Acute and long-term cytotoxicity of cigarette smoke and e-liquid vapour on cultured human lung cells

Thomas Alexander Mrva[1], **Oliver Okle**[2], **Peter C. Dartsch**[3]

[1]Happy People GmbH, Germany; [2]hjs consulting, Germany; [3]Dartsch Scientific GmbH, Germany;

Keywords: e-cigarette, human lung cells, cytotoxicity, long-term exposure, Happy Liquid

Introduction

In contrast to cigarette smoking, the vapour of e-cigarettes is not the result of a combustion process. However, the risks of e-cigarette use are uncertain which is due to the limited amount of scientific data regarding their health effects related to the variability of vaporisers, e-liquid ingredients and their quality (Dawkins and Corcoran 2014; Farsalinos and Polosa 2014; Grana et al. 2014). There are also limited amounts of studies looking on the in vitro toxicity profile of e-liquids and e-cigarettes by using cultured cells of the lung (Misra et al. 2014), mammalian fibroblasts (Romagna et al. 2013) and myocardial cells (Farsalinos et al. 2013).
Here, we present data on the acute and long-term toxicity of cigarette smoke and e-liquid vapour on cultured human lung cells.

Materials and methods

The investigations were done by using (1) a common cigarette brand of medium strength with 10 mg tar, 0,8 mg nicotine and 10 mg carbon monoxide, and (2) several e-liquids with different amounts of nicotine of the brand Happy Liquid produced by Happy People GmbH, München, Germany. In a specially designed apparatus (Figure 1) cigarette smoking and vaping was simulated (10 puffs with a duration of 3-5 seconds and a pause of 30 seconds between two puffs). For e-cigarette, a vaporiser 2,2 Ω and rechargeable battery 3,7 V (Evod, KangerTech) was used. The smoke or vapour was piped into 10 ml of HEPES-buffered cell culture medium. For examination of acute cytotoxicity, this primary extract was added at 0 to 100 vol% to mass cultures of human lung cells (cell line A-549; DSMZ, Braunschweig, Germany) with an initial seeding density of 5,000 cells/well in 96 well-plates. After 24 hours cell vitality was measured enzymaticaly by cleavage of XTT by mitochondrial dehydrogenases activity. For examination of long-term cytotoxicity, cells were seeded as clone cultures (seeding density: 1,000 cells/flask 25 cm^2) and were exposed to the primary extract at 0 to 10 vol% for 12 days. After this time period the number of clones resulting from at least 5-6 population doublings of a single cell were examined by using a digital image analysis system (Wimasis Image Analysis; ibidi, München).

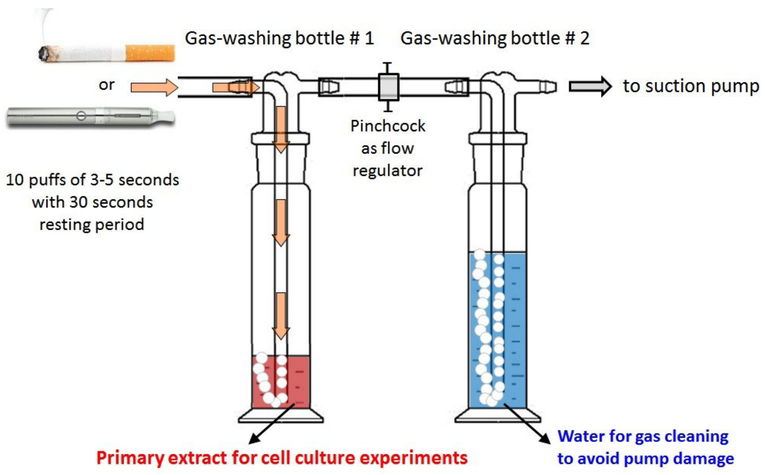

Fig. 1: Experimental setup for simulation of cigarette smoking or vaping. The suction pump on the right generates an adjustable underpressure which aspirates the smoke or vapour and bubbles it into the culture medium in the left gas-washing bottle # 1. This yields the primary extract.

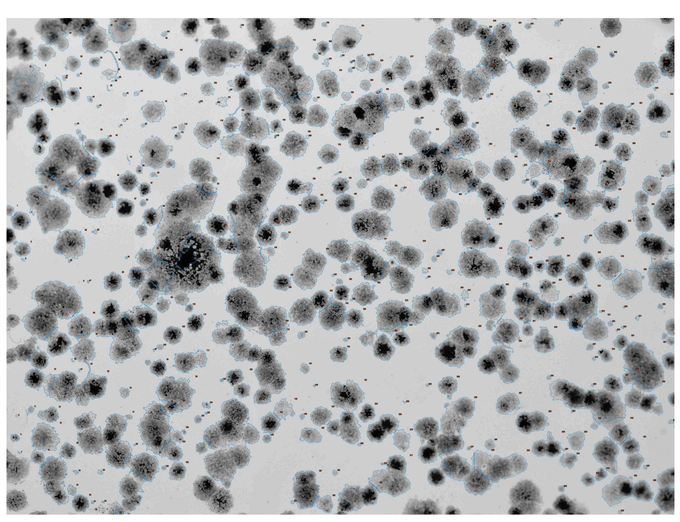

Fig. 2: Representative example of the cell clones in the reagent control. Clones have developed by the continuous mitotic activity of the human lung cells (A-549) within 12 days of cultivation. The cloning efficiency was examined by digital image analysis (Wimasis Image Analysis).

