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Mainstream smoke of the waterpipe: Does this environmental matrix reveal as significant source of toxic compounds?

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ABSTRACT

In recent years the number of waterpipe smokers has increased substantially worldwide. Here we report on the concentrations of tobacco-specific nitrosamines (TSNAs) and polycyclic aromatic hydrocarbons (PAHs) in waterpipe smoke and the analysis of selected biomarkers indicative for the body burden in waterpipe users. We further identify high amounts of unburned humectants (glycerol and propylene glycol) in the waterpipe smoke as main part of the so-called "tar" fraction. These results give cause for serious concern. For standardization we applied a machine smoking protocol. Smoke was collected on glass fiber filters and analyzed for nicotine, water, humectants, TSNAs, and PAHs. In addition, we determined carbon monoxide and found high amounts in the smoke being causative for high levels of carboxyhemoglobin (COHb) in the blood of smokers. In comparison to the reference cigarette 3R4F, the nicotine contents were 10-times higher, but TSNA levels were found lower in waterpipe smoke. This finding explained the low levels of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol detected in the urine of waterpipe smokers. Finally, the levels of benzo[*a*]pyrene were three times higher in waterpipe smoke compared to the reference cigarette. Altogether, the data presented in this study point to the health hazards associated with the consumption of waterpipes.

1. Introduction

The waterpipe is a traditional aid for tobacco consumption in Asia and Northern Africa (Maziak et al., 2004a). Although exact numbers are missing to date, web blogs, "hookah bar" advertisements and the advent of popular waterpipe stores indicate that both the general interest and the number of young people smoking waterpipes have considerably grown in recent years in European countries and North America (BZgA, 2008; Jackson and Aveyard, 2008; Primack et al., 2008).

There are major differences in the consumption of waterpipes and cigarettes: The flavored tobacco, mainly used for waterpipes in Europe and the US, contains huge amounts of humectants such as glycerol and propylene glycol. The humectants prevent the tobacco from burning thereby yielding a smooth and pleasant smoke. Furthermore, the heat for the waterpipe is generated using charcoal, which is placed on top of the tobacco head. Studies from Lebanon showed that smoking habits differ greatly between waterpipe and cigarette smokers (Shihadeh et al., 2004). For instance, average inhalation volumes were about 530 ml for single waterpipe puffs whereas the puff volumes found for cigarette smoking were in the range of 35–60 ml (Hammond et al., 2007). Furthermore, the smoking time differs greatly. For a waterpipe with 10 g tobacco the smoking time amounted to almost 60 min and 171 puffs whereas the time for a cigarette is between 5 and 10 min (6–11 puffs). Shihadeh and colleagues established a machine-smoking protocol, which is based on the investigations of smoking behavior of waterpipe smokers in Lebanon, and investigated several constituents of waterpipe smoke such as carbon monoxide (CO), polycyclic aromatic hydrocarbons (PAHs), various aldehydes (e.g., formaldehyde, acetaldehyde, acrolein), and certain metals (e.g., lead, chromium, arsenic) (Al Rashidi et al., 2008; Monzer et al., 2008; Sepetdjian et al., 2008; Shihadeh, 2003; Shihadeh and Saleh, 2005).

In the present study three major questions were addressed: firstly, we determined the levels of tar, nicotine and CO in the waterpipe smoke. To assess the internal body burden of these toxins, biomonitoring of nicotine and carboxyhemoglobin (COHb) in the blood and of cotinine in the urine of smokers was performed. Since the amounts of tar were high in waterpipe smoke, we investigated its composition and found high amounts of humectants. Secondly, the levels of tobacco-specific nitrosamines (TSNAs) were measured in tobacco, tobacco smoke and in the urine of consumers. Since *N*′-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) have been

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classified as human carcinogens (IARC, 2007), we determined the contents of these compounds in waterpipe tobacco and smoke. As biomarker of NNK its metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) was analyzed. Thirdly, various PAHs such as benzo[*a*]pyrene were measured in the waterpipe smoke. Benzo[*a*]pyrene has been classified as human carcinogen (IARC, 2010) and serves as well-established carcinogenic lead compound of environmental PAH mixtures since decades.

2. Materials and methods

2.1. Reagents

All chemicals used in this study were of analytical grade. A list of potential suppliers is given in the supplementary part (see Table S-1).

