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ORIGINAL ARTICLE

Effects of mesenchymal stromal cells play a role the oxidant/antioxidant balance in a murine model of asthma

M.A.S. Malaquias[a,*], L.A. Oyama[a], P.C. Jericó[a], I. Costa[b], G. Padilha[b], S. Nagashima[a], M. Lopes-Pacheco[b], C.L.K. Rebelatto[c], P.V. Michelotto[c], D.G. Xisto[b], P.R.S. Brofman[c], P.R.M. Rocco[b], L. de Noronha[a]

[a] *Pathology Experimental Laboratory, School of Medicine, Pontifical Catholic University of Paraná, Curitiba, Brazil*
[b] *Laboratory of Pulmonary Investigation, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*
[c] *Experimental Laboratory for Cell Culture, School of Medicine, Pontifical Catholic University of Paraná, Curitiba, Brazil*

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Abstract Asthma is a heterogeneous disease characterised by chronic airway inflammation. One of the most devastating consequences of this inflammatory process is the generation of reactive oxygen and nitrogen species responsible for oxidative stress. The aim of this study is to analyse the efficiency of treatment with human bone marrow-derived mesenchymal stromal cells (hMSC) in maintaining the oxidative balance in a murine model of allergic asthma by quantifying nitrotyrosine in lung tissues. After confirmation of asthma in the experimental model, samples of lung parenchyma were submitted to immunohistochemical assessment. Intravenous administration of hMSC reduced the levels of nitrotyrosine in the ASTHMA-hMSC group compared to those in the ASTHMA-SAL group. In conclusion, therapeutic administration of hMSC had a beneficial effect on oxidative stress, reducing the levels of nitrotyrosine in lung tissues in a model of allergic asthma.

Abbreviations: MSC, mesenchymal stromal cells; hMSC, human bone marrow-derived mesenchymal stromal cells; ROS, reactive oxygen species; RNS, reactive nitrogen species; OVA, ovalbumin; HE, haematoxylin and eosin; NO, nitric oxide; GPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; FEV1, forced expiratory volume in one second; FVC, forced vital capacity.

* Corresponding author.
E-mail address: mi.mv@hotmail.com (M.A.S. Malaquias).

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Introduction

Asthma is a serious public health problem that affects an estimated 300 million individuals worldwide.[1–4] Approximately 5–10% of those affected do not respond to conventional treatments and have a poor quality of life as a result.[5,6]

Asthma is defined as a chronic inflammatory disease that predominantly affects the large airways where multiple cells, both inflammatory and structural, play roles in its pathogenesis.[7,4] It is a multifactorial disease resulting from the interaction of genetic and environmental factors and involving an imbalance of oxidants and antioxidants factors.

Reactive oxygen species (ROS) are produced in response to many physiological conditions during the normal functioning of the human body. However, the production of ROS can have negative effects, as a consequence, the body has an antioxidant system. When there is an imbalance between the oxidant and antioxidant systems and oxidants predominate, oxidative stress occurs.[8]

There is strong evidence that the oxidative state in asthma is marked by an imbalance in the antioxidant and oxidant systems. ROS and reactive nitrogen species (RNS) play a role in inflammation of the airways and are determinants of disease severity.[9]

Oxidative stress can harm lung functioning causing hyperplasia of goblet cells with mucus hypersecretion, hypertrophy of smooth muscles of the airways and subepithelial fibrosis, vascular exudation, as well as other changes that exacerbate inflammation and consequently remodel airways.[10]

Stem cells are a possible treatment for asthma. Cell therapies can be described as a set of technological approaches that use stem cells for the treatment of diseases. Cell therapies have shown surprising results in different conditions and diseases related to airways.[11–15]

Oxidative stress plays a role in the pathophysiology of asthma, and it seems that it can be minimised with the use of stem cells.[16] One method to quantify oxidative damage is the measurement of nitrotyrosine, a marker of nitration of proteins by peroxynitrite.[17–19]

Thus, the aim of this work was to evaluate the efficacy of treatment with human bone marrow-derived mesenchymal stromal cells (hMSC) in maintaining the oxidative balance in an experimental model of allergic asthma by quantifying nitrotyrosine in lung tissues.

Methods

This study was approved by the Ethics Committee of the Carlos Chagas Filho Institute of Biophysics, Health Sciences Centre, Federal University of Rio de Janeiro. All animals received humane care in compliance with the ''Principles of Laboratory Animal Care'' formulated by the National Society for Medical Research and the ''Guide for the Care and Use of Laboratory Animals'' prepared by the U.S. National Academy of Sciences.

Animal preparation and experimental protocol

BALB/c mice from the laboratory of the Pontifical Catholic University of Paraná were used in experiments.

Thirty-two adult female BALB/c mice, weighing 24–34 g were randomly divided into the following groups:

- **CTRL-SAL**: The animals were sensitised and challenged with saline solution, and then received a saline solution treatment through the tail vein 24 h after the last allergenic challenge ($n = 8$).
- **CTRL-hMSC**: The animals were sensitised and challenged with saline solution, and then received an injection of hMSC (1×10^6 cells in 50 μL of IMDM) through the tail vein 24 h after the last allergenic challenge ($n = 8$).
- **ASTHMA-SAL**: Animals were sensitised by two subcutaneous injections containing 5 μg of ovalbumin (OVA) and 5 mg of aluminium hydroxide, and then challenged with nasal instillation of 25 μg of OVA diluted in 25 μL of sterile saline solution. The saline solution was administered through the tail vein 24 h after the last allergenic challenge ($n = 8$) (Fig. 1).
- **ASTHMA-hMSC**: The animals were sensitised by two subcutaneous injections containing 5 μg of OVA and 5 mg of aluminium hydroxide, and then challenged with nasal instillation of 25 μg of OVA diluted in 25 μL of sterile saline solution. hMSC (1×10^6 cells in 50 μL of IMDM) were injected into the tail vein 24 h after the last allergenic challenge ($n = 8$).

hMSC isolation

Mononuclear cells isolated from human bone marrow were used to obtain hMSC.[20] IMDM (Gibco, Invitrogen, NY, USA) supplemented with 15% foetal bovine serum (Gibco, Invitrogen, NY, USA) and 1% of antibiotic solution with penicillin (Gibco Invitrogen, NY, USA) and streptomycin (Gibco Invitrogen, NY, USA).

Asthma confirmation

Pulmonary mechanics

Seven days after treatment, the animals were sedated (diazepam, 1 mg IP), anaesthetised (sodium thiopental 20 mg/kg intraperitoneal), tracheotomised, paralysed (vecuronium bromide, 0.005 mg/kg intravenous), and ventilated with a ventilator for small animals (Samay VR15, Universidad de la Republica, Montevideo, Uruguay) with the following parameters: frequency of 100 cycles/min, tidal volume (TV) of 0.2 mL, and fraction of inspired oxygen of 0.21. The anterior chest wall was surgically removed and a positive end-expiratory pressure of 2 cm H_2O was applied. Pulmonary mechanics were measured using the end-inspiratory airway occlusion method.[21] In animals with open chests, tracheal pressure reflected the transpulmonary pressure (PL). After airway occlusion, there was a rapid fall in the PL (ΔP1) corresponding to maximum pressure

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