Results and conclusions

The data of the short-term cytotoxicity clearly showed that cigarette smoke extract had a marked acute toxic effect with no cell survival at concentrations > 10 vol%. In contrast, the vapour of the tested e-liquids of Happy People GmbH exhibited no cytotoxic effects even when the vapour extract was used undiluted 100 vol%. Long-term exposure of cigarette smoke extract increased cytotoxicity and resulted in complete cell death at concentrations ≥ 2.5 vol%. Long-term exposure of e-liquid vapour did not cause a cytotoxic effect.
Although vaping of e-liquid might not be harmless to human's health, it is far less toxic than inhalation of cigarette smoke.

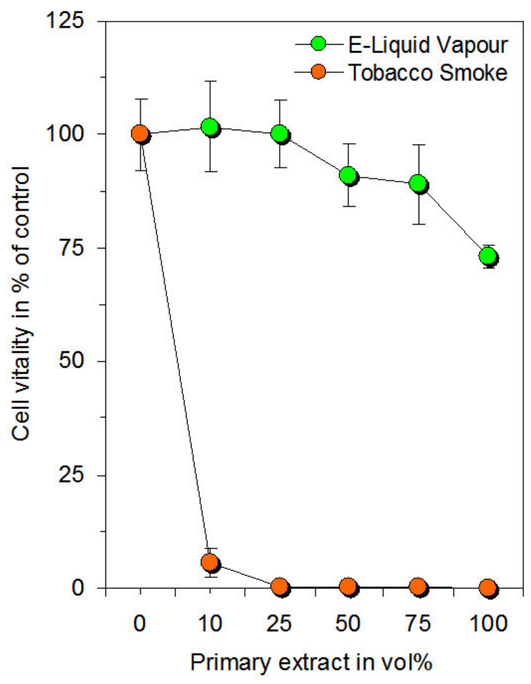

Fig. 3: Graphical presentation of the summarised experimental results on the short-term toxic effect of tobacco smoke extract in comparison to e-liquid vapour extract "Menthol" of the brand Happy Liquid containing 18 mg/ml of nicotine. The 1:10 diluted primary extract of tobacco smoke causes death of nearly all cultured human lung cells, whereas the undiluted (!) primary extract of e-liquid vapour does not cause a mark-ed loss in cell vitality. Thus, tobacco smoke is considerably more toxic to the lung cells than e-liquid vapour. Data represent mean value ± standard deviation of three experiments.

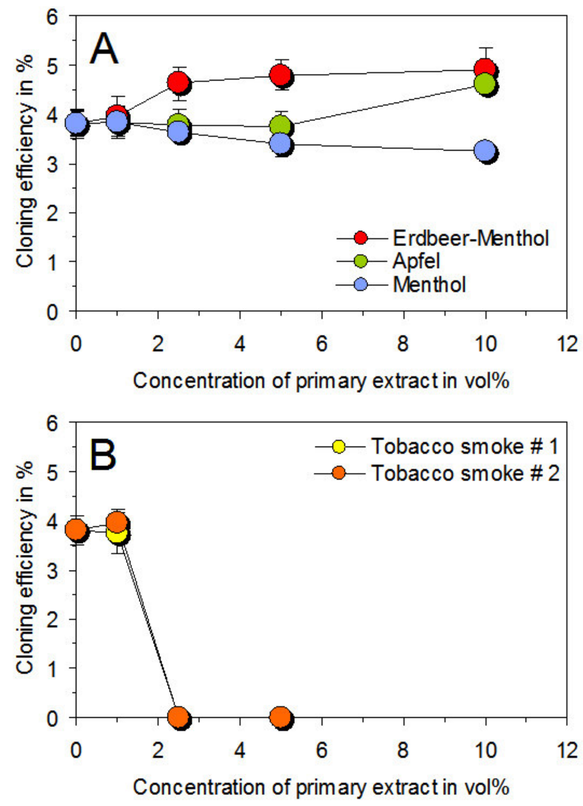

Fig. 4: Graphical presentation of the long-term toxicity results for three vapour extracts of the brand Happy Liquid (A) and the smoke extract of two tobacco cigarette brands (B). Only the cloning efficiency as the most meaningful value for vitality and proliferation of human lung cells after long-term exposure is depicted. Data represent mean value ± standard error of the mean of three experi-ments.

References

Dawkins L and Corcoran O (2014): Acute electronic cigarette use: nicotine delivery and subjective effects in regular users. Psychopharmacology 231: 401-407.

Farsalinos KE and Polosa R (2014): Safety evaluation and risk assessment of electronic cigarettes as tobacco cigarette substitutes: a systematic review. Ther Adv Drug Saf 5: 67-86.

Grana R, Benowitz N and Glantz SA (2014): E-Cigarettes. A Scientific Review. Circulation 129: 1972-1986.

Misra M, Leverette RD, Cooper BT, Bennett MB and Brown SE (2014): Comparative in vitro toxicity profile of electronic and tobacco cigarettes, smokeless tobacco and nicotine replacement therapy products: e-liquids, extracts and collected aerosols. Int J Environ Res Public Health 11: 11325-11347.

Romagna G, Allifranchini E, Bocchietto E, Todeschi S, Esposito M and Farsalinos KE (2013): Cytotoxicity evaluation of electronic cigarette vapor extract on cultured mammalian fibroblasts (ClearStream-LIFE): comparison with tobacco cigarette smoke extract. Inhal Toxicol 25: 354–361.

Farsalinos K, Romagna G, Allifranchini E, Ripamonti E, Bocchietto E, Todeschi S et al. (2013): Comparison of the cytotoxic potential of cigarette smoke and electronic cigarette vapour extract on cultured myocardial cells. Int J Environ Res Public Health 10: 5146–5162.