Biomedicine & Pharmacotherapy 102 (2018) 160–168

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Atorvastatin dose-dependently promotes mouse lung repair after emphysema induced by elastase

Adriana Correa Melo[a], Isabella Cattani-Cavalieri[a], Marina Valente Barroso[b], Nicolas Quesnot[a], Lycia Brito Gitirana[a], Manuella Lanzetti[a], Samuel Santos Valença[a,*]

[a] Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
[b] Pós-graduação em Imunologia e Inflamação, Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

ARTICLE INFO

Keywords:
Emphysema
Elastase
Mouse
Atorvastatin
Lung repair

ABSTRACT

Emphysema results in a proteinase – antiproteinase imbalance, inflammation and oxidative stress. Our objective was to investigate whether atorvastatin could repair mouse lungs after elastase-induced emphysema. Vehicle (50 µL) or porcine pancreatic elastase (PPE) was administered on day 1, 3, 5 and 7 at 0.6 U intranasally. Male mice were divided into a control group (sham), PPE 32d (sacrificed 24 h after 32 days), PPE 64d (sacrificed 24 h after 64 days), and atorvastatin 1, 5 and 20 mg treated from day 33 until day 64 and sacrificed 24 h later (A1 mg, A5 mg and A20 mg, respectively). Treatment with atorvastatin was performed via inhalation for 10 min once a day. We observed that emphysema at day 32 was similar to emphysema at day 64. The mean airspace chord length (Lm) indicated a recovery of pulmonary morphology in groups A5 mg and A20 mg, as well as recovery of collagen and elastic fibers in comparison to the PPE group. Bronchoalveolar lavage fluid (BALF) leukocytes were reduced in all atorvastatin-treated groups. However, tissue macrophages were reduced only in the A20 mg group compared with the PPE group, while tissue neutrophils were reduced in the A5 mg and A20 mg groups. The redox balance was restored mainly in the A20 mg group compared with the PPE group. Finally, atorvastatin at doses of 5 and 20 mg reduced nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and matrix metalloproteinase-12 (MMP-12) compared with the PPE group. In conclusion, atorvastatin was able to induce lung tissue repair in emphysematous mice.

1. Introduction

Chronic obstructive pulmonary disease (COPD) causes irreversible damage to lung structures, leading to progressive disability and death. Emphysema and chronic bronchitis are included in the group of diseases named COPD, but with differences in pathophysiology and symptoms [1]. Emphysema is the major cause of morbidity and mortality in COPD patients worldwide. It is characterized by chronic and permanent inflammation and destruction of the alveolar walls due to the breakdown of the extracellular matrix in the parenchyma. The parenchymal breakdown leads to enlargement of the alveoli, loss of elastic recoil, reduction of the gas exchange capacity, and pulmonary hyperinflation [2]. Inflammatory cells migrate to alveoli and, in addition to resident lung macrophages, release proteases that lead to an imbalance between them and proteinase inhibitory defenses or antiproteases [3]. Also associated with this condition are activated macrophages and neutrophils that release oxidants that, under conditions of oxidative stress, lead to a redox imbalance and consequent cellular damage [4]. Cigarette smoking is the main risk factor for pulmonary emphysema because it leads to a respiratory burst and consequent predominance of oxidants compared with antioxidants [5].

Studies using animal models of cigarette smoke-induced emphysema have demonstrated the importance of metalloproteinases, especially matrix metalloproteinase-12 (MMP-12). MMP-12 plays a pivotal role in the inflammatory process that leads to lung injury [6,7]. Genetic deficiency of α1-antitrypsin (α1-ATP) also causes pulmonary emphysema due to an elastolytic imbalance [8,9]. Both conditions cause destruction of lung tissue, yet the morphologies of these lesions differ, as emphysema is caused by cigarette smoke with a centrilobular characteristic, and emphysema is caused by α1-ATP deficiency with a panacinar characteristic [10]. Experimental investigations are being conducted using cigarette smoke or elastolytic enzymes to propose new treatment strategies since morphological emphysema in patients is similar in those mouse models [11,12].

* Corresponding author at: Laboratório de Biologia Redox, ICB/CCS/UFRJ, Av. Carlos Chagas Filho 373, Bloco J – sala 27, Cidade Universitária, Rio de Janeiro, R.J. CEP 21.941-902, Brazil.
E-mail address: samuelv@icb.ufrj.br (S.S. Valença).

https://doi.org/10.1016/j.biopha.2018.03.067
Received 31 January 2018; Received in revised form 12 March 2018; Accepted 12 March 2018
0753-3322/ © 2018 Elsevier Masson SAS. All rights reserved.

Pharmacological treatment for emphysema is limited, but clinical symptoms improve, thus leading to an improved quality of life [13]. Long-term inhaled bronchodilators are used, which are effective for relieving symptoms [14], while corticosteroids have limited effectiveness [15]. To date, no drug can repair the lung damage caused by cigarette smoking or α1-ATP deficiency. Recent studies [16–19] have reported the pleiotropic effects of statins (inhibitors of 3-hydroxy-3-methyl glutaryl coenzyme A reductase), going beyond their ability to lower cholesterol, including anti-inflammatory effects (suppression of pro-inflammatory cytokines, chemokines, adhesion molecules, and matrix metalloproteinases) and antioxidant effects (including reactive oxygen species (ROS) scavengers). Our group recently published a study in which we used two inhaled statins (atorvastatin and simvastatin) to repair the emphysematous lesions in the lungs of mice that were chronically exposed to cigarette smoke and found that repair occurred in both treated groups [20]. Our objective in the present study was to evaluate whether treatment with inhaled atorvastatin could promote lung repair in a mouse model of severe emphysema caused by elastase.

