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Methylene blue attenuates ischemia–reperfusion injury in lung transplantation

Marcus da Matta Abreu, MD, PhD,[a,*] *Rogerio Pazetti, PhD,*[a] *Francine Maria de Almeida, MSc,*[a] *Aristides Tadeu Correia, BSc,*[a] *Edwin Roger Parra, MD, PhD,*[b] *Laís Pereira da Silva,*[a] *Rodolfo de Paula Vieira, PhD,*[c] *Paulo Manuel Pêgo-Fernandes, MD, PhD,*[a] *and Fabio Biscegli Jatene, MD, PhD*[a]

[a] *Department of Cardiopneumology, Heart Institute (InCor), Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil*

[b] *Department of Pathology, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil*

[c] *Department of Research, Nove de Julho University (UNINOVE), São Paulo, Brazil*

ARTICLE INFO

Article history:
Received 2 May 2014
Received in revised form
13 July 2014
Accepted 18 July 2014
Available online 24 July 2014

Keywords:
Animal model
Ischemia–reperfusion injury
Methylene blue
Lung transplantation

ABSTRACT

Background: Ischemia–reperfusion injury (IRI) is one of the principal obstacles for the lung transplantation (LTx) success. Several strategies have been adopted to minimize the effects of IRI in lungs, including *ex vivo* conditioning of the grafts and the use of antioxidant drugs, such as methylene blue (MB). We hypothesized that MB could minimize the effects of IRI in a LTx rodent model.

Methods: Forty rats were divided into four groups ($n = 10$) according to treatment (saline solution or MB) and graft cold ischemic time (3 or 6 h). All animals underwent unilateral LTx. Recipients received 2 mL of saline or MB intraperitoneally before transplantation. After 2 h of reperfusion, arterial blood and exhaled nitric oxide samples were collected and bronchoalveolar lavage performed. Then animals were euthanized, and histopathology analysis as well as cell counts and cytokine levels measurements in bronchoalveolar lavage fluid were performed.

Results: There was a significant decrease in exhaled nitric oxide, neutrophils, interleukin-6, and tumor necrosis factor-α in MB-treated animals. PaO_2 and uric acid levels were higher in MB group.

Conclusions: MB was able in attenuating IRI in this LTx model.

1. Introduction

Lung transplantation (LTx) is a well-established therapeutic option for the treatment of end-stage lung diseases such as chronic obstructive pulmonary disease, cystic fibrosis, idiopathic pulmonary fibrosis, bronchiectasis, and primary pulmonary hypertension [1]. However, ischemia–reperfusion injury (IRI) remains one of the principal obstacles for LTx success. In its severe clinical presentation, IRI is known as primary graft dysfunction (PGD), which is associated with high morbidity and mortality in the first days after LTx [2].

* *Corresponding author.* Department of Cardiopneumology, Heart Institute (InCor), Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, Av. Dr. Enéas de Carvalho Aguiar, 44 – Bloco II, 5° andar - CEP 05403-900 – São Paulo, Brazil. Tel./fax: +55 11 26615197.

E-mail address: marcusdiretoria@hotmail.com (M.daM. Abreu).

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http://dx.doi.org/10.1016/j.jss.2014.07.043

A PGD is a situation that involves complex cellular, molecular, and biochemical changes. Several mechanisms contribute at the same time to the formation of morphologic and functional changes that are characterized by an increase in pulmonary vascular resistance, increased pulmonary capillary permeability and edema, leading to an impaired gas exchange with an increase in the alveolar–arterial oxygen gradient and a decrease in PaO_2. The morphologic response of the endothelium is characterized by the presence of apoptosis and infiltration by macrophages and polymorphonuclear cells. The hypoxia leads to an increased expression of cell adhesion molecules and the production of reactive oxygen species (ROS) leading to an activation of microvascular endothelial cells that become even more dysfunctional. At the same time, the pulmonary surfactant undergoes changes in its composition, function, and metabolism, leading to a reduction of lung compliance [3].

The etiology of PGD primarily involves the increased formation of ROS [1]. Briefly, a decreased oxygen supply reduces the synthesis and resynthesis of adenosine triphosphate, creating an ionic gradient in the cell membrane due to decreased extracellular active calcium transport. The accumulation of cytoplasmic calcium leads to the activation of a protease that converts xanthine dehydrogenase to xanthine oxidase [3]. Concurrent with these events, there is an accumulation of adenosine monophosphate, which decomposes into substances such as adenosine, inosine, and hypoxanthine. During the reperfusion process, in the presence of oxygen, xanthine oxidase converts hypoxanthine into ROS such as superoxide, peroxide, and hydroxyl radicals [3,4].

