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N-acylhydrazone derivative ameliorates monocrotaline-induced pulmonary hypertension through the modulation of adenosine AA2R activity

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ABSTRACT

Background: Pulmonary arterial hypertension (PAH) is a disease that results in right ventricular (RV) dysfunction. While pulmonary vascular disease is the primary pathological focus, RV hypertrophy and RV dysfunction are the major determinants of prognosis in PAH. The aim of this study was to investigate the effects of (E)-*N*′-(3,4-dimethoxybenzylidene)-4-methoxybenzohydrazide (LASSBio-1386), an *N*-acylhydrazone derivative, on the lung vasculature and RV dysfunction induced by experimental PAH.

Methods: Male Wistar rats were injected with a single dose (60 mg/kg, i.p.) of monocrotaline (MCT) and given LASSBio-1386 (50 mg/kg, p.o.) or vehicle for 14 days. The hemodynamic, exercise capacity (EC), endothelial nitric oxide synthase (eNOS), adenosine A_{2A} receptor (A_{2A}R), sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA2a), phospholamban (PLB) expression, Ca^{2+}-ATPase activity and vascular activity of LASSBio-1386 were evaluated.

Results and conclusions: The RV systolic pressure was elevated in the PAH model and reduced from 49.6 ± 5.0 mm Hg (MCT group) to 27.2 ± 2.1 mm Hg (MCT + LASSBio-1386 group; $P < 0.05$). MCT administration also impaired the EC, increased the RV and pulmonary arteriole size, and promoted endothelial dysfunction of the pulmonary artery rings. In the PAH group, the eNOS, A_{2A}R, SERCA2a, and PLB levels were changed compared with the control; in addition, the Ca^{2+}-ATPase activity was reduced. These alterations were related with MCT-injected rats, and LASSBio-1386 had favorable effects that prevented the development of PAH. LASSBio-1386 is effective at preventing endothelial and RV dysfunction in PAH, a finding that may have important implications for ongoing clinical evaluation of A_{2A}R agonists for the treatment of PAH.

1. Introduction

Pulmonary arterial hypertension (PAH) is a subset of pulmonary hypertensive syndromes, defined by a resting mean pulmonary arterial pressure (PAP) >25 mm Hg, pulmonary vascular resistance (PVR) >3 Wood units, and pulmonary wedge pressure <15 mm Hg, in the absence of other causes [1,2]. However, all are characterized by excessive pulmonary vasoconstriction and abnormal vascular remodeling processes that usually affect all vessel layers (intima, media, and adventitia) and result in severe loss of cross-sectional area and, therefore, increased right ventricular (RV) afterload [3]. Large pulmonary artery compliance is also decreased, contributing to strain on the RV [3]. Although the pathogenesis of PAH is incompletely understood, evidence suggests that PAH is associated with activation of inflammatory processes, endothelial damage and dysfunction, and abnormal coagulation [4]. The modification of pulmonary vascular structures causes an increase of resistance against which the RV has to work and induces RV dysfunction [5]. Still, our understanding of these pathobiological processes and pharmacotherapies to treat the disease is limited, and survival outcomes have remained poor over the past few decades [6].

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Adenosine is a potent modulator of cardiovascular function. When administered systemically, adenosine produces hypotension and bradycardia. These effects are thought to be mediated by different adenosine receptors localized in the periphery (heart and vasculature), particularly the adenosine A_{2A} subtype [7]. Adenosine A_{2A}receptors (A_{2A}R) are located primarily in the vasculature where they mediate vasodilatation [8], and in the heart they promote cardioprotective effects [9]. Previous studies have shown that A_{2A}R activation also reduces endothelial dysfunction in monocrotaline (MCT)-induced PAH [10], and its constitutive overexpression in young mice was associated with elevated cardiac contractility, increased sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA2a) expression, and calcium uptake by the sarcoplasmic reticulum Ca^{2+}-ATPase, suggesting salutary benefits of the A_{2A}R on cardiac function and cardiac hemodynamics [9]. Current therapies for chronic PAH are designed to reduce pulmonary arterial resistance by inducing vasodilatation, but these therapies only provide symptomatic relief. Recently, we have shown that an *N*-acylhydrazone derivative from safrole, a substance present in sassafras oil, may contribute to the prevention of MCT-induced PAH by reversing pulmonary vascular remodeling, which in turn reduces RV hypertrophy [10]; but our understanding of these pathological processes and the available pharmacotherapies remains limited. In the present study, we investigated the efficacy and a possible molecular mechanism of (E)-*N*′-(3,4-dimethoxybenzylidene)-4-methoxybenzohydrazide (LASSBio-1386), a new compound of the *N*-acylhydrazone class synthesized by our group (Fig. 1A), in MCT-induced PAH rats.

2. Methods

2.1. Drugs and reagents

LASSBio-1386 was synthesized by Laboratório de Avaliação e Síntese de Substâncias Bioativas at Universidade Federal do Rio de Janeiro, Brazil. LASSBio-1386 was dissolved in dimethylsulfoxide (DMSO) which was purchased from Merck (Darmstadt, Germany). Phenylephrine (Phe), acetylcholine (ACh), and ZM 241385 were dissolved in distilled water and were purchased from Sigma Aldrich (St. Louis, MO, USA). MCT was dissolved in 1 N HCl, neutralized with 0.5 N NaOH and diluted with phosphate-buffered saline (PBS).

Primary antibodies to A_{2A}R, eNOS, SERCA2a, phospholamban (PLB), and GAPDH were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-rabbit IgG-HRP secondary antibody was obtained from Abcam Corporation (Cambridge, MA, USA). Anti-alpha-smooth muscle actin antibody was purchased from Sigma (A5228, St. Louis, MO, USA).

