehicle or treatment, the investigators administered 13.33 ml/kg of the resultant solutions b.i.d.

During the treatment period, the rats were given food and water ad libitum. They were observed daily for behavior and general health status, and had blood samples, body weights, and body temperature taken weekly.

On Day 35, anesthetized animals were instrumented. Hemodynamic parameters were recorded continuously for at least 5 min or until loss of quality in the PAP signal. These recordings involved moving a flexible fluid filed catheter attached to a pressure transducer from the right ventricular (RV) cavity, through the pulmonary valve, into the pulmonary artery and observing the clear transitions in diastolic pressures and general pressure waveforms, as the catheter transitioned from the ventricle into the artery. At the end of the recording, a blood sample was collected. After the animal was exsanguinated, the pulmonary circulation was flushed with 0.9% NaCl, and tissues were harvested for further analysis.

2.2. Parameters measured on surgery day

On Day 35, a variety of parameters were obtained. Cardiac activity was monitored using three electrocardiographic (ECG) contact electrodes (Harvard BioSciences Inc., Holliston, MA) placed in a lead-I/II configuration and connected to an IsoDam8 differential amplifier (World Precision Instruments, LLC, Sarasota, FL). Heart rate (HR) was recorded using duplicate systems: from the ECG records (RR-intervals) and using an N-595 pulse oximeter (Nellcor, Plymouth, MN) attached to the left front paw of the animal. Blood oxygen saturation (SO_2) was measured using a pulse oximeter signal attached to the left front paw of the animal. Saturation values were measured in percentages using cursor readings in Clampfit 10.2.014.

Systemic arterial blood pressure (SAP) was monitored using an intra-arterial fluid-filled catheter connected to a pressure transducer (AD Instruments, Colorado Springs, CO), with diastolic and systolic pressures values measured in mm Hg using Clampfit 10.2.0.14. Calculation of mean SAP (mSAP) and pulse pressure (PP) used the following formulas: 1— mSAP = diastolic systemic pressure + ([systolic systemic pressure − diastolic systemic pressure] / 3); and 2— PP = systolic systemic pressure − diastolic systemic pressure.

RV systemic pressure (RVSP) and PAP were measured using an intraventricular fluid-filled catheter connected to a pressure transducer (AD Instruments). Diastolic and systolic pressures were measured in mm Hg using Clampfit 10.2.0.14 readings. Calculation of mean RVSP (mRVSP) and mean PAP (mPAP) values was performed as described for the mSAP. All hemodynamic parameters were digitized using a Digidata 1440A interface (Axon Instrument Inc., Foster City, CA), and acquired/displayed using Axoscope 10.2.0.14 (Axon Instrument Inc).

Organ weights were expressed as relative percentages and were calculated as follows: Relative organ weight = (organ weight × 100) / body weight. For the heart, Fulton's index (RV vs. left ventricle ratio) was calculated as such: Fulton's index = RV weight / (left ventricular + septum (LV+S) weight).

2.3. Histological preparation and categorization

Trachea, lungs, and heart were removed together; the liver was extracted separately. All tissues were weighed, including the right and left lobes of the lung, as well as the wet weights of the RV and LV+S. All tissues were fixed by perfusion in 10% neutral buffered formalin (NBF).

A transversal section of the middle left lobe was cut and forwarded in 10% NBF to the Institute for Research in Immunology and Cancer (Montreal, Quebec, Canada). Tissues were embedded, sliced (5-μm thickness), mounted, and conventionally stained with hematoxylin and eosin (H & E). Glass slides containing fixed and stained tissues were then visualized at 200 × magnification (Eclipse T100 microscope, Nikon, Melville, NY). At least 15 non-overlapping view fields/lung were selected for microphotographs (Nikon DS-Fi1 digital camera with Nikon NIS Elements 4.30, Nikon, and Melville, NY).

All vessels in a tissue section were analyzed, from largest to smallest, with no threshold or limit set in vessel size. Intra-acinar vessels were included. Vessels associated with terminal bronchioles and all larger airways were excluded. Vessels were divided based on lumen diameter: 1— *small* (10–50 μm); 2— *medium* (50–100 μm); and 3— *large* (> 100 μm). Diameters were measure using Infinity Analyze software v5.03 (Lumenera Corp., Ottawa, Ontario, Canada) at the widest point of the lumen, measured perpendicular to the long axis of