Several strategies have been adopted to minimize the effects of IRI in lungs, including *ex vivo* conditioning of the grafts and the use of antioxidant drugs, in both clinical and experimental settings [5,6]. Some studies have recently investigated the antioxidant properties of methylene blue (MB) [7,8]. MB has been used successfully as an inhibitory drug in lung lesions caused by intestinal ischemia followed by reperfusion in rats [4] and in the treatment of secondary hemodynamic changes to reperfusion in liver transplantation [9]. MB prevents ROS production by acting as an alternative xanthine oxidase electron receptor, competing with molecular oxygen for electron transfer [4]. The electrons are transferred to MB from the iron-sulfur center of xanthine oxidase, thus preventing the conversion of molecular oxygen into superoxide [4].

Another action mechanism related to MB and the ischemia–reperfusion (IR) process is the inhibitory action on nitric oxide (NO). The inducible nitric oxide synthase, which is expressed through the action of inflammatory mediators, can give rise to radical peroxynitrite and peroxynitrous acid, which participate in lipid peroxidation processes and increased endothelial cell adhesion. In the lungs, NO can lead to the formation of toxic peroxynitrite, resulting in an increased inflammatory response [3].

Our aim was to evaluate the effects of MB as an inhibitor of IRI in rats after LTx.

2. Material and methods

Eighty female Sprague–Dawley rats (300–350 g) were used in this study (40 donor and/or 40 recipients). Recipient rats were divided into four groups ($n = 10$) according to graft cold ischemic time of 3 or 6 h, and treatment with saline solution (SAL) or MB: 3SAL, 6SAL, 3 MB, and 6 MB. This study was approved by our institutional research committee (CAPPesq 3387/09/138) and performed according to the Guide for the Care and Use of Laboratory Animals [10].

2.1. Surgical procedure

2.1.1. Donor

Animals were anesthetized with isoflurane 5% (Isothane, Baxter, San Juan, Porto Rico), orotracheally intubated and mechanically ventilated (model 683; Harvard Apparatus, Holliston, MA) with 10 mL/kg and 80 cycles/min. General anesthesia was maintained with isoflurane 2% (isovapor mod. 1224, K. Takaoka, São Bernardo do Campo, Brazil). After median laparotomy, 500 U of heparin was injected into inferior vena cava. After 1 min, a median sternotomy was performed and pulmonary artery cannulated for anterograde perfusion with 20 mL of low-potassium dextran solution (LPD) (Perfadex, Vitrolife, Sweden) at 4°C with constant pressure (20 cm H_2O). Before perfusion, inferior vena cava was sectioned to decrease venous return, and the left atrial appendage was amputated to drain the LPD. Animals were euthanized by exsanguination, according to the Report of the American Veterinary Medicine Association Panel on Euthanasia [11].

After perfusion, trachea was tied at the end of the inspiratory flow and the cardiopulmonary block was excised and placed in a petri dish with cold LPD for back table step. Left hilum was dissected and cuffs fixed in the artery, vein, and bronchus, as previously described [12]. Grafts were maintained inflated during the ischemia period (3 or 6 h) and were stored in cold LPD till implantation.

2.1.2. Recipient

Recipient animals were anesthetized, intubated, and ventilated as described previously. Immediately before graft implantation, animals were intraperitoneally injected with 2 mL of either SAL 0.9% or MB 1%. Then they were placed in the right lateral recumbence and subjected to left thoracotomy at the fourth intercostal space. Subsequently, graft implantation was performed using a stereomicroscope (model SZ61; Olympus, Tokyo, Japan) at ×8 magnification [12]. In brief, the left hilum was dissected and clamped as proximal as possible. Then, graft implantation was performed by introducing graft cuffs into a little hole made in ventral wall of the artery, vein, and bronchus, respectively. After cuffs fixation using a 7.0 polypropylene silk, bronchus clamp was slowly opened and air flow reestablished. In sequence, vein clamp was removed for retrograde circulation establishment and, finally, artery clamp was gently opened aiming a soft graft perfusion. The closure of the recipient incision was performed in separate layers using 2.0 monofilament nylon sutures. After surgery completion, animals received analgesia (dipyrone 400 mg/kg) by gavage and were placed under spontaneous ventilation in individual cages with free access to water and food.