Pilot study of bronchial hyper-reactivity in two strains of mice exposed to an over-the-counter air freshener

William J. Meggs, Kori L. Brewer, Dorcas O'Rourke, Cecile Baccanale, Amy Fuller, Jeffrey Eells, Jennifer Parker-Cote and Dianne M. Walters

1Department of Emergency Medicine; 2Department of Comparative Medicine; 3Department of Cell Biology; 4Physiology Department, Brody School of Medicine, East Carolina University, Greenville, NC, USA.

ABSTRACT

Susceptible individuals with asthma and rhinitis report exacerbations with exposure to air fresheners. Experimental models may help shed light on the mechanism. We hypothesized mice will have increased bronchial reactivity after a period of air freshener exposure. Two strains of 25-30 gm 10-week-old male mice (C57BL/6J and BALB/cByJ) were exposed to an over-the-counter air freshener for 45 days, then mice were anesthetized and intubated by surgical tracheostomy. Newtonian resistances (Rn) were measured at baseline, after challenge with phosphate buffered saline (PBS) and acetylcholine (ACh) using FlexiVent. Statistical analysis was performed comparing means by student’s t-test. ACh challenge resulted in increased Rn in air freshener-exposed BALB/cByJ mice compared to controls. In the C57BL/6J strain, the mean Rn in mice exposed to air freshener was 0.62 ± 0.21 cmH2O/mL/sec versus 0.50 ± 0.08 in controls: p = 0.11. For the BALB/cByJ strain, the difference was 0.96 ± 0.26 cmH2O/mL/sec in air freshener-exposed versus 0.62 ± 0.28 cmH2O/mL/sec in controls: p = 0.02. BALB/cByJ mice had significantly higher Rn than C57BL/6J mice (p = 0.04) after ACh challenge. A mouse model of air freshener exposure may be used to study bronchial hyper-reactivity from exposure to air fresheners. Mouse strain is an important consideration.

KEYWORDS: air freshener, asthma, bronchial hyper-reactivity, respiratory irritants.

INTRODUCTION

Odorous volatile and semi-volatile organic chemicals are ubiquitously added to a host of consumer products, including perfumes, soaps, detergents, shampoos, deodorants, and air fresheners, to produce pleasant smells and counteract unpleasant ones. Positive benefits reported for air fresheners include ameliorating the adverse effects of noxious odors while improving mood, reducing stress, and enhancing memory [1]. However, survey and surveillance data report that susceptible individuals may have adverse reactions to air freshener exposure, including induction or exacerbation of asthma and other respiratory complaints [2, 3, 4]. Possible mechanisms by which air fresheners cause increased respiratory symptoms in some individuals with asthma include IgE sensitization, irritant triggered reactions, and odor-induced bronchospasm through conditioning or other pathways. Because multiple factors, including genetic predisposition, contribute to susceptibility to asthma, bronchial hyper-reactivity, and other airway disorders, we sought to establish a mouse model to study the effects of volatile organic chemicals (VOCs) in air fresheners and other fragranced products on bronchial hyper-reactivity. In this pilot study, we compared two common inbred mouse strains known to differ in airway responses to allergens [5] and evaluated bronchial
reactivity after exposure to an over-the-counter automobile air freshener. Development of a mouse model of fragrance-induced airway hyper-reactivity could be used to elucidate the mechanism of air fresheners in exacerbating asthma, and provide a platform for identifying which specific chemicals in air fresheners are most likely to produce bronchial hyper-reactivity in susceptible humans such as those with asthma.

METHODS

Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee. Mice were individually housed with standard mouse chow and water ad libitum, 12 hours of light and dark, and constant temperature of 78°F.

A randomized controlled trial was conducted in C57BL/6J and BALB/cByJ mice. Ten-week-old male mice weighing 25-30 gm were either exposed to a commercially available over-the-counter automobile air freshener by suspending the air freshener in their cages for 45 days (n = 6 per strain) or housed normally with no exposure to the air freshener (n = 4 per strain).

Lung function measurements

At the end of the study period, mice were anesthetized with tribromoethanol and intubated by surgical tracheostomy. Mice were mechanically ventilated with 10 ml/kg room air at 150 breaths/minute and a positive end expiratory pressure of 3 cm H2O using a FlexiVent system (SCIREQ, Montreal, QC, Canada). A script was used to ensure consistent timing of perturbations relative to standardization of volume history with total lung capacity maneuvers. The constant phase model was employed to measure airway Newtonian resistance (Rn). Three quick-prime measurements were taken prior to a ten-second aerosol of PBS or ACh (12.5 mg/mL) and approximately every 15 seconds post aerosol challenge for 3 minutes. Data points with a coefficient of determination (COD) ≥ 0.9 were averaged for baseline, PBS, and ACh in each individual subject.

Bronchoalveolar lavage

Immediately after lung function measurements, mice were exsanguinated, the left bronchus was clamped, and the right lung was lavaged with 4 successive aliquots of 26.25 ml/kg cold Hank’s Buffered Saline Solution. Bronchoalveolar lavage (BAL) fluid was centrifuged at 500 g for 10 min at 4°C and cell counts were made with a hemacytometer to estimate the total number of recovered cells. Slides were prepared by cytocentrifugation at 600 rpm for 5 min (Shandon Cytospin III, Thermo Fisher Scientific, Waltham, MA, USA) and stained with a three-step stain set (Richard-Allan, Kalamazoo, MI, USA). Cell differential counts were performed on 300 cells/slide using standard morphological criteria.