CO calibration gas was obtained from Air Liquide (Berlin Germany) and 92 mm glass fiber filter pads were from Borgwaldt KC (Hamburg, Germany). Waterpipe tobacco was purchased from Nakhla Tobacco (Two Apples flavor, Nakhla Tobacco, Egypt). Perforated aluminum foil (Ø 15.5 cm, 25 holes) was obtained from Falu, Ballingen, Germany. Quick lighting charcoal (Ø 40 mm) was purchased from Three Kings, The Netherlands. 3R4F reference cigarettes were purchased from the University of Kentucky (Kentucky Tobacco Research & Development Center, Lexington, KY, USA).

2.2. Smoking protocol and smoke collection

Smoking was simulated by connecting a Borgwaldt Shisha Smoker machine to a standard laboratory waterpipe (Borgwaldt KC) using a plastic hose. According to a topographical study each smoking session consisted of 171 puffs of 530 ml each and 2.6 s duration every 20 s and therefore resulted in a total length of 57 min and a total puff volume of 90.63 l (Shihadeh et al., 2004). Ten grams of waterpipe tobacco were transferred into the head of the pipe and covered with perforated aluminum foil. A single quick lighting charcoal disk was lit and, after 60 s, placed atop the perforated foil to start the smoking session. The total particulate matter (TPM) was collected by aspirating the smoke of an entire session through a 92 mm glass fiber filter pad. For determination of humectants, TSNAs and PAHs independent smoking sessions were conducted to address the varying extraction conditions (see Sections 2.4, 2.5 and 2.6), whereas levels of nicotine and water were determined from the same smoking session (see Section 2.3).

After a conditioning period of at least 24 h at 22 °C and 60% relative humidity according to German industrial standard norms (DIN-ISO, 2000a), 3R4F reference cigarettes were vaporized in a rotary RM 20 H smoking machine (Borgwaldt KC) by applying the following puff parameters: 60 s interval, 2 s duration, 35 ml volume (DIN-ISO, 2000b).

2.3. Determination of nicotine, water and carbon monoxide

Sample preparation for nicotine determination was according to DIN-ISO, 2000c with slight modifications. In brief, the filter pads were extracted with 100 ml of the extraction solution. The extract was then filtered through a 0.45 μm PTFE syringe filter and analyzed for nicotine. Nicotine analysis by GC-FID was performed on an HP 6890 gas chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with an FID detector and an autosampler (HP 6890 liquid injector). The chromatographic parameters are provided in the supplementary section (see Table S-2).

For the quantification of water, aliquots of the extraction solution utilized for nicotine analysis were used. Analysis was performed on a Mettler DL 18 Karl-Fischer-Titrator (Mettler-Toledo GmbH, Giessen, Germany).

For CO determination the whole gas phase was collected and quantified by using a non-dispersive infrared absorption (NDIR) CO analyzer (CO/CO_2-Analyzer C24, Borgwaldt KC).

2.4. Determination of humectants

The filter pads were spiked with the internal standard solution (1,4-butanediol) and 50 ml of methanol were added subsequently. Subsequently, the filter pads were agitated for 1 h on an HS 250 basic shaker (IKA Labortechnik, Staufen, Germany). The extract was then filtered into autosampler vials through a 0.45 μm PTFE syringe filter and analyzed by GC-FID. The chromatographic parameters are provided in the supplementary section (see Table S-3).

2.5. Determination of TSNAs

The filter pads were spiked with the internal standard solutions (NNN-$^{13}C_6$, NNK-$^{13}C_6$), 75 ml of 100 mM ammonium acetate were added subsequently and the pads were agitated for 1 h. The extract was then filtered into autosampler vials through a 0.45 μm PTFE syringe filter and analyzed by LC–MS/MS.

Waterpipe and cigarette tobacco (3R4F) were analyzed after transferring 0.2 g of a well-homogenized sample into a 20 ml flask, the addition of the internal standard solutions and subsequent extraction with 15 ml of 100 mM ammonium acetate for 1 h. The extract was filtered into autosampler vials using a syringe filter (0.45 μm PTFE) and analyzed by LC–MS/MS.

For sample analysis a Shimadzu LC-20AD prominence (Shimadzu, Duisburg, Germany) HPLC system coupled with an API 4000 Q TRAP mass spectrometer (AB Sciex Instruments, Applied Biosystems, Darmstadt, Germany) was used. The HPLC system comprised two pumps (LC-20AD), a column oven (CTO-20AC HT), a degasser (DGU-20A5), a controller (CBM-20A), and a temperature controlled autosampler (SIL-20ACHT). The chromatographic parameters are provided in the supplementary section (see Tables S-4.1, S-4.2 and S-4.3).

