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

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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 vapourisers, 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 vapouriser 2.2 V 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 enzymatically by cleavage of XTT by mitochondrial dehydrogenases activity. 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 enzymatically 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 cm²) and were exposed to the primary extract at 0 to 100 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).

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.

References