Effects of cigarette smoke and e-liquid vapour on mass cultures of human lung cells: Continuous monitoring by time-lapse videography

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Background

In previous studies we demonstrated that exposure of mass cultures of human lung cells to diluted cigarette smoke extracts resulted in decreased cell vitality and cell death within 24 hours. E-liquid vapour did not cause significant effects on cell vitality and morphology. To understand the mode of action of cigarette smoke and e-liquid vapour on cellular level, we monitored their effects on cell vitality and morphology continuously by using time-lapse videography.

Materials and methods

Human lung cells (cell line A-549; DSMZ, Germany) were cultured in µ-dishes, 35 mm high with standard bottom (ibidi, Germany) 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% fetal bovine serum and 100 Units/ml of penicillin & 100µg/ml of streptomycin (all reagents from GE Healthcare Life Sciences, Cölbe, Germany). After 2 days (50% cell confluency), culture medium was exchanged and 2 ml of Leibovitz L-15 medium was added containing 10 % fetal bovine serum and 10 vol% cigarette smoke extract or 50 vol% e-liquid vapour extract obtained by use of a e-cigarette with a vaporiser of 2,2 Ω and a 3.7 V rechargeable battery (Evod; KangerTech). Dishes were transferred to a temperature-controlled heating chamber for live cell imaging (ibidi, München, Germany) mounted on the stage of an Olympus IX50 inverted microscope. Cell reactions were recorded for 24 hours at 37°C by a Basler high resolution video camera acA1600-20uc at 1440 x 1080 pixels operated by the Basler pylon camera software 4.2 (Rauscher, Germany) at a speed of 1 frame per 30 seconds with an Olympus 10x planachromate objective.

Results and conclusions

10 vol% cigarette smoke primary extract caused a rapid decrease in cell migratory and mitotic activity within 4 hours after exposure, followed by a rounding of the vast majority of the lung cells. Numerous cells detached after cell membranes had been damaged or exhibited intracellular vacuolisation. After 12 hours, no living cells could be detected. The time course and the kind of cell damage resembled very much previous observations on other cell types after exposure to an excess of free superoxide anion radicals. Cells being exposed to 50 vol% of the primary extract of e-liquid vapour did not show such alterations in morphology. The results indicate that vaping does not produce an excess of free radicals which might cause acute cell death in the lung or the respiratory tract.

