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Research article

Health Risks of Heating Tobacco Instead of Burning it. Evaluation of Different Tobacco Heating Sticks Vs. Conventional Tobacco Cigarettes by Use of Cultured Human Lung Cells at The Air-Liquid Interface

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Abstract

Cigarette smoking which is one of the main causes of preventable death globally, is due to a combustion process with temperatures up to 900 °C which yield several thousands of more or less harmful compounds. Therefore, heating of tobacco at temperatures at around 300 °C instead of burning tobacco might be less harmful. The present study was designed to investigate the possible differences in acute inhalation toxicity between a tobacco heating device with different flavours in comparison to burning a conventional tobacco cigarette. Tobacco heating stick extracts were generated by using a Borgwaldt LM4E smoking machine according to the Coresta method no. 81 together with a cellulose filter and an elution with phosphate-buffered saline. For a concentional tobacco cigarette a manual procedure was used. Cultivated human lung cells (cell line A-549) were exposed as (1) submerged cultures and (2) by an aerosol of the extracts at the air-liquid interface. The results clearly demonstrate that heating of tobacco by use of different flavoured tobacco heating sticks is significantly less harmful to the lung cells than burning tobacco as done with a conventional tobacco cigarette. Thus, we conclude that heating of tobacco instead of burning it might be an alternative for those who cannot quit smoking, but are seeking for a possibility to reduce unwanted health effects.

Keywords: tobacco, heat-not-burn, health effects, submerged cell cultures, air-liquid interface

Introduction

Cigarette smoking is one of the main causes of preventable death globally. When lightning a tobacco cigarette, the combination of tobacco and oxygen in the air generates a continuing combustion process that consumes tobacco. During the period between two puffs, temperatures ranging from 600 to 800 °C in the center of the combustion zone are achieved, whereas the temperature increases during a puff to more than 900 °C at the periphery of the combustion zone [1]. The smoke of this combustion process is a complex aerosol mixture containing several thousands of compounds [2,3].

Since more and more smokers are looking for a way to replace tobacco cigarettes, they can find alternatives which heat tobacco instead of burning it. As a matter of fact, over the years, there have been proposed numerous smoking products which use various forms of energy to vaporize or heat tobacco. Heating of genuine tobacco is performed at much lower temperatures at 300 °C to 350 °C [4]. This is the primary difference to the smoking of conventional cigarettes. Thus, a largely reduced number of unwanted by-products might be the result of heating tobacco.

The present study was designed to investigate the possible differences in acute inhalation toxicity between tobacco heating sticks with different flavours in comparison to burning a conventional tobacco cigarette. For this purpose, human lung cells were used as submerged cultures and were also exposed at the air-liquid interface (ALI). Especially, the latter test system is very similar to the real situation in vivo.

Materials and Methods

Experimental procedure for getting extracts for aerosol application

The following 6 different flavoured tobacco heating sticks were examined versus a conventional tobacco cigarette. A total of 50 puffs for each of the tobacco heating sticks were collected on a cellulose filter and extracted by vigorous shaking for 15 min in 100 ml of phosphate-buffered saline with calcium and magnesium (PBS+). A Borgwaldt LM4E smoking machine was used according to the Coresta method no. 81 (June 2015) with the following parameters: puff volume 55 ml; duration 3 seconds; interval 30 seconds; profile rectangle [5]. A similar manual procedure was done by smoking 5 conventional tobacco cigarettes (condensate: 10 mg; nicotine: 0.8 mg) with 10 puffs each and bubbling of the smoke directly into 100 ml of PBS+.

Cultivation of A-549 cells

The investigations were done with human lung adenocarcinoma cells (cell line A-549; ECACC, Salisbury, UK) which are widely used in current scientific research all over the world [for example, see 6-10]. Cells were routinely cultured as mass cultures in a CO_2 incubator at 37 °C with a moist atmosphere of 5 % CO_2 and 95 % air. Culture medium was DMEM/Ham's F12 (1:1) supplemented with 10 % growth mixture and 0.5 % gentamycin.

Exposure of cells as submerged cultures

For the experiments, A-549 cells were taken from 80 to 90 % confluent mass cultures and were seeded into 96-well plates (200 µl/well). Cell densities were chosen that cell cultures did not reach confluency during the total experimental period. 24 hours after seeding, cells were completely attached and spread to the bottom of the wells. Then, culture medium was discarded and replaced by fresh culture medium containing the extracts with the following concentrations in the test: 0 – 10 – 20 – 40 – 60 – 80 vol% with 0 vol% as internal control (= only culture medium without primary extract).

