Lodenafil treatment in the monocrotaline model of pulmonary hypertension in rats*

Tratamento com lodenafila no modelo de hipertensão pulmonar induzida por monocrotalina em ratos

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Abstract

We assessed the effects of lodenafil on hemodynamics and inflammation in the rat model of monocrotaline-induced pulmonary hypertension (PH). Thirty male Sprague-Dawley rats were randomly divided into three groups: control; monocrotaline (experimental model); and lodenafil (experimental model followed by lodenafil treatment, p.o., 5 mg/kg daily for 28 days) Mean pulmonary artery pressure (mPAP) was obtained by right heart catheterization. We investigated right ventricular hypertrophy (RVH) and IL-1 levels in lung fragments. The number of cases of RVH was significantly higher in the monocrotaline group than in the lodenafil and control groups, as were mPAP and IL-1 levels. We conclude that lodenafil can prevent monocrotaline-induced PH, RVH, and inflammation.

Keywords: Hypertension, pulmonary; Monocrotaline; Interleukin-1.

Resumo

Avaliamos os efeitos da lodenafila na hemodinâmica e inflamação no modelo experimental de hipertensão pulmonar (HP) induzida por monocrotalina em ratos. Trinta ratos Sprague-Dawley foram randomicamente distribuídos em três grupos: controle, monocrotalina (modelo experimental) e lodenafila (modelo experimental e tratado com 5 mg/kg lodenafila v.o. por 28 dias). A pressão média de artéria pulmonar (PAPm) foi obtida por cateterismo cardíaco direito. Foram determinados a hiperтроfia ventricular direita (HVD) e os níveis de IL-1 em fragmentos de pulmão. O grupo monocrotalina apresentou valores significativamente maiores de PAPm, HVD e IL-1 em comparação aos grupos controle e lodenafila. Concluímos que a lodenafila pode prevenir o desenvolvimento de HP, HVD e inflamação.

Descritores: Hipertensão pulmonar; Monocrotalina; Interleucina-1.

Pulmonary arterial hypertension (PAH) is a poor prognosis disease, which is characterized by endothelial cell proliferation, hypertrophy and proliferation of muscle cells of the media of the pulmonary arteries, reduction of the vascular lumen, and development of plexiform lesions. The reduction of the vascular lumen leads to an increase in pulmonary vascular resistance, causing right ventricle (RV) hypertrophy, cor pulmonale, and death. In addition, PAH is a public health problem, since schistosomiasis, which is one of its causes, reaches epidemic proportions in developing countries. These pathways have been discovered using experimental models of PAH, chief among which is the monocrotaline model. Many of the drugs available for the treatment of PAH have been tested using this model.

The monocrotaline model is simple, inexpensive, and feasible, being routinely used in the initial analysis of drugs with potential effects on pulmonary circulation. Monocrotaline is an alkaloid derived from the seeds of the plant Crotalaria spectabilis; after undergoing oxidation in the

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liver, monocrotaline produces its toxic metabolite that will cause vasculitis and medial thickening of the pulmonary arteries and arterioles.\(^{(4)}\) Within 22 days after injection of monocrotaline, there is significant PAH.\(^{(5)}\)

Lodenafil carbonate is a new phosphodiesterase-5 inhibitor consisting of two lodenafil molecules attached to a carbonate bridge that behaves as a pro-drug, releasing lodenafil as an active metabolite. Its safety in treating erectile dysfunction is well established in preclinical and clinical studies; however, it has never been tested in treating PAH.\(^{(6)}\)

The objective of the present study was to assess the response to administration of lodenafil, in terms of hemodynamics and inflammation, in an experimental model of monocrotaline-induced PH.

All animals were handled humanely, in accordance with international standards for animal care.\(^{(7)}\) The study was approved by the Research Ethics Committee of the University of São Paulo School of Medicine, located in the city of São Paulo, Brazil.

Thirty male Sprague-Dawley rats (weight, 250–300 g) were randomly divided into three groups: control group, in which the animals were given a subcutaneous injection of saline (1 mL/kg) at the study outset (D0); monocrotaline group, in which the animals were given a subcutaneous injection of monocrotaline (60 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) on D0; and lodenafil group, in which the animals were given a subcutaneous injection of monocrotaline (60 mg/kg; Sigma-Aldrich) on D0 and were given lodenafil p.o. (5 mg/kg) once daily between D0 and day 28 of the study (D28).

On D28, after deep sedation with xylazine hydrochloride (i.p., 0.3 mg/kg; Rompun\(^*\); Bayer, Leverkusen, Germany) and ketamine hydrochloride (i.p., 10 mg/kg; Ketalar\(^*\); Pfizer, New York, NY, USA), the animals were weighed. Subsequently, hemodynamic measurements were performed, being followed by euthanasia (abdominal aortic bleeding) and removal of heart and lung tissue.

The hemodynamic measurements were performed by inserting an umbilical catheter into the external jugular vein, the catheter being connected to a pressure transducer (HP 1295C; Hewlett-Packard, Palo Alto, CA, USA) coupled to a hemodynamic monitor (Monitox Dx 2020; Hewlett-Packard), in accordance with a technique described in a previous study.\(^{(8)}\) Mean pulmonary artery pressure (mPAP) was thus measured.

The RV was dissected from the left ventricle (LV), the interventricular septum (S) having remained attached to the LV (LV+S). The ratio of RV weight to LV+S weight (i.e., RV/LV+S) was taken as the index of RV hypertrophy.\(^{(8)}\)

To assess the degree of inflammation, IL-1 levels were determined with a capture ELISA using a commercial IL-1 kit (R&D System Inc., Minneapolis, MN, USA).\(^{(9)}\) Peptide levels were measured in frozen lung fragments.

For the statistical analysis, ANOVA with post hoc Bonferroni correction was used to compare continuous variables among the groups. Values of p < 0.05 were considered significant.

Rats in the monocrotaline group developed PAH, as shown in Figures 1 and 2, as well as experiencing a significant increase in mPAP and RV hypertrophy.

Rats in the lodenafil group had significantly lower mPAP than did those in the monocrotaline group, and there was no significant difference between the former and those in the control group, i.e., lodenafil prevented the development of PAH (Figure 1). The same pattern was observed for the remodeling of the RV and for IL-1 levels (Figure 2).

To our knowledge, the present study is the first to demonstrate that lodenafil was able to prevent the development of PAH in an experimental model of monocrotaline-induced disease.

It is clear that experimental PAH models do not mimic human PAH cases closely enough. There are several factors that may be related to this limitation, among which are the speed of the onset of PAH, which occurs over years in humans but progresses rapidly in animal models, and

![Figure 1](http://dx.doi.org/10.1590/S1806-37132014000400010)
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In conclusion, lodenafil prevented the development of PAH and the remodeling of the RV in rats subjected to an experimental model of PAH. Our findings provide the first basis for the development of clinical studies to investigate the potential of lodenafil in the treatment of human PAH.

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