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The orl rat is more responsive to methacholine challenge than wild type

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ABSTRACT

Background: This study presents an animal model of native airway hyperresponsiveness (AHR). AHR is a fundamental aspect of asthma and reflects an abnormal response characterized by airway narrowing following exposure to a wide variety of non-immunological stimuli. Undescended testis (UDT) is one of the most common male congenital anomalies. The orl rat is a Long Evans substrain with inherited UDT. Since boys born with congenital UDT are more likely to manifest asthma symptoms, the main aim of this study was to investigate the alternative hypothesis that orl rats have greater AHR to a methacholine aerosol challenge than wild type rats.

Methods: Long Evans wild type ($n = 9$) and orl ($n = 13$) rats were anesthetized, tracheostomized, and mechanically ventilated at 4 weeks of age. Escalating concentrations of inhaled methacholine were delivered. The methacholine potency and efficacy in the strains were measured. Respiratory resistance was the primary endpoint. After the final methacholine aerosol challenge, the short-acting β_2-adrenoceptor agonist albuterol was administered as an aerosol and lung/diaphragm tissues were assayed for interleukin (IL)-4, IL-6, and tumor necrosis factor (TNF)-α. Histological and histomorphometrical analyses were performed.

Results: The methacholine concentration–response curve in the orl group indicated increased sensitivity, hyperreactivity, and exaggerated maximal response in comparison with the wild type group, indicating that orl rats had abnormally greater AHR responses to methacholine. Histological findings in orl rats showed the presence of eosinophils, unlike wild type rats. β_2-Adrenoceptor agonist intervention resulted in up-regulation of IL-4 diaphragmatic levels and down-regulation of IL-4 and IL-6 in the lungs of orl rats.

Conclusion: orl rats had greater AHR than wild type rats during methacholine challenge, with higher IL-4 levels in diaphragmatic tissue homogenates. Positive immunostaining for IL-4 was detected in lung and diaphragmatic tissue in both strains. This model offers advantages over other pre-clinical murine models for studying potential mechanistic links between cryptorchidism and asthma. This animal model may be useful for further testing of compounds/therapeutics options for treating AHR.

Abbreviations: AHR, airway hyperresponsiveness; *R*, respiratory resistance; Cdyn, dynamic compliance; EI, expansion index; GM, geometric means; IL, interleukin; IHC, immunohistochemistry; TNF α, tumor necrosis factor-α; V_g, total gas exchange area; V_p, parenchyma area; UDT, undescended testis.

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1. Introduction

The orl rat is an inbred Long Evans substrain with inherited cryptorchidism or undescended testis (UDT) [1,2]. This is a distinctive model of UDT since the phenotype is similar to that seen most commonly in humans. UDT is one of the most common male congenital anomalies in humans; between 2 and 5% of full-term and 30% of premature male infants are born with UDT [3]. In orl rats, UDT prevalence is approximately 60% [4]. It is likely that clinical UDT is the result of gene–hormone-environment interactions [2,5,6]. A case–control study demonstrated that boys born with UDT were more likely to present with asthma [7]. This study interviewed mothers of affected (83 UDT cases) and unaffected boys (129 controls), and reported a 9.6% proportion of at least one episode of asthma in cases vs. 1.6% in controls. In addition, we have observed respiratory distress, a wheezing-like pattern, and a higher mortality in the orl rat, than wild type rats. A respiratory phenotype in orl rats strain has not been described. Because with an asthma-like condition, the diaphragm may be spasmodically in contraction to force air in and out the lungs, we were interested also in the evaluation of the diaphragmatic tissue.

Asthma is currently defined by the Global Initiative for Asthma as a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, and the chronic inflammation is associated with airway hyperresponsiveness (AHR) that leads to recurrent episodes of wheezing [8]. AHR is a fundamental aspect of asthma, but also may be present with other respiratory disorders. AHR reflects an abnormal response characterized by airway narrowing following exposure to a wide variety of non-sensitizing stimuli of a chemical or physical origin. AHR reveal the ease with which airway narrowing is achieved (the sensitivity), and the extent of that response, which is represented by the hyperreactivity and maximal response [9] in the concentration–response curve. AHR may be due to the presence of airway inflammation, abnormal airway mechanics, or a combination of both; however additional mechanisms which lead to an augmentation of the stimuli (pre-junctional mechanisms) and mechanisms which lead to an increased response (post-junctional mechanisms) are complex possible mechanisms [10].

