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Short-term hyperprolactinemia decreases allergic inflammatory response of the lungs☆

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ABSTRACT

Aims: Prolactin is a major immunomodulator. The present study evaluated the effects of short-term hyperprolactinemia induced by domperidone before ovalbumin antigenic challenge on the lung's allergic inflammatory response.
Main methods: To induce hyperprolactinemia, domperidone was injected in rats at a dose of 5.1 $mg \cdot kg^{-1}$ per day, i.p., for 5 days from 10th to 14th day after OVA immunization. Total and differential leukocyte counts from bronchoalveolar lavage (BAL), femoral marrow lavage (FML), and blood were analyzed. The percentages of mucus and collagen production were evaluated. Levels of corticosterone and prolactin in serum, interleukin-4 (IL-4), IL-6, IL-10, tumor necrosis factor α (TNF-α) in lung explants supernatants were measured and interferon gamma (IFN-γ) in bronchiolar lavage cells suspensions (BAL) was measured.
Key findings: The rats that were subjected to short-term hyperprolactinemia exhibited a decrease in leukocyte counts in bronchoalveolar lavage, cellularity decrease in femoral marrow lavage fluid, a lower percentage of mucus, and an increase in lung IL-4, IL-6, IL-10, TNF-α and IFN-γ expression.
Significance: Hyperprolactinemia induced before antigenic challenge decreased allergic lung inflammation. These data suggest that prolactin may play a role in the pathophysiology of asthma. The present study demonstrates a prospective beneficial side effect of domperidone for asthmatic patients.

1. Introduction

Gastro-esophageal reflux disease (GERD) and asthma are frequently associated with each other. It is estimated that GERD is present in 60–80% of asthmatic adults and 50–60% of asthmatic children. There is considerable debate regarding the nature of this association, but GERD appears to be an important trigger for asthma and a risk factor for worsening symptoms, asthma-related hospitalization, and the requirement for oral steroids. Some studies have shown that anti-reflux therapy with domperidone improves asthma control in patients with symptomatic GERD [40]. Few studies of dopamine antagonist treatment for asthma have been conducted in humans, but case reports have described the use of domperidone in patients with GERD associated with asthma [21,42]. Several explanations have been made for the apparently paradoxical findings that medical antireflux therapy improves asthma symptoms, but not lung function [21]. However, improvements in lung function have been reported, attributable to the inhibition of bronchial hyperresponsiveness [22]. Thus, considering the possible therapeutic use of domperidone, the mechanisms by which it affects the lungs' allergic inflammatory response and moderates immune and inflammatory processes in asthma need to be explained.

Asthma is an inflammatory disease caused by repeated immediate-phase hypersensitivity and late-phase allergic reactions in the lungs, leading to the clinicopathologic triad of intermittent and reversible airway obstruction, chronic bronchial inflammation with eosinophils, bronchial smooth muscle cell hypertrophy, and hyperreactivity to bronchoconstrictors. The pathophysiologic sequence of atopic asthma is likely initiated by mast cell activation in response to allergen binding to immunoglobulin E (IgE) and Th2 cells that react to allergens [1] and produce inflammation. The inflammation is controlled by cytokines of the Th2 cluster. These cytokines include interleukin 4 (IL-4) and IL-13 (which regulates IgE production), IL-5 (which is heavily implicated in eosinophilia), and the counterregulatory cytokines IL-10 and

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transforming growth factor-β (TGF-β) that suppress T-cell function but activate other cells that contribute to the remodeling of airways [40]. Increased mucus secretion results from the action of cytokines, mainly IL-13, on bronchial epithelial cells [1].

Prolactin (PRL) is a peptide hormone that is secreted from the anterior pituitary gland under tonic inhibition by the hypothalamus via dopamine [5,36]. Prolactin is involved in more than 300 different functions, [6] and its functionality depends on the type of cells that express the PRL receptor. Based on its molecular and functional characteristics, it is a cytokine [36] and participates in innate and adaptive immune responses [23]. During an immune response, PRL promotes the proliferation and differentiation of T cells and influences the expression of CD69 and CD154 in CD4 T cells [6]. Prolactin possesses significant immunomodulatory properties because regulatory T cells constitutively express the PRL receptor [13]. This study was designed to test if hyperprolactinemia induced before antigenic challenge interferes with allergic lung inflammation.

