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A study on particles and some microbial markers in waterpipe tobacco smoke

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HIGHLIGHTS

- Waterpipe tobacco and smoke contain LPS and fungal biomass.
- A strong correlation was found between TPM and LPS in MS waterpipe smoke.
- Exposure to SH waterpipe smoke leads to deposition of particles in the airways.

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ABSTRACT

Waterpipe smoking is becoming increasingly popular worldwide. Research has shown that cigarette smoke, in addition to hundreds of carcinogenic and otherwise toxic compounds, may also contain compounds of microbiological origin. In the present study we analyzed waterpipe smoke for some microbial compounds. Both of the two markers studied, viz 3-hydroxy fatty acids of bacterial lipopolysaccharide (LPS) and ergosterol of fungal biomass, were found in waterpipe tobacco, in amounts similar as previously found in cigarette tobacco, and in smoke. Waterpipe mainstream smoke contained on average 1800 pmol LPS and 84.4 ng ergosterol produced per session. An average concentration of 2.8 pmol/m^3 of LPS was found in second hand smoke during a 1–2-h waterpipe smoking session while ergosterol was not detected; corresponding concentrations from smoking five cigarettes were 22.2 pmol/m^3 of LPS and 87.5 ng/m^3 of ergosterol. This is the first time that waterpipe smoking has been shown to create a bioaerosol. In the present study we also found that waterpipe smoking generated several polycyclic aromatic hydrocarbons, carbon monoxide, and high fraction of small (<200 nm) particles that may have adverse effects on human health upon inhalation.

1. Introduction

Cigarette tobacco contains large amounts of Gram-positive and Gram-negative bacteria as well as molds (Larsson et al., 2008). Also cigarette smoke is rich in microbial compounds. The presence of endotoxin, viz the biologically active lipopolysaccharide (LPS) of Gram-negative bacteria, in cigarette smoke was demonstrated already in 1999 (Hasday et al., 1999). This finding was important since endotoxin is a strong pro-inflammatory agent. Later, gas chromatography-tandem mass spectrometry (GC-MSMS) was used to identify LPS and fungal biomass marker ergosterol in mainstream (MS) smoke (Larsson et al., 2004, 2008). A positive relationship was found between the amounts of LPS and ergosterol in the tobacco of a studied cigarette and the amounts of the same substances in MS smoke (Larsson et al., 2008). A positive relationship was also found in second hand (SH) smoke between the number of cigarettes smoked indoors over a certain period of time and air concentrations of ergosterol and LPS (Sebastian et al., 2006). Sidestream (SS) smoke contains much fewer quantities of microbiological compounds than MS smoke probably due to thermal degradation (Larsson et al., 2012). While the microbial compounds in the smoke stem from the microbes in the tobacco, other chemicals in the smoke are largely formed by combustion during the smoking. These chemicals include for example carbon monoxide (CO) and numerous hazardous organic compounds (Shihadeh et al., 2012).

Waterpipe smoking (see Fig. 1) is considered by many tobacco users as being less harmful than cigarette smoking and has gained wide popularity in Europe and the US (Akl et al., 2011). However, the available data show that smoking waterpipe results in SH smoke emissions of appreciable amounts e.g. of ultrafine particles, polycyclic aromatic hydrocarbons (PAHs), and aldehydes (Daher et al., 2010). Several of the PAHs in waterpipe smoke are carcinogenic (Sepetdjian et al., 2008).

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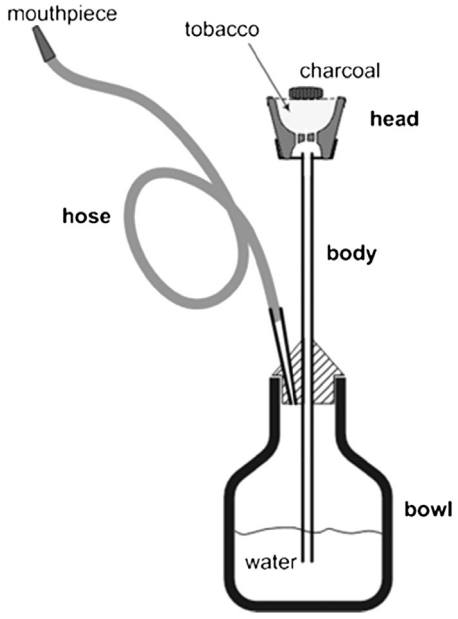

Fig. 1. A narghile waterpipe. The waterpipe consists of a head, body, water bowl, and hose. A moistened, flavored tobacco mixture is placed in the head and covered with a piece of perforated aluminum foil. Burning charcoal is placed on top of the aluminum foil to provide the heat needed to generate the smoke. When a user takes a puff, air and hot charcoal fumes are drawn through the tobacco mixture, and eventually through the water bubbler, hose and mouthpiece. Between puffs, sidestream toxicants are emitted directly from the head to the surrounding environment. Similar quantities of charcoal and tobacco mixture are consumed in a typical 1 hour café use session (Figure adapted from Monzer et al. (2008)).

