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Protective effects of phosphodiesterase inhibitors on lung function and remodeling in a murine model of chronic asthma

H.S. Campos[1], D.G. Xisto[1], M.B.G. Oliveira[1], I. Teixeira[1], E.M. Negri[5], T. Mauad[6], D. Carnielli[6], L.M. Lima[4], E.J. Barreiro[4], D.S. Faffe[2], W.A. Zin[2], J.R. Lapa e Silva[3] and P.R.M. Rocco[1]

[1]Laboratório de Investigação Pulmonar, [2]Laboratório de Fisiologia da Respiração, Instituto de Biofísica Carlos Chagas Filho, [3]Instituto de Doenças do Tórax, Hospital Universitário Clementino Fraga Filho, [4]Laboratório de Avaliação e Síntese de Substâncias Bioativas (LASSBio), Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde, Rio de Janeiro, RJ, Brasil
[5]Laboratório de Biologia Celular (LIM 59), [6]Departamento de Patologia, Universidade de São Paulo, São Paulo, SP, Brasil

Abstract

The aim of the present study was to compare the efficacy of a novel phosphodiesterase 4 and 5 inhibitor, LASSBio596, with that of dexamethasone in a murine model of chronic asthma. Lung mechanics (airway resistance, viscoelastic pressure, and static elastance), histology, and airway and lung parenchyma remodeling (quantitative analysis of collagen and elastic fiber) were analyzed. Thirty-three BALB/c mice were randomly assigned to four groups. In the asthma group (N = 9), mice were immunized with 10 μg ovalbumin (OVA, *ip*) on 7 alternate days, and after day 40 they were challenged with three intratracheal instillations of 20 μg OVA at 3-day intervals. Control mice (N = 8) received saline under the same protocol. In the dexamethasone (N = 8) and LASSBio596 (N = 8) groups, the animals of the asthma group were treated with 1 mg/kg dexamethasone disodium phosphate (0.1 mL, *ip*) or 10 mg/kg LASSBio596 dissolved in dimethyl sulfoxide (0.2 mL, *ip*) 24 h before the first intratracheal instillation of OVA, for 8 days. Airway resistance, viscoelastic pressure and static elastance increased significantly in the asthma group (77, 56, and 76%, respectively) compared to the control group. The asthma group presented more intense alveolar collapse, bronchoconstriction, and eosinophil and neutrophil infiltration than the control group. Both LASSBio596 and dexamethasone inhibited the changes in lung mechanics, tissue cellularity, bronchoconstriction, as well as airway and lung parenchyma remodeling. In conclusion, LASSBio596 at a dose of 10 mg/kg effectively prevented lung mechanical and morphometrical changes and had the potential to block fibroproliferation in a BALB/c mouse model of asthma.

Correspondence
P.R.M. Rocco
Laboratório de Investigação Pulmonar
Instituto de Biofísica Carlos
Chagas Filho, CCS, UFRJ
21949-900 Rio de Janeiro, RJ
Brasil
Fax: +55-21-2280-8193
E-mail: prmrocco@biof.ufrj.br

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Asthma is an inflammatory disease that involves large and distal airways, as well as lung parenchyma (1), and steroid treatment is the "gold" standard therapy for this condition. Concerns regarding the long-term use of steroids, particularly in young children, have stimulated the discovery of novel anti-inflammatory molecules with high tolerability and clinical efficacy (1). Furthermore, because lung remodeling is associated with an accelerated rate of deterioration in respiratory function and with perpetuation of symptoms (2), a drug with the capacity to act not only in the inflammatory process but also by preventing airway remodeling would be useful in the treatment of asthma. In this context, phosphodiesterase 4 (PDE4) inhibitors could be effective therapeutic options for asthma (3) because they increase intracellular concentrations of cyclic AMP (cAMP), which has a broad range of anti-inflammatory and anti-fibrotic effects on various key effector cells involved in asthma (3,4). A number of potent PDE4 inhibitors have undergone clinical trials with moderate success. Tolerability and clinical efficacy issues dampened enthusiasm for this application (3). However, the prospect of new potent and side effect-free inhibitors on the horizon has given this area a guarded optimism. LASSBio596, designed as a hybrid of thalidomide and aryl sulfonamide, is a new agent (structure given in Ref. 5) that exhibits potent inhibitory effects on PDE types 4 and 5 (5,6), which are the main isozymes distributed in the lungs (7). The present study was undertaken to test the effects of LASSBio596 on respiratory mechanics, lung morphometry, and airway and lung parenchyma remodeling in a murine model of chronic asthma.

