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Recovery of pulmonary structure and exercise capacity by treatment with granulocyte-colony stimulating factor (G-CSF) in a mouse model of emphysema

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ABSTRACT

Emphysema is a chronic obstructive pulmonary disease characterized abnormal dilatation of alveolar spaces, which impairs alveolar gas exchange, compromising the physical capacity of a patient due to airflow limitations. Here we tested the effects of G-CSF administration in pulmonary tissue and exercise capacity in emphysematous mice. C57Bl/6 female mice were treated with elastase intratracheally to induce emphysema. Their exercise capacities were evaluated in a treadmill. Lung histological sections were prepared to evaluate mean linear intercept measurement. Emphysematous mice were treated with G-CSF (3 cycles of 200 μg/kg/day for 5 consecutive days, with 7-day intervals) or saline and submitted to a third evaluation 8 weeks after treatment. Values of run distance and linear intercept measurement were expressed as mean ± SD and compared applying a paired *t*-test. Effects of treatment on these parameters were analyzed applying a Repeated Measures ANOVA, followed by Tukey's *post hoc* analysis. $p < 0.05$ was considered statistically significant. Twenty eight days later, animals ran significantly less in a treadmill compared to normal mice (549.7 ± 181.2 m and 821.7 ± 131.3 m, respectively; $p < 0.01$). Treatment with G-CSF significantly increased the exercise capacity of emphysematous mice (719.6 ± 200.5 m), whereas saline treatment had no effect on distance run (595.8 ± 178.5 m). The PCR cytokines genes analysis did not detect difference between experimental groups. Morphometric analyses in the lung showed that saline-treated mice had a mean linear intercept significantly higher ($p < 0.01$) when compared to mice treated with G-CSF, which did not significantly differ from that of normal mice. Treatment with G-CSF promoted the recovery of exercise capacity and regeneration of alveolar structural alterations in emphysematous mice.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a slowly progressive respiratory disease due to an exacerbated inflammatory process in lungs triggered by exposure to noxious particles or gases, especially cigarette smoke [1]. Characterized by airflow limitation, COPD is not a fully reversible condition, and is one of the major causes of chronic disability and permanent impairment. The prevalence of COPD will increase in the coming years to become the fifth most common cause of morbidity and the third most common

Abbreviations: G-CSF, granulocyte-colony stimulating factor; COPD, chronic obstructive pulmonary disease; Lm, mean linear intercept; ATRA, all-trans-retinoic acid.

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chronic disease worldwide [2]. According to new estimates for 2030, COPD is predicted to become the third leading cause of death [3].

Belonging to the group of COPD, emphysema is characterized by destruction of alveolar extracellular matrix, leading to airspace enlargement of the distal airspaces and a reduction in the alveolar capillary exchange area. These results in a largely irreversible airflow obstruction, usually progressive, associated with an abnormal inflammatory response of the lungs [1]. Pulmonary emphysema is notoriously unresponsive to medical treatment and no currently available pharmacological intervention has been shown to slow or halt the progression of the disease. Therefore, lung transplantation remains the only definitive therapeutic option for patients with advanced emphysema [4].

Granulocyte colony-stimulating factor (G-CSF) is a 20-kDa glycoprotein known to induce granulopoiesis. Since it acts as a critical regulator of myeloid progenitor cell proliferation, differentiation and survival, the G-CSF is currently used therapeutically for the treatment of leukopenia associated to chemotherapy [5]. More recently, different and interesting pleiotropic actions of G-CSF were reported, such as restoration of cardiac function and tissue repair both in ischemic heart disease [6–8] and in chronic chagasic cardiomyopathy [9], as well as in lung tissue [10,11].

Based on the data described in the literature about the potential therapeutic use of G-CSF, in the present study we aimed to investigate the therapeutic potential of G-CSF in emphysematous lung. Using an experimental model for emphysema developed in C57Bl/6 mice by intratracheal administration of porcine pancreatic elastase [12], we tested the effects of G-CSF in tissue repair of the lungs and functional recovery of the animals.

2. Material and methods

2.1. Animals

Two-month-old female C57BL/6 mice, raised and maintained in the animal facilities at the Gonçalo Moniz Research Center, Oswaldo Cruz Foundation (Salvador, Bahia, Brazil) were used in the experiments, and were provided with rodent diet and water *ad libitum*. All animals were sacrificed in a CO_2 chamber, and handled according the National Institutes of Health guidelines for ethical use of laboratory animals. This study was approved by Ethics Committee of Animal Use of Gonçalo Moniz Research Center.