2. Materials and methods

2.1. Animals

Male Wistar rats were obtained from the Department of Pathology Animal House, School of Veterinary Medicine, University of São Paulo. The animals were housed in rooms with ventilation at a constant temperature of 22–23 °C under a fixed 12 h/12 h light/dark cycle (lights on at 6:00 AM) with free access to food and water. All of the procedures were performed in strict accordance to the guidelines of the Colegio Brasileiro de Experimentação Animal and National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Allergic lung inflammation model in rats

The rats were sensitized with 10 μg ovalbumin (OVA; Egg Albumin Grade II, Sigma-Aldrich, St. Louis, MO, USA) [14,25] and 10 mg aluminum hydroxide (EMS Pharmaceuticals, Brazil) dissolved in phosphate-buffered saline (PBS) and administered subcutaneously on day 0 at a dose of 0.1 $mg \cdot kg^{-1}$. One week later (day 7), rats were boosted subcutaneously with the same treatment. For the challenge with OVA aerosol (1% in PBS), animals were individually placed in an inhalation chamber connected to an ultrasonic nebulizer for 15 min per day for 3 consecutive days (days 15, 16, and 17) according to previous studies [19,26].

2.3. Experimental hyperprolactinemia

Hyperprolactinemia was induced by the dopamine receptor blocker domperidone (Johnson & Johnson, Brazil). Although domperidone does not cross the blood–brain barrier,[28] it acts on hypophysis and increases PRL secretion [12,20,34]. Domperidone was administered at a dose of 1.7 mg/kg (i.p.) three times per day (6:30 AM, 2:00 PM, and 9:00 PM) for 5 consecutive days [34,35] from 10th to 14th day after OVA immunization. This treatment protocol has effectively produced stable hyperprolactinemia for more than 30 days [2].

2.4. Experimental outline

Male rats, 60–90 days of age, were randomly divided into four groups: naive group with no treatment and no lung allergy (N), control group (C), vehicle group (V), and domperidone group with induced lung allergy and respective treatments (D).

On day 0, the C, V, and D groups were injected with OVA and boosted on day 7. Between days 10 and 14, the D group was treated with domperidone as described above, whereas the V group was administered 0.9% NaCl. On days 15, 16, and 17, the C, V, and D groups received OVA in aerosol form to induce lung allergy. The experimental outline is depicted in Fig. 1.

2.5. Sample collection

On day 18, 24 h after the last OVA challenge, the animals were anesthetized with 5 $mg \cdot kg^{-1}$ of 2% xylazine hydrochloride (Konig; i.p.) and 30 $mg \cdot kg^{-1}$ of 5% ketamine (Ketalar; Konig; i.p.). The peritoneal cavity was opened, and blood was collected through the abdominal aorta in plastic syringes that contained 50 μl of 8% ethylenediamine tetraacetic acid (EDTA). Blood was set aside until clot formation and then immediately centrifuged for serum collection, which was stored at −80 °C. All blood collections were performed with the same schedule to avoid circadian rhythms effects. Subsequently, the lungs were washed four times with 5.0 ml heparinized PBS (20 ml) through a polyethylene cannula (1 mm inner diameter) inserted by tracheotomy. Bronchoalveolar lavage (BAL) was performed according to previous data [18]. Recovered BAL fluid was centrifuged at 170 × *g* for 10 min at 4 °C. The supernatant was discarded, and the resulting pellet was resuspended in PBS (1 ml). Rat femurs were removed, and a needle connected to a plastic syringe containing 5 ml PBS was inserted into each femoral marrow to allow cell collection by flushing. The femoral marrow lavage (FML) fluid was centrifuged at 170 × *g* for 10 min, and the cell pellet was resuspended, processed, and analyzed for total leukocyte counts [19,26] The adrenal glands were then removed and weighed. The relationship between the wet weight of the two adrenal glands (in milligrams) and body weight (in grams) was also analyzed, calculated, and compared among groups. Lung samples were also collected for explant culture and fixation for histochemistry.

2.6. Total and differential whole-blood counts

Samples were diluted 1:20 in Turk liquid (3% acetic acid) and counted in a Neubauer chamber. Blood smears were stained with May–Grünwald–Giemsa, and differential leukocytes were counted by light microscopy [19,26].

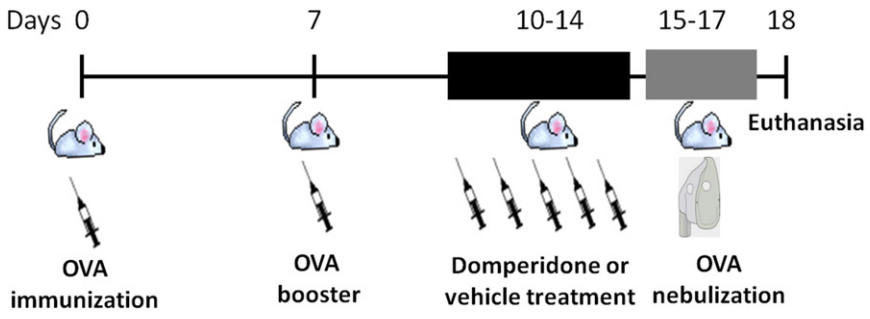

Fig. 1. Experimental outline.