The exposure time of the different concentrations of the extracts was 24 hours. Thereafter, culture medium of the 96-well plates was replaced by a mixture of 180 µl/well of culture medium and 20 µl/well of XTT (Xenometrix, Allschwil, Switzerland). Multiwell plates were incubated for another 120 min at 37 °C in the incubator and the optical density of each well was examined by a difference measurement at ΔOD = 450 – 690 nm using a double-wavelength elisa reader (BioTEK Elx 808). XTT is the sodium salt of 2,3-bis[2-methoxy-4-nitro-5-sulfopheny]-2H-tetrazolium-5-carboxy-anilide and has a yellowish colour. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring of XTT yielding orange formazan crystals which are soluble in aqueous solutions. The intensity of the resulting orange solution is directly correlated with cell vitality and metabolic activity. For further information on the use of XTT, see Roehm et al. [11]. Experiments were done in triplicate.

In addition, we also checked cell size distributions of cells exposed to the extracts by using a CASY cell counter and analyzer system (OLS-OMNI Life Science, Bremen, Germany) which quantifies cells and particles passing a measuring pore exposed to a low voltage electrical field. Based on a cells size and conductivity, a resistance signal is generated and recorded.

Exposure of cells at the air-liquid interface (ALI)

A-549 cells were seeded on transwell inserts (12-well cell culture inserts, porous size 0.4 µm; Corning/Sigma-Aldrich, Taufkirchen, Germany) and grown to confluency within two days. Then, transwells were transfered to a Cultex® RFS exposure module (Fig. 1) and exposed to the aerosol of the extracts in PBS+ for 15 and 30 minutes. Aerosols were generated using a vaporizer (AGK 2000, PALAS). Compressed air according to DIN EN 12021 (Linde Gases Division, Pullach, Germany) was used as a carrier gas in a main flow of 1.5 l/min and a chamber flow of 5 ml/min exposing 3 cell culture inserts at a time in each experimental run [12,13]. In order to obtain a constant pH value during the exposure period, cells were exposed in Leibowitz L-15 medium. After exposure, medium was exchanged to routine culture medium and cells were cultivated for another 24 hours in the incubator. Thereafter, cell vitality was checked using XTT as already described for submerged cultures.

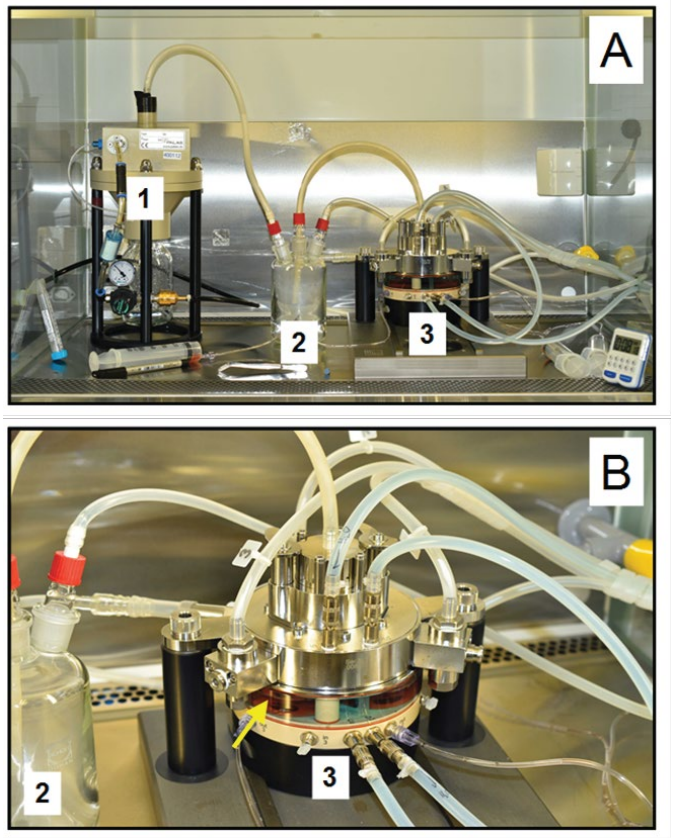

Figure 1. Experimental setup representing the different device units used for exposure of A-549 cells at the air-liquid interface. (1) Vaporizer to produce aerosol, (2) aerosol control chamber, (3) Cultex® RFS exposure module. The yellow arrow indicates the location of the transwells for an experimental run with each extract.