With regard to the rationale of the inflammatory biomarkers selected in this study, the overproduction of interleukin (IL)-4 has been associated with atopic and non-atopic asthma [11]. Tumor necrosis factor (TNF)-α is a pro-inflammatory cytokine that is also produced in increased amounts in asthmatic airways and may be an amplifying mediator in AHR/asthma [12,13]. IL-6, an interleukin that acts as the pro-inflammatory and anti-inflammatory cytokine, is a muscle-derived cytokine found in high levels in the tissues rats exposed to respiratory muscular work [14].

Native or intrinsic AHR (not antigen-induced) may be determined genetically. Most studies of inbred mice strains and backcrosses have suggested strong genetic control of airway narrowing [15–17]. It seems likely that animal models of native AHR may hold considerable potential for understanding the genetics of asthma and associated diseases. A detailed understanding of the concentration–response curves and their determinants – altered airway sensitivity, airway reactivity and maximal degree of airway narrowing – is fundamental for the translation of functional and drug response findings to the bed-side.

The aims of this pre-clinical translational study were to compare respiratory mechanics (under resting conditions, during methacholine), and following β_2-adrenoceptor agonist treatment, biomarkers of lung inflammation, and tissue structural differences between orl rats and wild type rats. We hypothesized that orl rats would have greater AHR than wild type rats, and this outcome would be associated with biomarkers of tissue inflammation/injury and structural alterations in the lung and diaphragmatic tissues.

2. Material and methods

2.1. Animal preparation

This animal model of inhaled methacholine (methylcholine chloride; Methapharm Inc., Ontario, CA) challenge-induced constriction was approved by the Institutional Animal Care and Use Committees. Two groups, Long Evans wild type rats ($n = 9$) five females and four males, and orl rats ($n = 13$) five females and eight males, were studied at 4 weeks of age (equivalent to early puberty/late childhood in humans). A control group of animals [sham-orl rats ($n = 6$)] was used. The sham-orl rats underwent the same procedures, but there were not challenged with inhaled methacholine, neither received β_2-adrenoceptor agonist treatment. Animals were anesthetized with intraperitoneal injections of a ketamine–xylazine (1.5:1 ratio) combination (0.05 mL/10 g), without the need of a paralytic agent. Their trachea was opened and a 19-gauge commercial cannula (Buxco Research Systems, Wilmington, NC, USA) was inserted (quarter of its length) and secured with suture to avoid leak and disconnections. Oxygen saturation and heart rate were monitored using a pulse oximeter (MouseSTAT, Smiths Medical, Waukesha, WI). Body temperature was monitor using a laser thermometer (ThermoWorks, Alpine, UT) and maintained using a far infrared warming pad (Kent Scientific Corporation, Torrington, CT).

2.2. Baseline respiratory mechanics

Animals were immediately connected to the pneumotachograph of the plethysmography/ventilator chamber FinePointe RC (Buxco Research Systems, Wilmington, NC, USA) and mechanically ventilated. The volume-targeted ventilator is built-in the system. Respiratory function was measured using this commercial plethysmography system, which collects invasive respiratory resistance and compliance data in anesthetized animals that are tracheostomized. Assessment herein involved measurements of flow and pressure at the tracheal opening. ECG leads were placed to monitor the heart rate. Body temperature was maintained using the heated table of the chamber. Following instrumentation, a 10-min acclimation period was performed to obtain a stable period inside the plethysmography chamber through 3 LPM of O_2, tidal volumes = 0.05 mL/10 g, respiratory rate of 90 breaths/min, and 2 cm H_2O of positive end-expiratory pressure. A baseline recording without nebulization (resting mechanics) was done at the end of the acclimation period, followed by a control recording using normal saline.

2.3. Methacholine challenge and short-acting β_2-adrenoceptor agonist administration

Once a stable control-saline measurement was recorded, an aerosol methacholine challenge was started with 0.3, 0.7, 1.5, 3.1, 6.2 and 12.5 mg/mL of methacholine during 5 min, at a volume of 0.05 mL using a standard commercial clear Plexiglass in-line aerosol block (Buxco Research Systems, Wilmington, NC, USA) and nebulizer head (Aerogen, Inc., Galvan, Ireland). Respiratory resistance, dynamic compliance (Cdyn), respiratory rate and tidal volume were recorded every 5 min during the methacholine administration. At the end of the methacholine challenge, animals were treated with a short-acting β_2-adrenoceptor agonist, albuterol sulfate inhalation solution, 0.5% (Bausch & Lomb Incorporated, Tampa, FL) at a concentration of 0.238 μg/mL using the same