Statistical analysis

Mean Newtonian resistance was compared pre- vs. post-aerosol challenge within each strain of mice using paired t-tests with p<0.05 indicating significance. Average resistance post-ACh challenge was compared across the 2 strains of mice using unpaired t-test. All statistical analysis was performed using Graphpad Prism (v.8.0).

RESULTS AND DISCUSSION

In both strains of mice, there was no significant difference in Newtonian airway resistance between air freshener-exposed and air control groups at baseline and after PBS aerosol challenge, although BALB/cByJ mice showed slightly elevated values with exposure to air freshener. However, air freshener-exposed BALB/cByJ mice showed a marked increase in airway reactivity to ACh over air controls, while exposed C57BL/6J mice had only a small increase (Figure 1). The average Rn values for each strain and exposure group at baseline, and after PBS and ACh aerosol challenges are shown in Table 1. In the C57BL/6J strain, the mean Rn in mice exposed to air freshener was 0.62 ± 0.21 cmH2O/mL/sec versus 0.50 ± 0.08 in controls (p = 0.11, 95% confidence interval for difference of means = -13.6-126.8). For the BALB/cByJ strain, the difference was 0.96 ± 0.26 cmH2O/mL/sec in air freshener-exposed versus 0.62 ± 0.28 cmH2O/mL/sec in controls: p = 0.02, 95% CI for difference in means = 15.4 – 135.7. Newtonian resistance after ACh challenge was significantly higher in the BALB/cByJ versus C57BL/6J mice (p = 0.04, 95% confidence interval of difference of means = 0.03-0.89) exposed to air freshener. There were no significant differences between air freshener-exposed and control mice in total cell
Bronchial hyper-reactivity in mice exposed to air freshener and other scented products on humans with asthma and rhinitis are a public health concern given their common use. An online survey of adults in the USA found that that 64.3% of asthmatics reported adverse health effects from fragranced products, with reports of respiratory problems (43.3%), migraine headaches (28.2%), and asthma attacks (27.9%). Additionally, lost workdays or job loss due to air fresheners in the last year was reported by 35.4% of asthmatics [4].

Inability to use a public numbers recovered in BAL fluid, nor in percentages of differential cell types. BALB/cByJ mice had slightly elevated total cell numbers compared to C57BL/6J mice, but the difference was not significant (data not shown).

Air fresheners are a complex mixture of volatile organic chemicals. According to the manufacturer’s material safety data sheet, the air freshener used in this study contained over 20 volatile organic chemicals. The reported effects of air fresheners

![Figure 1](image)

**Figure 1.** Newtonian resistance in C57BL/6J and BALB/cByJ mice exposed to air or air freshener at baseline, after challenge with phosphate buffered saline (PBS), and after challenge with acetylcholine (ACh). Lung function measurements were after 45 days of exposure to air fresher in 6 mice per strain or 4 control mice per strain. *indicates a significant increase over C57BL/6J mice exposed to air freshener and challenged with ACh, p = 0.02, 95% CI for difference in means = 15.4 – 135.7.

**Table 1.** Newtonian resistances at baseline and post-PBS and ACh challenge in air control and air freshener-exposed C57BL/6J and BALB/cByJ mice. Unexposed vs. exposed for BalbC/cByJ: p = 0.02 (95% CI = 15.4 – 135.7). Unexposed vs. exposed for C57BL/6J: p = 0.11 (95% CI = -13.6-126.8).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Exposure</th>
<th>Baseline (± SEM)</th>
<th>Post-PBS (± SEM)</th>
<th>Post-ACh (± SEM)</th>
<th>% post-ACh increase over Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J</td>
<td>Room Air</td>
<td>0.36 (± 0.031)</td>
<td>0.42 (± 0.044)</td>
<td>0.50 (± 0.042)</td>
<td>39%</td>
</tr>
<tr>
<td></td>
<td>Air Freshener</td>
<td>0.309 (± 0.029)</td>
<td>0.42 (± 0.027)</td>
<td>0.619 (± 0.142)</td>
<td>101%</td>
</tr>
<tr>
<td>BALB/cByJ</td>
<td>Room Air</td>
<td>0.377 (± 0.013)</td>
<td>0.335 (± 0.086)</td>
<td>0.617 (± 0.094)</td>
<td>64%</td>
</tr>
<tr>
<td></td>
<td>Air Freshener</td>
<td>0.402 (± 0.030)</td>
<td>0.416 (± 0.091)</td>
<td>0.965 (± 0.109)</td>
<td>138%</td>
</tr>
</tbody>
</table>
The current study sought to identify an appropriate mouse strain in which to study bronchial reactivity in response to fragranced consumer product exposures. Since genetic susceptibility can play a role in bronchial hyper-responsiveness, we chose two genetically diverse strains based on studies that examined airway reactivity in response to methacholine exposure [5]. We anticipated that BALB/c mice would be more susceptible than C57BL/6 and our findings support this hypothesis. This preliminary study suggests that a BALB/c strain may be an effective model to study bronchial hyper-reactivity from exposure to air fresheners or other fragranced consumer products. Future studies are planned to examine specific volatile organic compounds commonly used in fragranced products and to elucidate mechanisms through which they may act.

CONCLUSIONS
A mouse model of air freshener exposure may be used to study bronchial hyper-reactivity from exposure to air fresheners. Mouse strain is an important consideration.

CONFLICT OF INTEREST STATEMENT
The authors have no conflict of interest to declare.

REFERENCES