BALB/c mice were assigned to four groups. In the asthma group, mice (N = 9; 20-25 g) were immunized using an adjuvant-free protocol by the intraperitoneal (*ip*) injection of 10 µg ovalbumin (OVA) on 7 alternate days. Forty days after the beginning of sensitization, an intratracheal challenge was performed according to the following protocol. Mice were anesthetized with sevoflurane, a 0.5-cm long midline cervical incision was made to expose the trachea, and 20 µg OVA in 20 µL warm (37ºC) sterile saline (0.9% NaCl) was instilled. The cervical incision was closed with 5.0 silk suture and the mice were returned to their cage. The animals recovered rapidly after surgery. This procedure was performed three times at 3-day intervals. For the dexamethasone and LASSBio596 groups (N = 8 each) the animals were sensitized and challenged as in the asthma group and treated for 8 consecutive days with *ip* injections of dexamethasone and LASSBio596, respectively, starting 24 h before the first intratracheal challenge with OVA. Later, they were challenged with intratracheal instillations of OVA. The control group (N = 8) received saline using the same protocol. Twenty-four hours after the last challenge, the animals were sedated with 5 mg diazepam, *ip*, and anesthetized with 20 mg/kg pentobarbital sodium, *ip*, and a snugly fitting cannula (0.8 mm ID) was introduced into the trachea. A pneumotachograph was connected to the tracheal cannula for the measurements of airflow (V') and changes in lung volume (V_T). The pressure gradient across the pneumotachograph was determined by means of a Validyne MP45-2 differential pressure transducer (Engineering Corp., Northridge, CA, USA). Tracheal pressure (Ptr) was measured with a Validyne MP-45 differential pressure transducer (Engineering Corp.). All signals were conditioned and amplified with a Beckman type R Dynograph (Schiller Park, IL, USA). Flow and pressure signals were also passed through 8-pole Bessel filters (902LPF, Frequency Devices, Haverhill, MA, USA) with the corner frequency set at 100 Hz, sampled at 200 Hz with a 12-bit analog-to-digital converter (DT2801A, Data Translation, Marlboro, MA, USA), and stored in a microcomputer. All data were collected using

LABDAT software (RHT-InfoData Inc., Montreal, Quebec, Canada). Muscle relaxation was achieved with vecuronium bromide (5 µg/kg body weight, *iv*), and a constant flow ventilator provided artificial ventilation (Samay VR15, Universidad de la Republica, Montevideo, Uruguay). Special care was taken to keep tidal volume (V_T = 0.2 mL) and flow (V' = 1 mL/s) constant in all animals in order to avoid the effects of different flows, volumes, and inspiratory durations on the measured variables. Pulmonary mechanics were measured by the end-inflation occlusion method (8). In an open chest preparation, Ptr corresponds to transpulmonary pressure. Pulmonary resistive (ΔP1), viscoelastic/inhomogeneous (ΔP2) pressures, ΔPtot (= ΔP1 + ΔP2), static elastance (Est), dynamic elastance (Edyn), and the difference between dynamic and static elastances (ΔE) were determined. The right lung was fixed for light microscopy. Four-micrometer thick tissue sections were stained with hematoxylin-eosin for morphometric analysis of lung architecture such as the fraction area of collapsed and normal alveoli, and the bronchoconstriction index (9). Specific staining methods were also used to quantify collagen (Picrosirius-polarization method (10)) and elastic fibers (Weigert's resorcin fuchsin method modified with oxidation (11)) in the airways and alveolar septa.

