Impact of low dose chlorine inhalation in healthy humans: a pilot study

Low dose chlorine inhalation in humans

Ojanguren I MD, PhD¹, Chaboillez S RT¹ Cloutier Y Ing², Panariti A PhD³, McGovern TK PhD³, Martin JG MD³, Lemiere C MD, MSc¹

¹Centre intégré universitaire de santé et de services sociaux du Nord-de-l'Île-de-Montréal, Hôpital du Sacré-Cœur de Montréal, Université de Montréal, Montréal, Quebec, Canada.
²Institut de Recherche Robert Sauvé en Santé et Sécurité au Travail, Canada.
³Meakins Christie Laboratories, Department of Medicine, McGill University, Research Institute of the McGill University Health Center, Canada.

Dr. C. Lemiere
Department of Chest Medicine
Centre intégré universitaire de santé et de services sociaux du Nord-de-l'Île-de-Montréal, Hôpital du Sacré-Cœur de Montréal,
5400 Gouin Ouest, Montreal, (Qc), Canada, H4J 1C5
E-mail: catherine.lemiere@umontreal.ca

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The airway injuries induced by chlorine inhalation have been mainly studied in humans after a high-dose accidental exposure or in animal models [1, 2]. Chlorine can induce a direct oxidative epithelial injury, but further damage may also occur with a migration and activation of inflammatory cells such as neutrophils within the airway, with a subsequent release of reactive oxygen species and proteolytic enzymes. The release of reactive species can contribute to airflow limitation and airway hyperresponsiveness. The release of matrix metallopeptidases (MMP) and cysteinyl leukotrienes induced by an airway epithelial insult may promote the development of airway remodeling [2].

A low-dose exposure to chlorine occurs frequently during activities such as routine attendance at swimming pools or usage of domestic and/or industrial cleaners. Although some human studies have looked at the effects of low-dose chlorine exposures on airway function, few studies have reported the impact of low-dose chlorine exposure on airway inflammation in humans [3,4]. A better understanding of these mechanisms is critical in improving the prevention and treatment of injuries induced by chlorine inhalation.

The objectives of the present study were to investigate the effect of low-dose chlorine exposure on lung function and airway inflammation in healthy subjects.
A pilot experimental crossover study comparing the effect of a low-dose chlorine exposure and fresh air exposure on respiratory function and airway inflammation was performed. The subjects were exposed to gaseous chlorine and fresh air with a two-week washout period at the Hôpital du Sacré-Coeur de Montréal (Montreal, Qc, Canada), between February and June 2017.

Eligible subjects were 18 years or older and healthy. All participants provided informed consent. The study was approved by the Research Ethics Committee of Sacré-Coeur Hospital.

Spirometry [5], methacholine inhalation challenge test [6] and sputum induction were performed at the first visit. Sputum was induced and processed as previously described [7] for total and differential cell counts. Supernatant was stored for subsequent measurements of oxidative stress markers, cysteinyl leukotrienes, anti-inflammatory prostaglandins, and potential mediators of remodeling. Forty-eight hours later, the subjects were exposed to either fresh air or to 1 ppm of gaseous chlorine for 15 minutes, the threshold value accepted as safe in Canada [8], using a previously described closed circuit apparatus [9]. Spirometry was repeated immediately after the end of the exposure period. Thirty minutes following the exposure, subjects underwent sputum induction. Twenty-four hours after the exposure, subjects once again underwent spirometry, methacholine challenge test, and sputum induction. After a washout period of two weeks, subjects repeated the different procedures, but were exposed to the alternate exposure condition. The order of exposures (chlorine first followed by clean air vs clean air first followed by chlorine) was randomized and balanced.
Forced expiratory volume in the first second (FEV$_1$), sputum total and differential cell counts and select soluble biomarkers were compared between baseline, immediate post-exposure and 24 hours post-exposure, whereas PC$_{20}$ and FeNO were compared at baseline and 24 hours post-exposure.

All statistical analyses including demographic and clinical variables, FEV$_1$, FeNO values, PC$_{20}$ and sputum total, differential cell counts and biomarkers were performed using Kruskal-Wallis tests for continuous variables and chi-square tests for categorical variables, as appropriate. Exposure condition and time-points were taken into account in a crossover design context. Statistical analyses were completed using STATA software.

Six healthy subjects were included, five of whom (83.3%) were women. The median (IQR) age of the recruited subjects was 27.5 (8.0) years old. Five (83.3%) subjects had never smoked while one of them was an ex-smoker with a median (IQR) of 0 (0.5) pack years. Five (83.3%) were atopic.

The median (IQR) of the FEV$_1$ (% predicted), FEV$_1$ (L) and the FEV$_1$/FVC (%) values were 114.0 (17.6) %, 3.2 (0.7) L and 83.7 (5.4) %, respectively.