2.2. Animals and experimental design

The protocols used were approved by the Animal Care and Use Committee at the Universidade Federal do Rio de Janeiro. Male Wistar rats (220–300 g) were randomly divided into four groups (6 rats each) housed at 20 ± 3 °C under a 12-h light/12-h dark cycle with free access to food and water as follows: (1) control rats were given saline, (2) MCT rats were given saline 0.2 mL, (3) MCT rats were given DMSO 0.2 mL, and (4) MCT rats were given LASSBio-1386 at 50 mg/kg body weight. MCT (60 mg/kg body weight) was administered intraperitoneally to induce PAH [10–12]. Sterile saline was used as the control. Two weeks after MCT or saline administration, rats were dosed via oral gavage once daily for 2 weeks with DMSO only or LASSBio-1386. Rats were weighed daily, and the dosages of LASSBio-1386 were adjusted appropriately.

2.3. Effects of LASSBio-1386 on isolated pulmonary arteries

The pulmonary artery rings were removed from normal male Wistar rats (220–300 g), cleaned of connective tissue, and prepared for isometric tension recording, as previously described [10]. After an equilibration period of 2 h at 1.5 g resting tension, the preparations were contracted with Phe (10 μM) and exposed to ACh (10 μM) to test the endothelium integrity. Vascular endothelium was considered intact when the ACh-induced relaxation was >60% of the Phe-induced contraction. For experiments in which the vascular endothelium was mechanically removed, the ACh-induced relaxation was <10%. Increasing concentrations of LASSBio-1386 (5×10^{-6} to 5×10^{-4} M) were added at the plateau of Phe-induced contraction. ZM 241385 (10^{-7} M), a selective antagonist of the adenosine A_{2A} receptor (A_{2A}R) [13], was used to evaluate the possible mechanism mediating the effects of the derivative. Experiments with vehicle alone were performed on endothelium-intact rings to eliminate possible interference to the contractile response. Treatment with the maximum concentration of DMSO (0.2% v/v) did not significantly alter the vascular contractility.

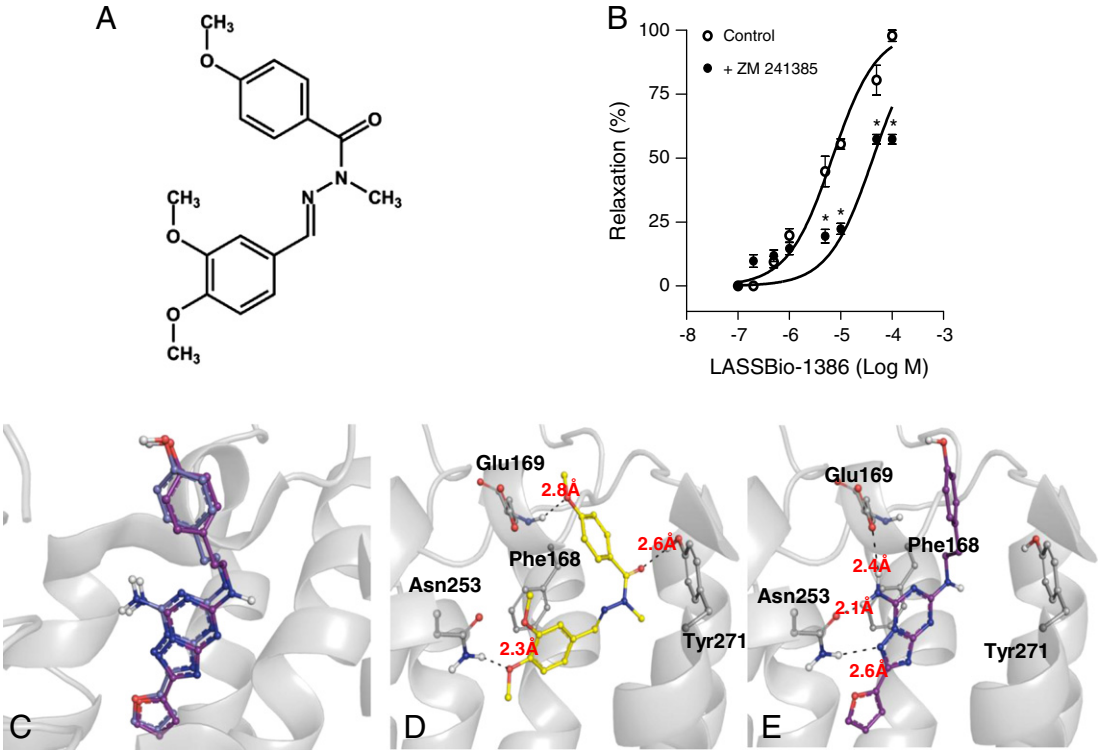

Fig. 1. (A) Chemical structure of (E)-*N*′-(3,4-dimethoxybenzylidene)-4-methoxybenzohydrazide (LASSBio-1386). (B) Concentration-response curves for LASSBio-1386 in pulmonary artery rings from normal Wistar rats, contracted with phenylephrine (10^{-5} mol/L), in the presence or absence of ZM 241385 (10^{-7} mol/L). Data are mean ± SEM ($n = 5$). *$P < 0.05$ compared to control. (C) Superposition of ZM241385 conformation in the crystal structure of A_{2A} receptor (purple) and that obtained after re-docking (light purple) using the program GOLD 5.2. RMSD = 0.63 Å. (D) Binding mode predicted of LASSBio-1386 and its interactions in A_{2A} adenosine receptor. (E) Interactions of the co-crystallized antagonist ZM 241385 in the A_{2A} adenosine receptor (PDB ID: 3EML).