The SigmaStat 2.0 statistical software package (Jandel Scientific Corporation, San Raphael, CA, USA) was used. Differences among the four groups were assessed by one-way ANOVA. If multiple comparisons were then required, the Tukey test was applied. A P value <0.05 was considered significant.

There were no statistically significant differences in flow or volume among all groups. Static elastances, and resistive and viscoelastic/inhomogeneous pressures were statistically higher in the asthma group than in the treated or control groups (Table 1).

Histological changes in the asthma group included alveolar collapse and narrower central airways (higher bronchoconstriction index), which were avoided by the administration of LASSBio596. Although dexamethasone inhibited the changes in central airways, lung parenchyma displayed areas of alveolar collapse (Table 2). The collagen fiber content in the alveolar septa and airways was greater in the asthma group than in control animals. Both dexamethasone and LASSBio596 inhibited lung parenchyma and airway fibrosis. There was no statistically significant difference in the amount of elastic fibers in the airways and alveolar septa among groups (Table 2).

The present study provides evidence that LASSBio596 is as effective as dexamethasone in inhibiting inflammatory changes in the airways and preventing lung parenchyma and airway remodeling in a murine model of chronic asthma. Additionally, LASSBio596 prevented alveolar collapse to a greater extent than dexamethasone, although there were no functional differences between the LASSBio596 and dexamethasone groups. The model of chronic asthma used in the present study has been reported to reproduce

Table 1. Mechanical respiratory parameters.

	Control (N = 8)	Asthma (N = 9)	Dexamethasone (N = 8)	LASSBio596 (N = 8)
Flow (mL/s)	1.01 ± 0.01	1.01 ± 0.01	1.00 ± 0.01	1.00 ± 0.01
Volume (mL)	0.20 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.20 ± 0.01
Est,L (cmH_2O/mL)	20.97 ± 0.72*	37.03 ± 1.55	21.90 ± 0.99*	24.02 ± 1.27*
ΔP1,L (cmH_2O)	0.43 ± 0.03*	0.76 ± 0.03	0.46 ± 0.05*	0.46 ± 0.04*
ΔP2,L (cmH_2O)	0.63 ± 0.04*	0.99 ± 0.06	0.68 ± 0.04*	0.76 ± 0.04*
ΔPtot,L (cmH_2O)	1.06 ± 0.03*	1.76 ± 0.07	1.14 ± 0.06*	1.21 ± 0.06*

Data are reported as means ± SEM. In the asthma group, mice were sensitized with ovalbumin and exposed to repeated challenges with intratracheal instillation of ovalbumin. In the dexamethasone and LASSBio596 groups, the animals of the asthma group were treated with 1 mg/kg dexamethasone disodium phosphate (0.1 mL, *ip*) or 10 mg/kg LASSBio596 dissolved in dimethyl sulfoxide (0.2 mL, *ip*), respectively, 24 h before the first intratracheal instillation of ovalbumin, for 8 days. The control group received 0.1 mL saline using the same protocol. Est,L = lung static elastance; ΔP1,L = lung resistive pressure; ΔP2,L = lung viscoelastic/inhomogeneous pressure; ΔPtot,L = total lung pressure.
*P < 0.05 compared to the asthma group (one-way ANOVA followed by Tukey test).

many characteristic features of the human disease such as eosinophilic infiltration, airway epithelium thickening, and goblet cell hyperplasia (12,13).

LASSBio596 exhibits important anti-inflammatory and immunomodulatory properties (5,6). This new thalidomide analog lacks the phthalimide ring (responsible for the teratogenic effects of thalidomide), thus suggesting the probable absence of an eventual teratogenic effect of this compound. LASSBio596 modulates the inflammatory process by inhibiting PDE types 4 and 5, regulators of the breakdown of the intracellular second messengers cAMP and cGMP, respectively.

