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The effect of chronic exposure to waterpipe tobacco smoke on airway inflammation in mice

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ABSTRACT

Purpose: Acute exposure of experimental animals to waterpipe tobacco smoke has been shown to induce lung inflammation and injury. The aim of this study was to investigate the effect of chronic exposure to waterpipe smoke on inflammatory markers and oxidative stress in the mouse lung.

Method: Using a whole-body exposure system, animals were exposed to waterpipe smoke for 6 weeks with a one-hour daily exposure for 5 days a week.

Results: Exposure to waterpipe tobacco smoke induced the recruitment of inflammatory cells to the airway. Significant elevation in macrophages, lymphocytes and neutrophils was detected in the bronchoalveolar lavage fluid of exposed animals ($P < 0.01$). Furthermore, levels of catalase, glutathione peroxidase (GPx) and superoxide dismutase (SOD) in the lung homogenates were elevated ($P < 0.05$). Finally, waterpipe smoking altered the levels of a panel of inflammatory cytokines including TNFα, IL-1 β, IL-6, IL-10 and IL-12 biomarkers in the lung of exposed animals ($P < 0.05$).

Conclusion: These results support the notion that waterpipe tobacco smoking exerts harmful respiratory health effects.

1. Introduction

Smoking is a major world-wide health problem. Globally, 5–6 million deaths each year are attributed to tobacco use and death rates may increase to 10 million within the next 20–30 years [1]. Worldwide, over 1 million people die from lung cancer each year [2]. Commonly, tobacco is consumed in different ways including cigarette, cigar, and waterpipe (hookah, narghile, or shisha) smoking. The popularity of waterpipe tobacco smoking (WTS) is growing in the eastern Mediterranean and throughout the world, especially among youth [3]. Most likely, this spread, in part, is due to the use of sweetened and flavored tobacco and the misperception that the water in the bowl of a waterpipe "filters" the smoke, rendering it less harmful and less addictive than cigarette smoke [4].

Compared to cigarette smoke, waterpipe tobacco smoke carries substantially higher amounts of toxicants that might augment the harmful effects of tobacco on lung health [5]. Studies on the mainstream smoke aerosol of the waterpipe showed that the "tar" of a single smoking session is typically two orders of magnitude greater than that produced from smoking a single cigarette [6]. In addition, the yields of polycyclic aromatic hydrocarbons (PAH) in one waterpipe use session are many-fold higher than those of a single cigarette [7]. WTS has been shown to cause DNA damage to buccal mucosal cells and to blood lymphocytes [8–11]. Also, the way waterpipe tobacco is smoked results in a dramatically higher exposure volume to smoke and a longer smoke inhalation period resulting in more tobacco consumption per use session [12]. Waterpipe tobacco smokers take an average of 100 puffs per session, and each puff involves inhalation of over 500 mL of smoke. Thus, a 45-min waterpipe smoking session involves inhalation of about 100 times the smoke as a single 5-min cigarette smoking session [13].

In a recent study, we showed that acute exposure (7 days) to waterpipe tobacco smoke induced inflammation and injury to mice lungs as it caused significant increases in the absolute count of neutrophils, macrophages, and lymphocytes. In addition, acute exposure caused elevation in proinflammatory markers such as TNF-α and IL-6 in bronchoalveolar lavage fluid and oxidative stress markers including

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GPx activity in mice lung [14]. The objective of this study was to investigate the effects of a chronic exposure to waterpipe tobacco smoke on the oxidative stress and inflammatory markers in the mouse lung.

2. Methods

2.1. Animals and housing

Young adult male and female Balb/c mice ($n = 10$ per group: 5 from each sex, age from 8 to 10 weeks old) were housed and treated in accordance to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023) and Jordan University of Science and Technology animal use ethics committee. Number of animals per group was based on previous published work [14]. Mice were kept on a 12 h light/dark cycle at room with controlled temperature (25 ± 1 °C) with free access to water and food. The mice were allowed to acclimatize for 10 days before starting the experiments. Mice were assigned randomly to receive fresh air or waterpipe tobacco smoke (WTS) for 6 weeks.

2.2. Waterpipe tobacco smoke (WTS) exposure procedure

Animals were exposed to WTS in a whole-body exposure chamber (38x25x25 cm, LxWxH) which was connected to a programmable waterpipe smoking machine, as described previously [14]. The smoking machine was programmed to puff in accordance with the Beirut Method [15] specifies 171 puffs of 530 mL volume, 2.6 s puff duration, and 17 s interpuff interval. The waterpipe used in this study was the same as that reported previously [14], and was loaded with 10 g of "Two Apples" flavor Nakhla brand ma'assel tobacco each exposure session. Quicklight charcoal briquettes (Shaban, Egypt) were used as the heat source. About 700 mL of tap water was used to fill the bowl of waterpipe prior to each exposure session.

