Effects of Nitrogen Dioxide on Non-Specific Airway Inflammation in Individuals with Allergic Asthma

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Introduction

- Nitrogen dioxide (NO$_2$) is a major air pollutant world-wide
- Environmental sources
  - combustion of fossil fuels
  - motor vehicle exhaust
  - stationary sources (industrial, heating, power generation)
- Residential
  - gas and kerosene appliances
- Occupational
  - welding
Introduction

- **Outdoor concentrations:**
  - California; up to 0.27-0.31 ppm (1 hr average)

- **Indoor concentrations:**
  - 0.12-1.09 ppm (1 hr average); peaks up to 2-4 ppm

- California air quality standard: 0.25 ppm for 1 hr

- **Precursor in the formation of tropospheric ozone via photochemical reactions**
**NO$_2$ Deposition**

- Up to 90% of inhaled NO$_2$ absorbed in the airways
- Deposited throughout the respiratory tract
- Maximum at the junction of conducting and respiratory airways
- Increases with exercise
NO\textsubscript{2} Toxicity

- Oxidant
- Partly water soluble, reacts with airway lining fluid to form nitric and nitrous acids
- Damage via lipid pre-oxidation, cell membranes, increase in cell permeability, bronchial epithelium, decrease in ciliary beat frequency
- Causes cough, throat irritation, dyspnea (15-25 ppm); pneumonia, bronchiolitis (25-100 ppm); fatal pulmonary edema (>150 ppm)
**NO$_2$ Health Effects: Asthma**

- Association between NO$_2$ exposure and increased respiratory symptoms, emergency department visits or hospitalization for asthma

- Asthmatics could be more susceptible due to:
  - Airway inflammation and hyper-reactivity
  - Allergy
**NO₂ Controlled Exposure: Asthma**

- \( n = 12; 1.0 \text{ ppm NO}_2; 3 \text{ hr}; \) bronchoalveolar lavage 1 hr post-exposure; no changes in cell distribution; changes in prostaglandins; decreased \( \text{FEV}_1 \)

- \( n = 8; 0.3 \text{ ppm NO}_2; 1 \text{ hr}; \) sputum-induction 2 hr post-exposure; no change in cell distribution; no change in pulmonary function
Hypothesis

It was hypothesized that exposure to 0.4 ppm NO\textsubscript{2}, compared to filter air, would induce non-specific airway inflammation, 6 hr and 26 hr post-exposure, in individuals with asthma.
Methods

Design

Controlled human exposure experiment: single-blind, counter-balanced, repeated-measures

Subjects

- Twelve atopic subjects with mild asthma:
  - 7 females, 5 males
  - age = 35.7 ± 9.4 yr
  - FEV₁ = 75.1 ± 9.1 % pred
  - MCh PC₂₀ = 2.0 ± 2.3 mg ml
Methods

Procedure

- Exposure: Filtered Air; 0.4 ppm NO₂ (0.4 ± 0.017) ppm (compressed gas); chamber, 3 hr, 3 x 30 min exercise
- Spirometry: pre- and post-exposure, and pre-S-6
- Sputum induction: 6 hr and 26 hr post-exposure
- Cells: total and differential counts
- Proteins: total protein, IL-6, IL-8, GMCSF

Statistical Analysis

- Wilcoxon Signed-Rank Test, significance set at p<0.05
Sputum-Induction

- Non-invasive, safe, inexpensive
- Nebulized 3% saline
- Inhaled via mouth-piece
- \( t = 20 \text{ min}; 2 \text{ min interval collection} \)
- Collect sputum and saliva separately
- Sputum homogenized
- Cell counts
- Proteins
- Sample derived from central airways
- Controlled human exposures: \( \text{NO}_2 \), ozone, particles, diesel exhaust
S-I Neutrophils: Ozone, Particles, Acid