Results

Cell vitality and cell size distribution of submerged cultures

In the case of tobacco cigarette extract, only the highest possible extract concentration of 80 vol% present in the culture medium of submerged cultures for 24 hours caused a significant decrease in cell vitality. Therefore, only this concentration was taken into further consideration for all products tested here.

As shown in Figure 2, tobacco cigarette extract caused a decrease in cell vitality by 50 % in comparison to the solvent control (= PBS+). This decrease was statistically significant ($p < 0.01$) as checked by Wilcoxon-Mann-Whitney test. In contrast, all 6 different tobacco heating sticks did not cause any significant loss in cell vitality. However, some slight differences between the flavours were observed with flavour "C" and "E" showing a decreased cell vitality by about 8 to 10 %. The enzymatical data with XTT were in accordance with the cell counter data demonstrating the largely decreased cell number in the case of tobacco cigarette extract in comparison to tobacco heating stick extracts (Figure 3). The average cell diameter of A-549 was in the range of 16 to 18 µm for the control and all

tobacco heating stick extracts. A distinct cell diameter for tobacco cigarette extract cannot be given, because the typical Gaussian distribution was no longer present. The tobacco cigarette extract resulted in a diameter shift and matching towards smaller diameters as typical for cell debris and cell fragmentation.

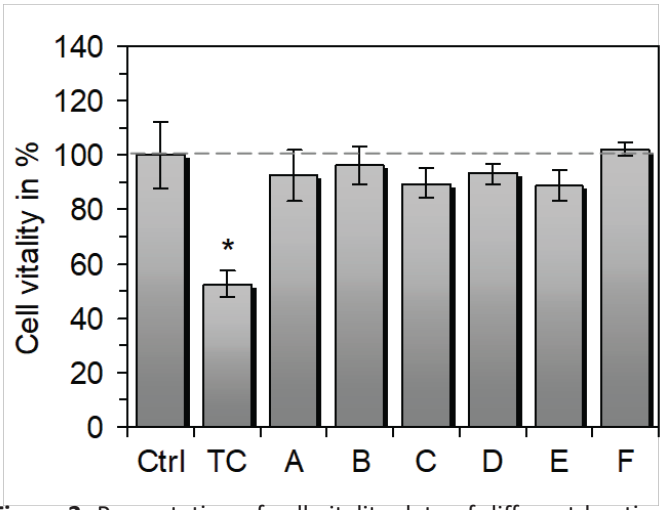

Figure 2. Presentation of cell vitality data of different heating tobacco stick extracts ("A" to "F") in comparison to solvent control (Ctrl; set as 100 % cell vitality) and tobacco cigarette extract (TC). An extract concentration of 80 vol% present in the culture medium was used. Data represent mean value ± standard deviation of 3 parallel experiments.

