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Repeated Domperidone treatment modulates pulmonary cytokines in LPS-induced acute lung injury in mice

Talita Rossetto Barreto, Carolina Costola-de-Souza, Rafael Oliveira Margatho, Nicolle Queiroz-Hazarbassanov, Sandra Campos Rodrigues, Luciano Freitas Felício, João Palermo-Neto, Adriano Zager*

Neuroimmunomodulation Research Group, Department of Pathology, School of Veterinary Medicine, University of São Paulo (USP), São Paulo, Brazil.

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ABSTRACT

The dopaminergic antagonist drug Domperidone has immunomodulatory effects. We investigated the effects of repeated Domperidone treatment in a model of Lypopolyssacharide (LPS)-induced acute lung inflammation. Adult C57BL/6J mice were treated with either Vehicle or Domperidone for 5 days, and challenged intranasally with LPS in the following day. The behavior of mice was analyzed in the open field and elevated plus-maze test before and 24 h after LPS challenge. The bronchoalveolar lavage fluid, blood and lung tissue were collected 24 h and 48 h after LPS challenge. Domperidone treatment increased LPS-induced tumor necrosis factor (TNF) and interleukin (IL)-6 production in the bronchoalveolar lavage fluid, without altering tissue damage and the number of immune cells in the lungs and circulation. Locomotor and anxiety-like behavior were unchanged after Domperidone and/or LPS treatment. Cytokine data indicate that Domperidone promotes a change in activity of other cell types, likely alveolar epithelial cells, without affecting immune cell migration in the present model. Due to the role of these cytokines in progression of inflammation, Domperidone treatment may exacerbate a subsequent inflammatory injury.

1. Introduction

Dopamine plays a pivotal role in motor, cognitive, behavioral and endocrine functions in central nervous system. Dopaminergic neurons cell bodies are mainly located in the substantia nigra *pars compacta*, ventral tegmental area and hypothalamus, and are identified by the expression of tyrosine hydroxylase (TH), the rate-limiting enzyme of catecholamine synthesis. The expression of dopamine receptors is distributed in the central nervous system, particularly on regions of dopaminergic projections, such as dorsal (nigrostriatal) and ventral (mesolimbic) striatum, prefrontal cortex (mesocortical) and anterior hypophysis (tuberoinfundibular).

Despite the well-known role of dopamine in brain and behavioral functions, accumulating evidence indicates that immune cells also express constitutively TH and secrete dopamine, as well as express dopamine receptors [1,2]. In T cells, stimulation of D1 dopaminergic receptor (D1R) impairs T regulatory cell function and inhibits the cytotoxicity of $CD8^+$ cells [3,4], whereas the stimulation of D2R by dopamine promotes IL-10 production by T cells [5]. In macrophages, dopamine inhibits the NLRP3 inflammasome via D1R [6], and alleviates an experimental spinal cord injury [7], and the blockage of D2R with Haloperidol increases macrophage activity [8].

In addition, the continuous use of antagonist drugs also elicits hyperprolactinemia due to blockage of D2R in the tuberoinfundibular pathway that influences the immune function per se. Previous research have demonstrated that continuous treatment with D2R antagonist Domperidone increased serum prolactin levels and decreased the production of reactive oxygen species by in vitro macrophages [9]. In addition, short-term Domperidone treatment increased the carrageenan-induced inflammatory paw edema, whereas the long-term treatment decreased inflammatory edema, indicating a dual effect of Domperidone treatment on inflammatory response [10,11]. In fact, Domperidone has been used as a preventive strategy against canine leishmaniosis in high prevalence areas, due to its effect on enhancing cell-mediated immunity [12,13]. A recent study from our group has revealed that repeated Domperidone treatment increased pulmonary secretion of pro-inflammatory cytokines induced by lung allergic inflammation, in spite of decreased inflammatory cell infiltrate in the lungs [14], suggesting that this drug treatment may have a relevant impact in pulmonary acute inflammatory response.

In this sense, the acute lung injury (ALI) and its severe form, acute respiratory distress syndrome, is a pulmonary disease characterized by

* Corresponding author.
E-mail address: adrianozager@hotmail.com (A. Zager).

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bilateral inflammatory infiltrates, alveolar-capillary injury, neutrophils accumulation and cytokine release [15]. The mortality rate of ALI is still at around 40%, due to persistent respiratory failure and increased susceptibility to multi-organ dysfunction, despite recent advances in intensive care medicine [16,17].

ALI is still a huge burden to public health systems and new therapeutical strategies are necessary to control or prevent the inflammatory process. Given the dual effect of Domperidone treatment and consequent hyperprolactinemia in immune-inflammatory response, the aim of the present study was to investigate the effects of repeated Domperidone treatment on acute lung injury induced by Lypopolyssacharide (LPS) in mice.

2. Methods

2.1. Animals

Adult (8 weeks old) C57BL/6J male mice from our colony, weighing 20–30 g each, were used. The animals were housed in standard polypropylene cages at a controlled room temperature (22 ± 2 °C) and humidity level (65–70%), with artificial lighting (12 h light/12 h dark cycle) and with free access to rodent chow (Nuvilab®, Nuvital company, São Paulo, Brazil) and filtered water. Sterilized and residue-free wood shavings were used as animal bedding. The animals were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo, Brazil (Ethical permit #7737251115).