Exposure

Neut. (%)
S-I Differential Cells: Zinc-Oxide

Filtered Air

Zinc-Oxide
Abbreviations: FA = filtered air; NO$_2$ = nitrogen dioxide; S-6 = sputum induction at 6 hr after exposure; S-26 = sputum induction at 26 hr after exposure
<table>
<thead>
<tr>
<th></th>
<th>S-6 FA</th>
<th>S-6 NO₂</th>
<th>S-26 FA</th>
<th>S-26 NO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leukocytes &lt;sup&gt;10⁴/ml&lt;/sup&gt;</td>
<td>59.5</td>
<td>82.8</td>
<td>57.5</td>
<td>58.5</td>
</tr>
<tr>
<td></td>
<td>41.5 – 119.3</td>
<td>50.5 – 122.5</td>
<td>44.0 – 88.0</td>
<td>51.0 – 66.0</td>
</tr>
<tr>
<td>% Macrophage</td>
<td>35.9</td>
<td>43.2</td>
<td>38.1</td>
<td>44.1</td>
</tr>
<tr>
<td></td>
<td>14.6 – 64.8</td>
<td>18.5 – 53.4</td>
<td>29.6 – 56.1</td>
<td>34.3 – 46.9</td>
</tr>
<tr>
<td>% Neutrophil</td>
<td>59.6</td>
<td>49.9</td>
<td>56.1</td>
<td>53.1</td>
</tr>
<tr>
<td></td>
<td>30.9 – 79.9</td>
<td>38.1 – 79.9</td>
<td>41.1 – 66.2</td>
<td>46.3 – 62.1</td>
</tr>
<tr>
<td>% Eosinophil</td>
<td>1.2</td>
<td>0.8</td>
<td>1.4</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>0.3 – 1.8</td>
<td>0.5 – 3.0</td>
<td>0.6</td>
<td>0.0 – 4.6</td>
</tr>
<tr>
<td>% Lymphocyte</td>
<td>0.25</td>
<td>0.4</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>0.0 – 3.1</td>
<td>0.2 – 1.2</td>
<td>0.3 – 1.8</td>
<td>0.0 – 3.0</td>
</tr>
<tr>
<td>Macrophage &lt;sup&gt;10⁴/ml&lt;/sup&gt;</td>
<td>28.6</td>
<td>27.9</td>
<td>21.9</td>
<td>25.1</td>
</tr>
<tr>
<td></td>
<td>7.7 – 57.6</td>
<td>14.6 – 31.5</td>
<td>15.0 – 39.8</td>
<td>9.5 – 29.1</td>
</tr>
<tr>
<td>Neutrophil &lt;sup&gt;10⁴/ml&lt;/sup&gt;</td>
<td>41.0</td>
<td>48.8</td>
<td>34.3</td>
<td>32.6</td>
</tr>
<tr>
<td></td>
<td>18.3 – 76.2</td>
<td>19.6 – 91.3</td>
<td>18.1 – 76.6</td>
<td>23.6 – 53.5</td>
</tr>
<tr>
<td>Eosinophil &lt;sup&gt;10⁴/ml&lt;/sup&gt;</td>
<td>0.9</td>
<td>0.9</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>0.3 – 2.1</td>
<td>0.5 – 2.6</td>
<td>0.4 – 1.3</td>
<td>0.0 – 2.2</td>
</tr>
<tr>
<td>Lymphocyte &lt;sup&gt;10⁴/ml&lt;/sup&gt;</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>0.0 – 6.2</td>
<td>0.05 – 1.1</td>
<td>0.1 – 0.6</td>
<td>0.0 – 1.5</td>
</tr>
</tbody>
</table>

FA: filtered air, NO₂: nitrogen dioxide, S-6 and S-26: sputum induction 6 and 26 hours after exposure. Values expressed as median; interquartile range.
Individual Differential Neutrophil Count in Induced Sputum at 6 hr (A) and 26 hr (B) Post-Exposure to Filtered Air and Nitrogen Dioxide
### Protein Analysis of Induced Sputum at 6 hr and 26 hr Post-Exposure to Filtered Air and Nitrogen Dioxide