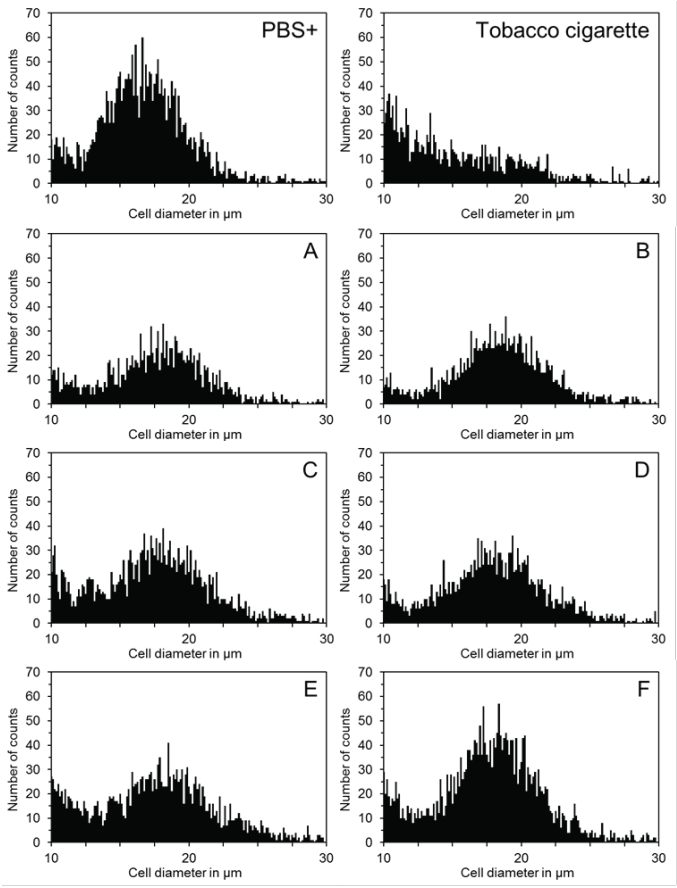

Figure 3. Graphical presentation of cell size distributions of A-549 exposed to the tobacco heating stick extracts "A" to "F" in comparison to tobacco cigarette extract and solvent control (= PBS+). Data are obtained with a CASY cell counter and analyzer system.

Cell vitality after exposure at ALI

As depicted in Figure 4, exposure of human lung cells at the ALI to the aerosols caused a time-dependent decrease in cell vitality only in the case of tobacco cigarette extract. Exposure for 15 min resulted in a decreased cell vitality of about 30 % and exposure for 30 min in a decreased cell vitality of approximately 45 %. Both values differed significantly from the solvent control and from the values obtained with tobacco heating stick extracts ($p < 0.01$; Wilcoxon-Mann-Whitney test). For all tobacco heating stick extracts, no significant time-dependent decrease in cell vitality in comparison to the solvent control was observed. The results were similar to those obtained under submerged culture conditions.

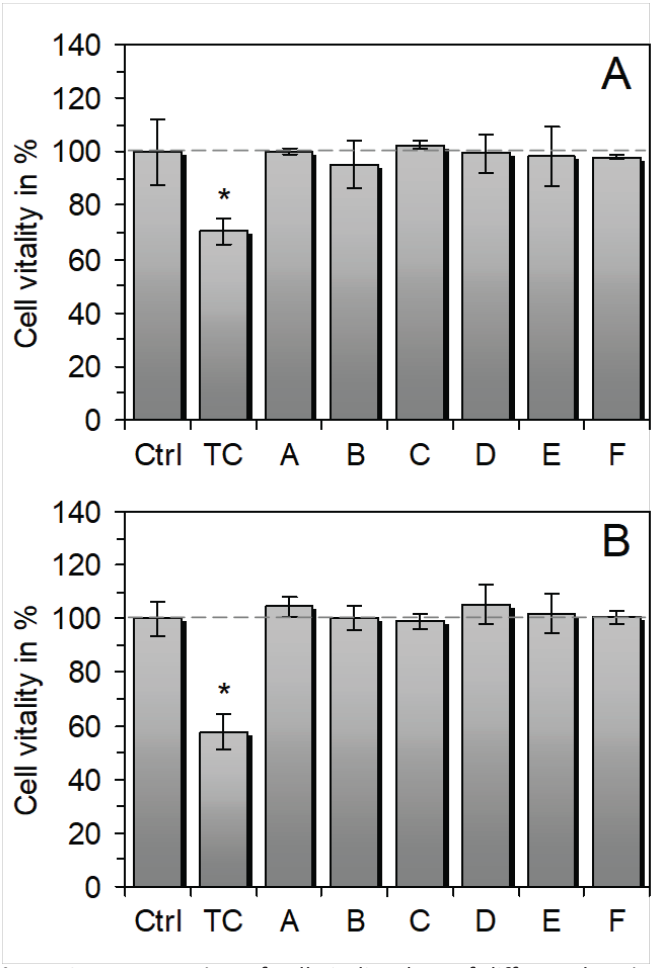

Figure 2. Presentation of cell vitality data of different heating tobacco extracts ("A" to "F") in comparison to solvent control (Ctrl; set as 100 % cell vitality) and tobacco cigarette extract (TC) after exposure at the air-liquid interface for 15 min (A) and 30 min (B). Data represent mean value ± standard deviation of four parallel experiments.

Discussion

As demonstrated in the present in vitro-study with cultured human lung cells by use of two different test systems, heating of tobacco by use of different flavoured tobacco heating sticks is significantly less harmful to lung cells than burning tobacco as done with a conventional tobacco cigarette.