Research has also shown that waterpipe MS smoke, even after having passed the water in the pipe bowl, contains high levels of CO, toxic metals as well as carcinogenic compounds (Sajid et al., 1993; Sepetdjian et al., 2013; Shihadeh et al., 2012), and that toxicants are effectively delivered to the bloodstream (Blank et al., 2011) inducing measurable acute health effects (e.g. changes in heart rate variability (Cobb et al., 2012)). However, there have been no studies on the possible presence of microbe-derived substances in waterpipe tobacco and smoke. Because of the significantly lower temperature of the tobacco in a waterpipe compared to a cigarette (Shihadeh, 2003), microbial substances may be more efficiently transferred intact to the smoke from the tobacco.

The aim of the present study was to measure some selected microbial compounds in waterpipe tobacco and smoke. LPS and ergosterol were determined in tobacco and in machine generated SS and MS smoke. SH smoke was studied following smoking in an aerosol chamber. Waterpipe smoke was also analysed for PAHs, CO, particle size, and particle concentration. Cigarette smoke was used for comparison. Both types of smoke were characterized with regard to particle size distribution and mass concentration in order to estimate and compare the exposure and deposited dose in the respiratory tract.

2. Materials and methods

2.1. Waterpipe tobacco

Two 100 g packages of eight brands of waterpipe tobacco were purchased at retail outlets in Beirut during the month of April, 2011. For each brand, the contents of the two packages were homogenized and approximately 100 g were sampled and ground manually using a mortar and a pestle. Two grams from each resulting mixture were then placed in a sterile, sealed plastic tube. The 16 tubes were coded for blinding and sent along with empty tubes to Lund University for analysis.

2.2. Machine generated mainstream and sidestream smoke

MS and SS waterpipe smoke were machine-generated at the American University of Beirut following the 171-puff Beirut Protocol (Katurji et al., 2010; Shihadeh, 2003). In brief, 10 g of "Two-Apples" Nakhla™ waterpipe tobacco mixture were loaded in the waterpipe head and the head was covered by an aluminum sheet which was then perforated using a standard hole-punch pattern. The waterpipe was of the design described in Shihadeh (2003) and the leather waterpipe hose had an infiltration rate of 1.6 liters per min (LPM) when measured as described elsewhere (Saleh and Shihadeh, 2008). A single lit 33 mm cylindrical charcoal briquette (5–6 g typical weight) for waterpipe smoking (Three Kings™, Netherlands) was placed on the top of the head at the start of the smoking session; an additional ½ briquette was added at the 105th puff. MS smoke was drawn by the smoking machine through four parallel 47 mm glass fiber filters (Gelman Type A/E), which were changed periodically (3–5 filter changes per session) during a given smoking session to avoid breakthrough overload. The filters were arranged in a parallel flow configuration using a 4-way aerosol splitter (TSI, Inc.) that was attached to the mouthpiece of the waterpipe (see Shihadeh et al. (2012) for details). To collect SS smoke, the waterpipe head was sealed in a 10 L cubical flow enclosure during smoking. Diluted SS smoke emissions were drawn at 16.7 LPM through a 47 mm glass fiber filter installed at the top of the enclosure, while HEPA-filtered makeup air entered through a flow port located on one side. All filters were conditioned for at least 72 hours at 22 °C and 60% humidity prior pre- and post-weighing to determine collected total particulate matter (TPM), and then sent to Lund University for analysis. Field blanks were included and all samples were coded for blinding. Filters from 10 replicate sessions were analyzed. In addition to collecting MS and SS smoke TPM for ergosterol and LPS analyses, CO yields were measured in the MS smoke as in Shihadeh and Saleh (2005).

In separate experiments ($n = 4$) MS smoke was collected as described above. Aliquots (50-ml) of the water in the waterpipe bowl (850-ml) were taken both before and after each smoking session, freeze-dried, and sent to Lund for analysis.

2.3. Second hand smoke

The experiments were conducted in an 21.6 m^3 exposure chamber at the Aerosol Laboratory at Lund University. The chamber's interior surfaces are made of stainless steel, and there is a 0.8 m^2 glass window in one of the walls. Detailed description is given elsewhere (Pagels et al., 2009). In the present study the air supplied to the chamber first passed through an air conditioning unit which allowed control of the air temperature and relative humidity. Then the supply air, before entering the chamber, passed through an activated carbon filter to remove volatile organic compounds and an ultra-low penetration air (ULPA) filter for particle removal. Air to the chamber was supplied from the roof while the exhaust was positioned in the opposite corner from the supply at a height of 0.5 m from the floor. The air exchange rate was set to 0.5 h^{-1}. A positive pressure difference of about 10 Pa between the chamber and surroundings was established to eliminate penetration of particles from the outside. To ensure complete mixing, a fan was operating in the chamber.

In three experiments comprising waterpipe smoking, a portion of tobacco ("Two-Apples" Nakhla™ approximately 10 g) was placed in the waterpipe head and covered by perforated aluminium foil. One liter of distilled water was added in the waterpipe bowl. Thereafter, the smoker entered the chamber and waited for approximately 10 minutes for removal of background particles and stabilization of air pressure. Then the smoker placed charcoal (quick lighting charcoal briquette, approximately 6–7 g) at the top of the waterpipe head, and began smoking. When the coal was finished, a new briquette was lit and added to the