<table>
<thead>
<tr>
<th></th>
<th>S-6</th>
<th></th>
<th>S-26</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FA</td>
<td>NO₂</td>
<td>FA</td>
<td>NO₂</td>
</tr>
<tr>
<td>IL-6 (pg ml)</td>
<td>106.0</td>
<td>78.1</td>
<td>64.3</td>
<td>66.1</td>
</tr>
<tr>
<td></td>
<td>82.6 – 251.6</td>
<td>31.6 – 98.8</td>
<td>28.4 – 129.2</td>
<td>31.1 – 171.1</td>
</tr>
<tr>
<td>IL-8 (pg ml)</td>
<td>2329.7</td>
<td>3045.1</td>
<td>3347.5</td>
<td>2349.4</td>
</tr>
<tr>
<td></td>
<td>1705.0 – 5822.4</td>
<td>2289.0 – 5250.0</td>
<td>1774.0 – 5951.6</td>
<td>1289.5 – 4014.3</td>
</tr>
<tr>
<td>GM-CSF (pg ml)</td>
<td>2.2</td>
<td>1.1</td>
<td>1.1</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>0.9-3.2</td>
<td>0.6-1.5</td>
<td>1.0-2.3</td>
<td>0.2-2.5</td>
</tr>
<tr>
<td>Total Protein (mg ml)</td>
<td>1.62</td>
<td>2.0</td>
<td>1.67</td>
<td>2.20</td>
</tr>
<tr>
<td></td>
<td>1.60 – 1.63</td>
<td>1.42 – 2.88</td>
<td>1.66 – 2.10</td>
<td>1.44 – 2.75</td>
</tr>
</tbody>
</table>

*IL-6: interleukin-6, IL-8: interleukin-8, TP: total protein. Values expressed as median and interquartile range*
### Spirometric Pulmonary Function Responses to Filtered Air and Nitrogen Dioxide

<table>
<thead>
<tr>
<th>SPF</th>
<th>Pre-Exposure</th>
<th>Post-Exposure</th>
<th>Pre-S-6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FA</td>
<td>NO₂</td>
<td>FA</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>3.61</td>
<td>3.66</td>
<td>3.86</td>
</tr>
<tr>
<td></td>
<td>3.0-4.3</td>
<td>3.2-4.2</td>
<td>3.0-4.3</td>
</tr>
<tr>
<td>FEV₁ (L)</td>
<td>2.97</td>
<td>2.79</td>
<td>2.92</td>
</tr>
<tr>
<td></td>
<td>2.2-3.3</td>
<td>2.4-3.4</td>
<td>2.3-3.4</td>
</tr>
<tr>
<td>FEF₂₅-₇₅ (L s)</td>
<td>2.39*</td>
<td>2.56</td>
<td>2.61</td>
</tr>
<tr>
<td></td>
<td>1.5-3.1</td>
<td>1.8-2.9</td>
<td>1.8-3.3</td>
</tr>
</tbody>
</table>

*SPF: spirometric pulmonary function, FA: filtered air, NO₂: nitrogen dioxide, S-6: sputum induction 6 hr post-exposure, FVC: Forced vital capacity, FEV₁: Forced expiratory volume in one second, FEF₂₅-₇₅: Mean forced expiratory flow rate between 25-75% of FVC, * = significantly different (p=0.04). Values as median; interquartile range*
Individual FEF25-75% Values at 6 hr Post-Exposure to Filtered Air and Nitrogen Dioxide
The results of this project indicate that in individuals with asthma, exposure to 0.4 ppm NO$_2$ results in:

- Decrease in spirometric pulmonary function
- No changes in inflammation-associated cells
- No changes in cytokine proteins
Discussion

- Maximal NO$_2$ deposition and S-I sampling region
- Sputum-induction variability:
  - With-in subjects
  - Between subjects
  - Asthma: disease status
Discussion

- Bronchoscopy
- Decrease in $\text{FEF}_{25-75\%}$ potentially in the region of higher $\text{NO}_2$ deposition
- Individual susceptibility
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