2.2. Emphysema induction

C57Bl/6 female mice ($n = 30$) were anesthetized via inhalation of isofluorane (0.5–2%). The anesthetic concentration offered was controlled by monitoring the heart rate, which was kept above 350 bpm. Anesthetized, the animals were placed in a supine position on a heated table to be submitted to instillation of 100 μl of elastase (2 U/100 g body weight porcine pancreatic elastase; Sigma, Aldrich, Taufkirchen, Germany) dissolved in saline ($n = 20$) by intratracheal route. Normal control animals were not manipulated ($n = 10$). The animals were submitted to anterior cervical incision and the muscle were divulsed in order to visualize the trachea and make the tracheal punction to administer the solutions. Afterwards, mice were sutured, kept on a warm plate (30 °C) until restoration of spontaneous breathing, after which they were extubated.

2.3. Treatment with G-CSF

Twenty-eight days following elastase-induced emphysema, the physical capacity of the animals was reevaluated during 5 consecutive days (treadmill challenge). After this, emphysematous mice were separated in two experimental groups, using the following treatment protocol. The G-CSF treated group ($n = 10$) received human recombinant G-CSF (200 μg/kg/d; Granulokine 30; Hoffman la Roche, Switzerland) via intraperitoneal route. This treatment was performed in 3 cycles, where each cycle lasted 5 consecutive days, with a 7 day interval between each cycle. The second group of emphysematous mice ($n = 10$) was submitted to the same treatment protocol, receiving saline, as opposed to G-CSF.

2.4. Treadmill

A motor-driven treadmill chamber for one animal (LE 8700 Panlab, Barcelona, Spain) was used to exercise the animals. The speed of the treadmill and the intensity in milliamps of the shock were controlled by a potentiometer (LE 8700 treadmill control, Panlab). Room air was pumped into the chamber at a controlled flow rate (600 ml/min) by a chamber air supplier (OXYLET LE 400, Panlab). The mean room temperature was maintained at 21 ± 1 °C. After an adaptation period of 40 min in the treadmill chamber the mice were exercised at different velocities, starting at 7.2 m/min and increasing the velocity 7.2 m/min every 10 min. The inclination of the treadmill was maintained at an uphill angle of 10°. Velocities were increased until the animal could no longer sustain a given speed and remained for more than 10 s on an electrified stainless steel grid, which provided an electrical stimulus (1 milliamp) to keep the mice running. Total running distance and running time were recorded. Treadmill tests were carried out on all mice prior to emphysema induction. The initial reevaluation of these mice took place 28 days after intratracheal administration of elastase/saline, and again 8 weeks after the conclusion of the administered treatment protocol.

2.5. Histological and morphometrical analysis

Mice were sacrificed 8 weeks after the end of the treatment, using CO_2. Opening the thorax and abdomen, the lungs were exposed. The trachea was cannulated with gelco number 18 and the lungs were perfused with buffered 4% formalin applying a constant transpulmonary pressure of 20 cm H_2O for 2 h. After this procedure the trachea was sutured in order to hold the intrapulmonary pressure at 20 cm H_2O and the entire cardiopulmonary tissue block was removed and fixed in formalin (4%). Lung histological sections were prepared to evaluate mean linear intercept (Lm). Analyses were performed on whole lung sections after slide scanning with 20× magnification using the Aperio ScanScope system (Aperio Technologies, Vista, CA). Morphologic examinations were performed following Thurbeck [13]. The Lm of each lung was determined using light microscopy on 20 randomly selected fields, originating from randomly selected tissue samples covering the entire lung and containing apical as well as basal areas of the organ using ImageScope software (Aperio Technologies). The Lm, as indicator of air space size, was calculated from counting lines of defined length that were randomly placed on each of the 20 lung sections of 5 μm-thick and the number of intercepts crossing the lines counted. The Lm was calculated from the length of the lines multiplied by the number of the lines divided by the sum of all counted intercepts.

2.6. Sample preparation – RNA isolation and cDNA synthesis

Immediately following the mice sacrifice, RNA was harvested from lung tissue, isolated with TRIzol reagent (Invitrogen) and the concentration was determined photometricly. The RNA quality was analyzed in 1.2% agarose gel. A High Capacity cDNA Reverse