All mechanical parameters increased in the present model of chronic asthma (Table 1). Static elastance and viscoelastic/inhomogeneous pressure changes were possibly associated with the increase in the fractional area of alveolar collapse and structural modifications in small airways and lung parenchyma (Table 2). Airway resistance increased probably because of bronchoconstriction and airway remodeling (Table 2). Both dexamethasone and LASSBio596 inhibited these mechanical changes (Tables 1 and 2). There is evidence that PDE4 inhibitors relax airway smooth muscle, reduce bronchial edema, modulate the activity of pulmonary nerves, and suppress the activation of the inflammatory cells (3,14,15). Airway remodeling reduces the distensibility of the airways, exaggerates narrowing of the airway lumen when smooth muscle shortens, leading to irreversible airflow obstruction. In the present study, LASSBio596 also modulated the extracellular matrix remodeling. In this respect, Kohyama and colleagues (4) reported that PDE4 inhibitors are able to suppress fibroblast activity and have the potential to block the development of progressive fibrosis. The absence of elastogenesis in airway and lung parenchyma in mice sensitized with OVA agrees with data reported by Xisto and colleagues (13). Inhibitors with adequate specificity for PDE5 may increase cGMP concentrations in the lungs, resulting in relaxation of pulmonary vascular smooth muscle (16). However, we cannot rule out the effect of the PDE5 inhibitor on airway inflammation and bronchoconstriction (17). In airway epithelial cells, PDE4 and PDE5 predominate, hydrolyzing 60 to 75% and 80% of the total cAMP and cGMP, respectively (18). Crosstalk exists between cAMP and cGMP because cAMP also inhibits PDE5, thereby allowing cGMP levels to rise and both cAMP and cGMP to activate protein kinase G (17).

Table 2. Lung morphometry.

	Normal (%)	Collapse (%)	Contraction index	Lung parenchyma		Airway	
				Collagen ($\mu m^2/\mu m$)	Elastic ($\mu m^2/\mu m$)	Collagen ($\mu m^2/\mu m$)	Elastic ($\mu m^2/\mu m$)
Control	92.28 ± 1.13*+	7.74 ± 1.12*+	0.85 ± 0.07*	0.010 ± 0.001*	0.28 ± 0.01	4.66 ± 0.77*	4.54 ± 0.64
Asthma	77.82 ± 0.79#	22.18 ± 0.79#+	1.50 ± 0.11	0.182 ± 0.36	0.31 ± 0.02	8.89 ± 1.02	5.07 ± 0.27
Dexamethasone	84.26 ± 2.22*#	15.74 ± 2.22*#	1.02 ± 0.08*	0.015 ± 0.001*	0.32 ± 0.02	4.64 ± 0.77*	4.43 ± 0.59
LASSBio596	88.80 ± 1.78*+	7.25 ± 0.09*+	1.11 ± 0.05*	0.013 ± 0.001*	0.32 ± 0.01	4.21 ± 0.74*	5.09 ± 0.68

Data are reported as mean ± SEM for 10 random, non-coincident fields per mouse. In the asthma group, mice were sensitized with ovalbumin and exposed to repeated challenges with intratracheal instillation of ovalbumin (N = 9). In the dexamethasone and LASSBio596 groups (N = 8 each), the animals of the asthma group were treated with 1 mg/kg dexamethasone disodium phosphate (0.1 mL, *ip*) or 10 mg/kg LASSBio596 dissolved in dimethyl sulfoxide (0.2 mL, *ip*), respectively, 24 h before the first intratracheal instillation of ovalbumin, for 8 days. The control group received 0.1 mL saline using the same protocol (N = 8).

*$P < 0.05$ compared to the asthma group; #$P < 0.05$ compared to the LASSBio596 group; +$P < 0.05$ compared to the dexamethasone group (one-way ANOVA followed by Tukey test).

In a murine model of chronic asthma, LASSBio596 prevented mechanical and histological lung changes and inhibited collagen deposition when administered to sensitized animals before they were challenged with OVA.

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