2.6. Determination of PAHs

Samples for PAH analysis were prepared according to a previously published method (Zha et al., 2002) with some modifications listed next. The filter pads were spiked with the internal standard solution and extracted with 50 ml of methanol. After shaking the filter pads for 1 h, 30 ml of the extraction solution were concentrated to 5 ml using an IR-Dancer 360 (Zinsser Analytic, Frankfurt, Germany) and filtered through a 0.45 μm PTFE syringe filter. Then 7 ml of deionised water was added. After SPE cartridges (Varian Bond Elut CH 500 mg/3 ml, Varian, Darmstadt, Germany) were pre-conditioned with 2 ml of methanol followed by 2 ml of 65:35 (v/v) water/methanol, the smoke extract was loaded and washed with 4.8 ml of water followed by 1.6 ml of methanol. Then the cartridges were dried under nitrogen and the PAHs were eluted with 2 ml of cyclohexane. The cyclohexane extracts were concentrated to 0.5 ml and analyzed by GC–MS.

Analysis of 3R4F reference cigarettes was performed slightly different. Here only 25 ml of methanol were used for extraction and 10 ml were concentrated to a volume of 5 ml. Subsequent sample clean-up was similar to the clean-up of waterpipe samples.

GC–MS analyses were performed on an HP 6890 gas chromatograph equipped with an Agilent MSD 5975C mass spectrometer (Agilent Technologies), a Gerstel Multi Purpose Sampler (MPS-2), and a Gerstel Cold Injection System (CIS) (Gerstel, Mühlheim an der Ruhr, Germany). The chromatographic parameters are provided in the supplementary section (see Tables S-5.1 and S-5.2).

2.7. Biomonitoring

The 22 participants were age 18 and older. The groups consisted of 10 non-smokers, 10 waterpipe smokers and two cigarette smokers. All participants were interviewed before starting the experiments. Only waterpipe smokers with no additional cigarette consumption were considered and asked for their tobacco consumption during the preceding 48 h. Non-smokers were asked for possible exposure to environmental tobacco smoke during the preceding 48 h. The study was approved by the Ethics committee of the Charité Medical School, Berlin, Germany.

After taking one blood sample from non-smokers, total urine was subsequently collected for 24 h. Two blood samples were taken from smokers, one sample prior to the smoking of one cigarette or of one waterpipe, and one sample immediately after smoking. Waterpipe smokers consumed 5 g of a flavored waterpipe tobacco (Nakhla Tobacco) in a traditional waterpipe (height: 68 cm) with one piece of quick lighting charcoal (Three Kings). In a well-ventilated room solitary waterpipe smokers inhaled at will for a total time of 30 min and then were requested to abstain from further smoking during the urine collection period. Smoking was finished after 30 min since the smoke lost its specific taste at that time. The total urine volume was recorded and samples were stored at −20 °C until further analysis.

COHb and nicotine levels were determined by the private and accredited analytical laboratory "Labor 28" (Berlin, Germany) using GC for nicotine and headspace GC for COHb. NNAL levels were determined by the Analytisch-Biologisches Forschungslabor, Munich, Germany as described (Kavvadias et al., 2009).

Determination of cotinine was performed as described (Voncken et al., 1989) with some modifications. In brief, 100 μl urine were transferred into a pear-shaped flask to which 0.3 ml of a 5 N NaOH solution and 0.79 μg phenanthrene-d_{10}, dissolved in 50 μl dichloroethane, were added. After shaking (1 min) and centrifugation (2 min at 3000 rpm), the aqueous phase was removed and an aliquot of 2 μl of the dried dichloroethane layer was used for GC–MS analysis. The chromatographic parameters are provided in the supplementary section (see Table S-6).

3. Results

3.1. Total particulate matter (TPM), nicotine, water, tar and carbon monoxide (CO)

Data on TPM, nicotine, water and CO contents in the smoke of waterpipes and 3R4F reference cigarettes are compiled in Table 1. The average values for TPM, tobacco and charcoal consumption (mean ± SD) for 15 replicate smoking sessions were 2.71 ± 0.20 g, 3.87 ± 0.36 g, and 8.22 ± 0.10 g, respectively. In comparison to the TPM values for 3R4F reference cigarettes (11.0 mg) reported in the literature (Liu et al., 2009), a waterpipe session yielded 250-