2. Materials and methods

2.1. Animals and reagents

Eight-week-old male C57BL/6 mice (18–22 g) were purchased from the Multidisciplinary Center for Biological Investigation on Laboratory Animal Science (CEMIB - UNICAMP, Campinas, Brazil). The mice were fed Purina Chow (Nuvilab®, Curitiba, Brazil) and were housed in a room with a controlled environment maintained at 18 °C–22 °C, 50%–70% relative humidity, and a 12-h light/dark cycle. The mice acclimated for two weeks prior to the experimental procedures. All reagents were purchased from Sigma-Aldrich (St. Louis, USA) except where otherwise specified.

2.2. Experimental design

Mice were divided into 6 groups: one control group treated with the vehicle (sham) and five others that were intranasally with 4 × 0.6 U of porcine pancreatic elastase (PPE) every other day (day 1, 3, 5 and 7). One PPE group was sacrificed 24 h after 32 days, and another PPE group was sacrificed 24 h after 64 days. We aimed to discover a possible difference in the recovery of lung elasticity at either 32 or 64 days. In addition, we used other PPE groups treated with atorvastatin at 3 different doses: 1 mg (A1 mg), 5 mg (A5 mg), and 20 mg (A20 mg). These last 3 groups were sacrificed 24 h after 64 days of intranasal PPE. This protocol was adapted from Ishii et al. [21]. Atorvastatin was administered via inhalation for 10 min. All groups contained 10 mice. This model characterizes a treatment since at 33 days the animals already had emphysema and treatment was initiated after disease was established (a figure showing our experimental design is available as supplementary data). Animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory animals. Additionally, this experimental protocol was approved by the Animal Ethics Committee of Universidade Federal do Rio de Janeiro under number 115/13.

2.3. Bronchoalveolar lavage and lung homogenates

Mice were euthanized by cervical displacement, and the thoracic cavity was opened; blood was collected directly from the right ventricle. Bronchoalveolar lavage (BAL) was performed in the right lung of all animals in each group (while the left lung was clamped). Briefly, a cannula was inserted into the trachea, the airspaces were washed six times with buffered saline solution (250 µl, 4 °C), and the flow-through was maintained on ice (final volume 1.2–1.5 ml). The BAL fluid was then centrifuged, and the supernatant was collected and stored at −20 °C. The total number of cells in the BAL fluid was determined using a Neubauer chamber. The right lungs of all animals were removed and homogenized in 1.0 ml of lysis buffer and centrifuged at 210 x *g* for 10 min, and the supernatants were collected for Western blot analysis. The lysis buffer consisted of 1 tablet of protease inhibitor (0.1% Triton X-100) in 100 ml of phosphate buffer. The total protein in the samples (tissues and BAL) was determined by the Bradford method.

2.4. Tissue processing and morphometry

The left lungs of all animals were inflated with 10% phosphate-buffered formalin (pH 7.2) at 25 cm H_2O pressure for 2 min and then ligated, removed, and weighed. Lungs were fixed for 48 h before embedding in paraffin. Sagittal 4-µm serial sections of the lungs were stained with hematoxylin and eosin, orcein, Sirius red, and Giemsa for histological analyses. Alveolus enlargement was quantified based on the mean linear intercept length (Lm) in 100 random fields per group [22]. The volume densities of the alveoli (Vv alveoli), elastic fibers (Vv elastic fibers), and collagen fibers (Vv collagen fibers) were estimated by counting the number of structures that were intersected by the test system (partial points, Pp) in 100 random fields per group [23]. Lm and Vv were counted in five sections, with each section containing three lung slices per mouse. Hematoxylin and eosin images were captured at 10× magnification. Orcein and Sirius red images were captured at 40× magnification. In addition, alveolar macrophages and neutrophils were counted in ten random fields at 100× magnification using Giemsa-stained sections.

2.5. Western blotting

Proteins (30 µg) were resolved by sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) and electrotransferred to polyvinylidene difluoride (PVDF) membranes. Primary antibodies against MMP-12 and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) were purchased from Santa Cruz Biotechnology (Santa Cruz, USA). Primary antibody against β-actin was purchased from Sigma-Aldrich (St Louis, USA). Protein detection was performed with the appropriate horseradish peroxidase-conjugated secondary antibody/ECL detection systems (GE Healthcare Bio-Sciences, USA).

2.6. Biochemical analyses

Superoxide dismutase (SOD) activity was assayed by monitoring the inhibition of adrenaline auto-oxidation at 480 nm [24]. Catalase (CAT) activity was measured based on the rate of the decrease in hydrogen peroxide concentration monitored at 240 nm [25]. Nitrite levels were determined by a method based on Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% *N*-[1-naphthyl] ethylenediamine dihydrochloride in water). The absorbance was measured with a microplate reader at 550 nm [26]. Reduced and oxidized glutathione (GSH and GSSG) levels were examined with 5,5′-dithio-bis(2-nitrobenzoic acid), which produces the 2-nitro-5-thiobenzoate chromophore detectable at 412 nm [27]. As an index of oxidative damage induced by lipid peroxidation, we used the thiobarbituric acid reactive substances (TBARS) method to analyze malondialdehyde (MDA) products during an acid-heating reaction. TBARS levels were determined by absorbance at 532 nm and expressed as MDA equivalents [28].

2.7. Statistical analyses

Values for all measurements are expressed as the mean ± standard error of the mean (SEM). All analyses were performed using one-way ANOVA followed by a Tukey post hoc test ($p < 0.05$ was considered significant), except for the GSH/GSSG ratio and Western blotting quantifications, which were performed with the Kruskal – Wallis test

Download English Version:

https://daneshyari.com/en/article/8525298

Download Persian Version:

https://daneshyari.com/article/8525298

Daneshyari.com