Impact of Low Dose Chlorine Inhalation in Healthy Humans: A Pilot Study

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Airway injuries induced by inhalation of chlorine have mainly been studied in humans after accidental high-dose exposure and in animal models [1,2]. Chlorine can induce direct oxidative epithelial injury, although further damage may also occur with migration and activation of inflammatory cells such as neutrophils within the airway, with 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 and cysteinyl leukotrienes induced by an epithelial insult in the airway may promote the development of airway remodeling [2].

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 exposure 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 for improving the prevention and treatment of injuries induced by chlorine inhalation.

The objective of the present study was to investigate the effect of low-dose chlorine exposure on lung function and airway inflammation in healthy individuals.

We performed a pilot experimental crossover study comparing the effect of low-dose chlorine exposure and fresh air exposure on respiratory function and airway inflammation. Participants were exposed to gaseous chlorine and fresh air with a 2-week washout period at Hôpital du Sacré-Coeur de Montréal, Montreal, Canada between February and June 2017.

Eligible participants 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 for total and differential cell counts as previously described [7]. 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 participants were exposed either to 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, participants underwent sputum induction. Twenty-four hours after the exposure, participants once again underwent spirometry, methacholine challenge test,

Table. Inflammatory Parameters Before and After Fresh Air and Chlorine Exposure[a]

	24 h Pre-exposure	30' Postexposure	24 h Postexposure	*P* Value	24 h Pre-exposure	30' Postexposure	24 h Postexposure	*P* Value
PC_{20}, mg/mL	96 (87.0)	--	93.5 (96.0)	.79	96.0 (72.0)	--	94.0 (78.0)	.93
FeNO, ppb	17.25 (6.0)	--	16.0 (5.3)	.93	15.5 (5.0)	--	15.3 (7.0)	.52
TCC, 106 c/mL	1.6 (1.3)	2.9 (3.8)	1.4 (0.8)	.11	1.1 (0.5)	3.68 (3.2)	1.4 (0.7)	.01
Eosinophils, %	0.0 (0.3)	0.0 (0.3)	0.1 (0.3)	.73	0.1 (0.3)	0 (0.25)	0 (0.25)	.81
Neutrophils, %	35.9 (49.3)	66.9 (30.3)	29.4 (30.8)	.16	36.8 (46.8)	64.6 (36.0)	45.9 (50.5)	.45
Macrophages, %	63.3 (47.3)	30.8 (30.0)	67.5 (31.8)	.19	43.0 (13.8)	33.9 (42.3)	53.5 (51.8)	.88
Lymphocytes, %	0.5 (1.3)	0.3 (0.5)	0.4 (1.0)	.86	0.9 (0.8)	0.3 (0.8)	0 (0.75)	.08
Bronchial cells, %	0.5 (0.5)	0.3 (1.3)	0.5 (2.0)	.80	0.8 (0.8)	0.3 (0.8)	0.5 (1.0)	.26
Epithelial cells, %	5.6 (8.4)	7.9 (7.9)	6.8 (7.0)	.93	5.0 (4.2)	5.1 (0.5)	12.8 (11.4)	.29

Abbreviations: FeNO, fractional exhaled nitric oxide; PC_{20}, provocative concentration of methacholine inducing a 20% fall in FEV_1; TCC, total cell count.

[a]Data are presented as median (IQR).

and sputum induction. After a washout period of 2 weeks, the procedures were repeated, but the participants 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 selected soluble biomarkers were compared between baseline, immediate postexposure, and 24 hours postexposure, whereas PC_{20} and FeNO were compared at baseline and 24 hours postexposure.

All statistical analyses including demographic and clinical variables, FEV_1, FeNO, PC_{20}, total sputum, differential cell counts, and biomarkers were performed using the Kruskal-Wallis test for continuous variables and the χ^2 for categorical variables, as appropriate. Exposure condition and time-points were taken into account using a crossover design. Statistical analyses were completed using STATA software.

Six healthy individuals were studied (5 women [83.3%]). Median (IQR) age was 27.5 (8.0) years. Five participants (83.3%) had never smoked, and 1 was an ex-smoker with a median (IQR) of 0 (0.5) pack years. Five participants (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 or after exposure to fresh air and before exposure to chlorine in terms of lung function and airway responsiveness. The total cell count increased slightly immediately after chlorine exposure. The same trend was observed after fresh air exposure, although the difference was not statistically significant. IL-8 seemed to increase slightly after both fresh air exposure and chlorine exposure. Neutrophils tended to increase after exposure to both fresh air and chlorine, although, once again, the difference was not statistically significant (Table).

D'Alessandro et al [3] reported significant reductions in FEV_1 (–350 mL) and increases in specific airway resistance in 5 healthy individuals and 5 patients with airway hyperresponsiveness after chlorine exposure at 1 ppm for an hour. Sastre et al [4] investigated 13 cleaners complaining of work-related asthma symptoms when exposed to cleaning agents, 3 asthmatic controls, and 3 healthy individuals. The authors 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 cleaners experienced an asthmatic reaction after chlorine exposure. A greater fall in FEV_1 was observed in the cleaners after exposure to chlorine than placebo, although no significant change was observed in PC_{20} 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), oxidative stress markers, cysteinyl leukotrienes, anti-inflammatory prostaglandins, or remodeling markers after exposure to 1 ppm of chlorine for 15 minutes compared with exposure to fresh air (Supplementary Table). IL-8 and neutrophils tended to increase after exposure both to chlorine and to 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 48 hours prior to the second sputum induction. The significance of this finding is uncertain.

In conclusion, exposure to 1 ppm 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 should be tested to study the impact of chlorine gas on airway inflammation.

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Conflicts of Interest

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