The entire volume of each puff was automatically discharged into the exposure chamber by the smoking machine. The chamber was also continuously fed with fresh air at a rate of approximately 1.5 L air changes per minute, resulting in mean CO concentrations of 967 ± 113 ppm (mean ± SD) in the chamber during each exposure session. CO was logged in one minute intervals using an electrochemical sensor (Monoxor II, Bacharach Inc. PA). Mice in the WTS group were exposed to WTS for one hour daily, 5 days per week for 6 weeks. The control group received standard care with no WTS exposure.

2.3. Analysis of bronchoalveolar lavage fluid (BALF)

Adult mice were sacrificed 20–24 h after the last exposure session by administering a high dose of thiopental (40 mg/kg). After cannulating the trachea, BALF was collected by instilling the lungs with 4 × 0.3 mL aliquots of sterile phosphate-buffered saline. All aliquots were combined for each animal. The collected BALF was centrifuged and the supernatant was saved for cytokines measurement. The cell pellet was cytocentrifuged using a Cytospin 4 (Thermo Electron Corporation, Waltham, MA) at 2000 rpm for 5 min. Total inflammatory cells were counted using Hemacytometer (Hausser Scientific, Horsham, PA). Hema-Gurr-stained cytospin kit was used to count number of differential inflammatory cells as described previously [16].

2.4. Preparation of lung homogenates

Extracted lung tissues were homogenized in lysis buffer and protease inhibitor cocktail (Sigma–Aldrich Corp., MI, USA) as described previously [14]. Homogenates were centrifuged at 14000 x g to remove cellular debris and the supernatants were used to assay the levels of inflammatory and oxidative stress biomarkers.

2.5. Measurements of oxidative and inflammatory biomarkers

Lung and BALF levels of tumor necrosis factor-α (TNFα), interleukin (IL)-1β, 2, 6, 10 and 12 were assayed using eBioscience (San Diego, CA) enzyme-linked immunosorbent assay (ELISA) technique following manufacturer instructions. Absorbance was read at 450 nm using an ELx800 plate reader (Bio-teak instruments, Winooski, USA).

Levels of anti-oxidative enzymes were measured in lung homogenates. Glutathione peroxidase (Sigma–Aldrich, MI, USA), catalase activity (Cell Biolabs, CA, USA), and superoxide dismutase activity (Sigma–Aldrich, MI, USA) were measured following manufacturers' instructions. Enzyme activities were expressed as unit/mg protein. Total protein concentration was estimated utilizing BioRAD kit (Hercules, CA, USA).

2.6. Statistical analysis

Data analysis was performed using GraphPad Prism software (La Jolla, CA). Two-group analysis was done using the unpaired *t*-test. $P < 0.05$ was considered statistically significant.

3. Results

As shown in Fig. 1, the exposure to WTS significantly increased the airway recruitment of inflammatory cells, specifically neutrophils, macrophages and lymphocytes ($P < 0.05$). In addition, chronic exposure to WTS increased the level of total protein in BALF as compared to unexposed mice ($P < 0.05$; Table 1). Further, higher levels of TNFα, IL-1β and IL-6 in BALF and lung were induced by WTS exposure ($P < 0.05$; Table 1). The level of IL-12 in BALF, but not lung tissue, was higher in WTS exposed mice than control (Table 1). On the other hand, a significant reduction in the level of IL-10 in BALF and lung tissues of WTS exposed mice as compared to control ($P < 0.05$; Table 1). No change in the level of IL-2 in BALF was detected by WTS exposure (Table 1). Finally, it is evident that chronic WTS exposure is associated with increased activities of SOD, GPx and catalase enzymes in lung tissue homogenates ($P < 0.05$; Fig. 2).

4. Discussion

In this study, we examined the chronic effects of WTS on lung inflammation in mice. The results strongly suggest that WTS is associated with lung injury as revealed by robust changes in inflammatory and oxidative stress biomarkers. The results from different countries point to a growing worldwide epidemic of WTS [17,18]. This epidemic is concerning given that waterpipe smoke contains and exposes users to, among other toxicants, polycyclic aromatic hydrocarbons and tobacco specific nitrosamines that cause cancer [5,6] as well as nicotine that causes dependence [19]. Moreover, because waterpipe users inhale 50–100 L of smoke with each use session, even occasional users are exposed to high toxicant levels. Despite these facts, many waterpipe tobacco smokers perceive WTS as a behavior that poses little risk of cancer or dependence [4].

The results presented here suggest a strong association between chronic waterpipe smoking and lung injury as demonstrated by changes in the levels of a panel of inflammatory and oxidative stress biomarkers. The data reported here represent a demonstration of the effect of waterpipe smoke, and are consistent with recent work examining the effects of WTS on cellular parameters implicated in the pathogenesis of COPD through impaired cellular growth and inflammation [20]. These changes may predispose the lungs to cancer if they persist, as is the case among chronic tobacco smokers.

Previous animal studies revealed that acute exposure to WTS induced airway inflammation [14], and major matrix metalloproteinases level [21], in mice lungs. In agreement with acute findings, results of the current study showed that chronic exposure to WTS for 6 weeks