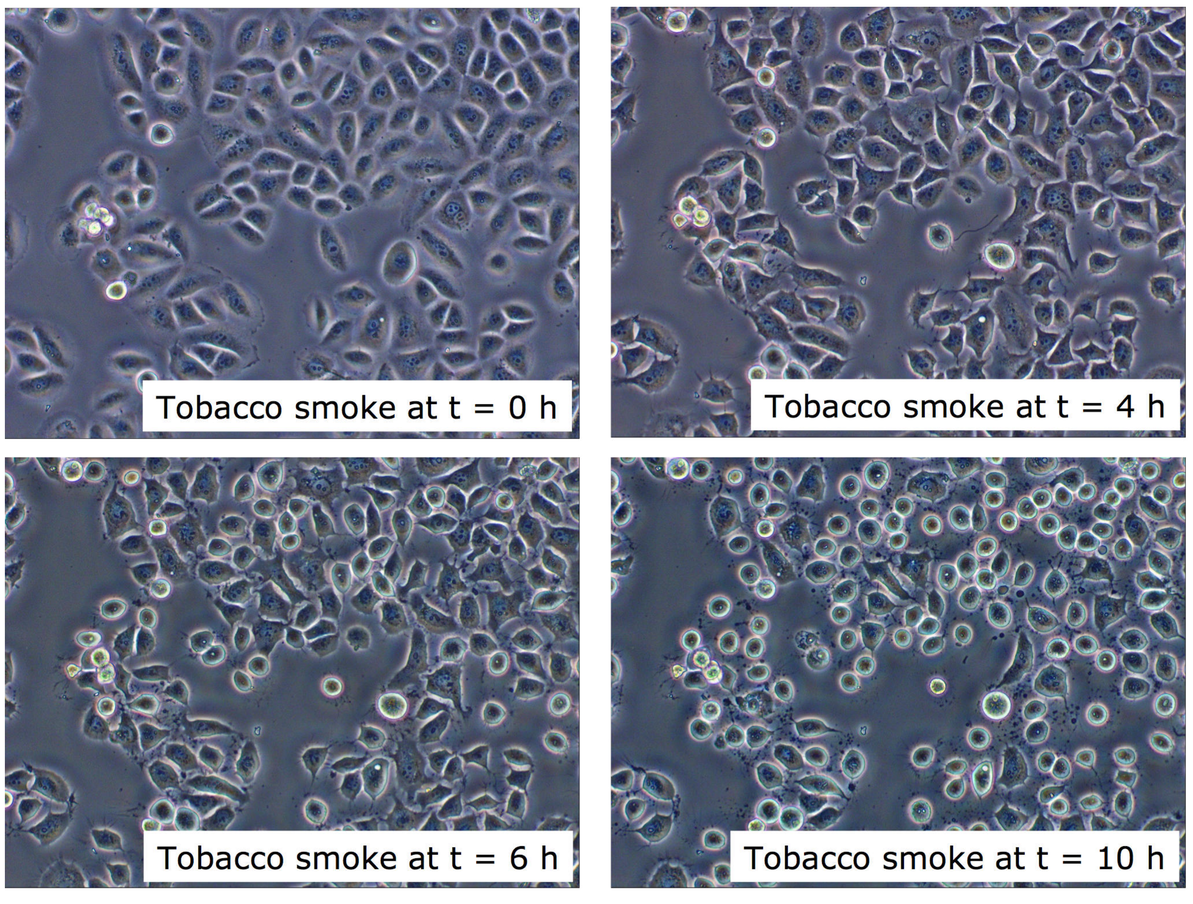

Fig. 1: Effect of the tobacco smoke primary extract (10 vol%) on cultured human lung cells. Within 4 hours cell migration and mitotic activity is stopped completely. Thereafter, cells become rounded (6 hours). Finally, cell membranes are partially destroyed and free floating cytoplasmic particles can be found in the culture medium. At that time point, most of the cells are dead and do not show any enzymatic activity of the mitochondria as a marker of cell vitality. The mode of cell death resembles very much the effect of free superoxide anion radicals on cells. Single pictures are taken from a time-lapse video micrography sequence as presented in the frame above.

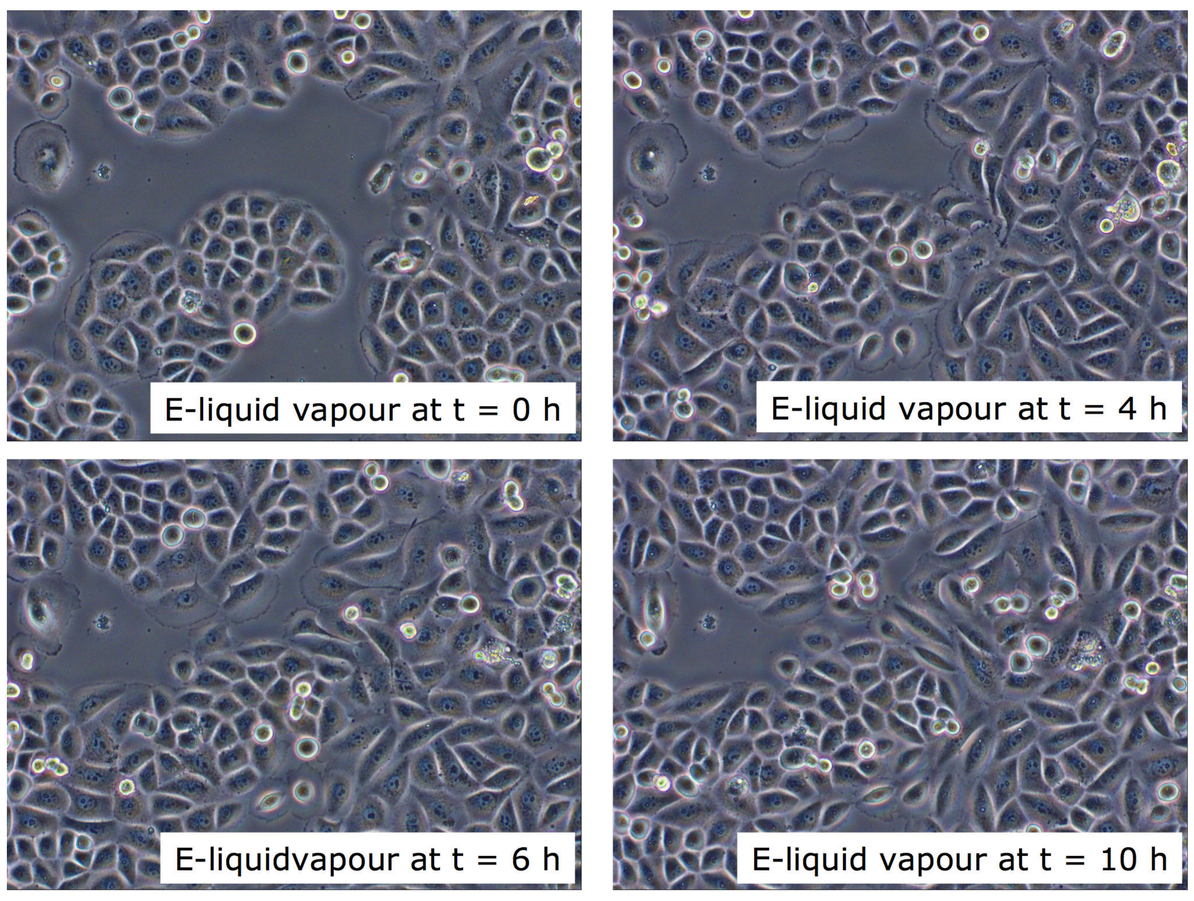

Fig. 2: Effect of the e-liquid vapour primary extract (50 vol%) on cultured human lung cells. In contrast to tobacco smoke extract there are no alterations in cell moprhology or vitality. Cells migrate and proliferate until subconfluency is reached. The rounded cells in the sequence above represent proliferating cells in various stages of mitosis. Single pictures are taken from a time-lapse video micrography sequence as presented in the frame above.

References

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