Thus, the present study confirms previous investigations demonstrating the reduction of the formation of harmful and potentially harmful constituents by heating devices in comparison to a tobacco (reference) cigarette. For example, Schaller et al. [14] have observed a reduction of about 90 % in

cytotoxicity in comparison to a 3R4F reference cigarette by the neutral red assay and examination of the mutagenic potency in the mouse lymphoma assay. Also Forster et al. [15] found that in comparison to the University of Kentucky 3R4F reference cigarette the toxicant levels in the emissions of the tobacco heating device used were significantly reduced across all chemical classes by more about 90 %.

To make this kind of comparative percentage quantification is quite problematic in our test assays presented here. Nevertheless, the emissions of a conventional tobacco cigarette reduced cell vitality to about 50 % of the cell vitality observed for heating stick extracts. It could be possible that the difference is much lower than described by Schaller et al. [14] and Forster et al. [15], because we made the extraction only in PBS+, i.e. an aqeous solvent and not an organic solvent. However, even the water-soluble emissions of heating sticks as tested here have a significantly lower cytotoxic effect than the water-soluble emissions of the conventional tobacco cigarette.

Abbreviations: ALI: air-liquid interface; PBS+: phosphate-buffered saline with calcium and magnesium

References

1. Baker RR. Temperature variation within a cigarette combustion coal during the smoking cycle. High Temp Sci. 1975; 7: 236-247.
2. Rodgman A, Perfetti TA. The Chemical Components of Tobacco and Tobacco Smoke. CRC press, Boca Raton, FL, USA. 2013.
3. Kurgat C, Jebet A, Kibet J. The chemistry of tobacco charcoal: a potentially harmful residue from tobacco burning. Africa Environ Rev J. 2018; 3: 118-124.
4. Pryor WA, Church DF, Evans MD, et al. A comparison of the free radical chemistry of tobacco-burning cigarettes and cigarettes that only heat tobacco. Free Radical Biol Med. 1990; 8: 275-279.
5. Adamson J, Jaunky T, Thorne D, et al. Characterisation of the borgwaldt LM4E system for in vitro exposures to undiluted aerosols from next generation tobacco and nicotine products (NGPs). Food Chem Toxicol. 2018; 113: 337-344
6. Ramage L, Jones AC, Whelan CJ. Induction of apoptosis with tobacco smoke and related products in A549 lung epithelial cells in vitro. J Inflammation. 2006; 3: 3-14.
7. Kode A, Yang SR, Rahman I. Differential effects of cigarette smoke on oxidative stress and proinflammatory cytokine release in primary human airway epithelial cells and in a variety of transformed alveolar epithelial cells. Respiratory Res. 2006; 7: 132-152.
8. Zhao H, Albino AP, Jorgensen E, et al. DNA damage response induced by tobacco smoke in normal human bronchial epithelial and A549 pulmonary adenocarcinoma cells assessed by laser scanning cytometry. Cytometry. A 75: 840-847.
9. Jorgensen ED, Zhao H, Traganos F, et al. DNA damage response induced by exposure of human lung adenocarcinoma cells to smoke from tobacco- and nicotine-free cigarettes. Cell Cycle. 2010; 9: 2170-2176.
10. Cervellati F, Muresan XM, Sticozzi C, et al. Comparative effects between electronic and cigarette smoke in human keratinocytes and epithelial lung cells. Toxicology in Vitro. 2014; 28: 999-1005.
11. Roehm NW, Rodgers GH, Hatfield SM, et al. An improved colorimetric assay for cell proliferation and viability utilizing the tetrazolium salt XTT. J lmmunol Meth. 1991; 142: 257-265.
12. Scheffler S, Dieken H, Krischenowski O, et al. Cytotoxic evaluation of e-liquid aerosol using different lung-derived cell models. Int J Environ Res Publ Health. 2015; 12:12466-12474.
13. Scheffler S, Dieken H, Krischenowski O, et al. Evaluation of e-cigarette liquid vapor and mainstream cigarette smoke after direct exposure of primary human bronchial epithelial cells. Int J Environ Res Publ Health. 2015; 12:3915-3925.
14. Schaller JP, Keller D, Poget L, et al. Evaluation of the tobacco heating system 2.2. Part 2: Chemical composition, genotoxicity, cytotoxicity, and physical properties of the aerosol. Reg Toxicol Pharmacol. 2016; 81: S27-S47.
15. Forster M, Fiebelkorn S, Yurteri C, et al. Assessment of novel tobacco heating product THP1.0. Part 3: Comprehensive chemical characterisation of harmful and potentially harmful aerosol emissions. Reg Toxicol Pharmacol. 2018; 93: 14-33.

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