2.2. Drugs

Domperidone was administered intraperitoneally (i.p.) at the dose of 1.7 mg/kg, three times per day (6:30 AM, 2:00 PM, and 9:00 PM), for five consecutive days. This treatment protocol has been shown to effectively produce stable hyperprolactinemia [14].

2.3. Acute lung injury (ALI)

The morning after the last vehicle or Domperidone treatment, mice were anesthetized with ketamine and xylazine (i.p., 100 and 10 mg/kg, respectively), and either sterile saline (naive) or Lypopolyssacharide (100 μg/mL of LPS from *E. coli* serotype O55:B5; Sigma-Aldrich, USA) were intranasally instilled at the volume of 1 μL/g of body weight, according to previous studies [18–20]. 24 and 48 h after the induction of inflammation, mice were anesthetized and killed by exsanguination of the inferior vena cava for the collection of blood, followed by bronchoalveolar lavage fluid (BAL) and tissue collection.

2.4. Open field test

The open field arena consisted of a round wooden arena (40 cm in diameter, 25.5 cm high walls) painted white with an acrylic washable covering. Each mouse was individually placed in the center of the apparatus, and the total locomotor activity (distance traveled in centimeters), mean velocity, and time spent in peripheral and central zones were automatically measured over a period of five minutes. The data was collected by a video camera mounted 100 cm above the arena and analyzed with the Ethovision XT software (Version 7.0 Noldus Information Technology, Leesburg, VA). The apparatus was cleaned with a 5% alcohol solution before placement of the animals to eliminate possible biasing effects from odor clues left by the previous subjects. Control and experimental mice were intermixed for observations that were performed from 08:00 am to 12:00 pm.

2.5. Elevated plus-maze test

The plus-maze consisted of 2 open arms (25 × 5 cm) and 2 closed arms (25 × 5 × 15 cm) arranged perpendicularly, painted white, and elevated 50 cm above the floor. Immediately after the open field test, each mouse was placed in the center of the apparatus and the time spent in open and closed arms were recorded for five minutes. The data was collected by a video camera mounted 100 cm above the arena and analyzed with the Ethovision XT software (Version 7.0 Noldus Information Technology, Leesburg, VA). The percentage of time spent by the mice in the open arms was calculated by the formula: [time in open arms / (time in open arms + time in closed arms)] × 100. The apparatus was cleaned with a 5% alcohol solution before placement of the animals to eliminate possible biasing effects from odor clues left by the previous subject.

2.6. Collection of bronchoalveolar lavage fluid (BAL) and leukocyte counting

For the BAL collection, the lungs were flushed with 1.5 mL of PBS solution using a cannula inserted by tracheostomy. After collection, the BAL fluid was centrifuged at 250 × *g* for 5 min. The supernatant was stored at − 80 °C, and the remaining cell pellet was resuspended in 1 mL of PBS solution. Total leukocyte counts were performed by adding 10 μL of crystal violet to 90 μL of the cell suspension. Neubauer chambers were used for cell counting under a light microscope. The differential cell count was done on the cytocentrifuged (250 x g for 5 min) (FANEM, São Paulo, Brazil) cell suspension (100 μL) stained with Rosenfeld's dye using standard morphological criteria for identification of neutrophils, macrophages and lymphocytes.

2.7. Leukocyte counting in the blood

The blood samples were collected from the abdominal vena cava with syringes containing 10 μL of 10% EDTA. The blood samples were subsequently diluted in Turk's fluid (1:20; 3% acetic acid) and the total number of cells was counted with the aid of a Neubauer chamber under a light microscope. Differential leukocyte counts were performed on blood smears stained with Rosenfeld's dye using standard morphological criteria for identification of neutrophils, macrophages and lymphocytes.

2.8. Quantification of cytokines

BAL supernatants were analyzed for the concentrations of interleukin (IL)-2, IL-4, IL-6, interferon (IFN)-γ, tumor necrosis factor (TNF), IL-17 and IL-10 by flow cytometry using the Cytometric Bead Array (CBA) Th1/Th2/Th17 mouse kit (BD Biosciences, USA) according to the manufacturer's instructions. IL-1β analysis was performed by ELISA (Biolegend, CA, USA).

2.9. Protein analysis in the BAL

The BAL supernatant aliquots were placed in a 96-well plate (10 μL/well) with 250 μL of Bradford reagent (Sigma-Aldrich, St. Louis, USA). After 30 min incubation period, the absorbance was measured at 595 nm. A standard curve was obtained using bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, USA).

2.10. Histological analysis

The lung tissue was collected and fixated in 10% formaldehyde solution, cut into 5 μm sections and stained with hematoxylin and eosin (H.E.). From each sample, four representative photos were taken (magnification × 200), and five high power fields were randomly assigned to each photo. The alveolar wall thickness was measured by ImageJ software (ImageJ, U.S. NIH, Bethesda, Maryland, USA). For each field, the degree of lung damage was determined, according to a modified ALI score [21,22]. In brief, (a) thickness of alveolar walls, (b)