No differences were found before and after fresh air and before chlorine exposures in terms of lung function and airway responsiveness. The total cell count increased slightly immediately after chlorine exposure; although not statistically significant, the same trend was observed after fresh air exposure. IL-8 seemed to increase slightly after both fresh air exposure and chlorine exposure. Although non-statistically significant, neutrophils tended to increase after exposure to both fresh air and chlorine (Table 1).
D’Alessandro et al. [3] described significant reductions in FEV$_1$ (-350ml) and increases in specific airway resistance in five healthy subjects and five subjects with airway hyperresponsiveness after chlorine exposure at 1 ppm for an hour. Sastre et al. [4] investigated 13 cleaning employees complaining of work-related asthma symptoms when exposed to cleaning agents, three asthmatic controls and three healthy subjects. They assessed sputum differential cell counts and FeNO levels in addition to pulmonary function after a 60 minute-exposure to 0.4 ppm of chlorine. Three cleaning employees experienced an asthmatic reaction after the chlorine exposure. There was a greater fall in FEV$_1$ in the cleaning employees after exposure to chlorine than placebo, but no significant change in PC$_{20}$ was observed after exposure to chlorine. Minimal increases in FeNO were observed after exposure to chlorine.

We did not find any changes in FEV$_1$, PC$_{20}$, FeNO (table 1), as well as in oxidative stress markers, cysteinyl leukotrienes, anti-inflammatory prostaglandins, or remodeling markers, after an exposure to 1ppm of chlorine during 15 minutes compared to fresh air exposure (supplementary table). IL-8 and neutrophils showed a trend towards increase after exposure to both chlorine and fresh air. Although this effect may have been induced by the repetition of saline inhalation used for sputum induction, it is usually observed when sputum collection is repeated within 24 hours. Our first sputum induction was performed 48h prior to the second sputum induction. The significance of this finding is uncertain.

In conclusion, exposure to 1ppm of chlorine for 15 minutes did not induce significant changes in airway function or inflammation compared with fresh air exposure in healthy
individuals. Higher doses of chlorine may be tested to study the impact of chlorine gas on airway inflammation.

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REFERENCES


Table 1. Inflammatory parameters before and after fresh air and chlorine exposure.

<table>
<thead>
<tr>
<th></th>
<th>Fresh air 1 ppm, 15 minutes</th>
<th>Chlorine 1 ppm, 15 minutes</th>
<th>p</th>
<th>Fresh air 1 ppm, 15 minutes</th>
<th>Chlorine 1 ppm, 15 minutes</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24h pre exposure</td>
<td>30' post exposure</td>
<td>24h post exposure</td>
<td>p</td>
<td>24h pre exposure</td>
<td>30' post exposure</td>
</tr>
<tr>
<td>PC&lt;sub&gt;20&lt;/sub&gt;, mg/ml</td>
<td>96 (87.0)</td>
<td>--</td>
<td>93.5 (96.0)</td>
<td>0.79</td>
<td>96.0 (72.0)</td>
<td>--</td>
</tr>
<tr>
<td>FeNO, ppb</td>
<td>17.25 (6.0)</td>
<td>--</td>
<td>16.0 (5.3)</td>
<td>0.93</td>
<td>15.5 (5.0)</td>
<td>--</td>
</tr>
<tr>
<td>TCC, 10&lt;sup&gt;6&lt;/sup&gt; c/ml</td>
<td>1.6 (1.3)</td>
<td>2.9 (3.8)</td>
<td>1.4 (0.8)</td>
<td>0.11</td>
<td>1.1 (0.5)</td>
<td>3.68 (3.2)</td>
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<tr>
<td>Eosinophils (%)</td>
<td>0.0 (0.3)</td>
<td>0.0 (0.3)</td>
<td>0.1 (0.3)</td>
<td>0.73</td>
<td>0.1 (0.3)</td>
<td>0 (0.25)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>35.9 (49.3)</td>
<td>66.9 (30.3)</td>
<td>29.4 (30.8)</td>
<td>0.16</td>
<td>36.8 (46.8)</td>
<td>64.6 (36.0)</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>63.3 (47.3)</td>
<td>30.8 (30.0)</td>
<td>67.5 (31.8)</td>
<td>0.19</td>
<td>43.0 (13.8)</td>
<td>33.9 (42.3)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>0.5 (1.3)</td>
<td>0.3 (0.5)</td>
<td>0.4 (1.0)</td>
<td>0.86</td>
<td>0.9 (0.8)</td>
<td>0.3 (0.8)</td>
</tr>
<tr>
<td>Bronchial cells (%)</td>
<td>0.5 (0.5)</td>
<td>0.3 (1.3)</td>
<td>0.5 (2.0)</td>
<td>0.80</td>
<td>0.8 (0.8)</td>
<td>0.3 (0.8)</td>
</tr>
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<td>Epithelial cells (%)</td>
<td>5.6 (8.4)</td>
<td>7.9 (7.9)</td>
<td>6.8 (7.0)</td>
<td>0.93</td>
<td>5.0 (4.2)</td>
<td>5.1 (0.5)</td>
</tr>
</tbody>
</table>

Data are presented as median (interquartile range [IQR]). List of abbreviations: FeNO: Fractional exhaled nitric oxide; PC<sub>20</sub>: Provocative concentration of methacholine inducing a 20% fall in FEV<sub>1</sub>; TCC: Total cell count.