Exposure of a human bronchial epithelial cell line with cigarette mainstream smoke in the CULTEX® Radial Flow System (RFS)

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Introduction

The respiratory tract is the main entry for airborne substances like gases, particles and complex mixtures (combustion products). The inhalation of air pollutants may affect human health, by acute cyto- and genotoxicity as well as chronic alterations, ranging from reversible cellular changes to persistent modifications of the airways. The toxicological evaluation of inhalable airborne substances is presently carried out by performing animal tests, mainly on rodents. Due to several changes in the EU legislation, the development of *in vitro* methods is strongly required. In the OECD guidelines for chemical testing, no method for the assessment of inhalable substances is recommended. Many scientific publications describe approaches for testing airborne substances *in vitro*, but mainly condensates, dilutions or suspensions of the test materials are analyzed. Only a few papers deal with the direct exposure of cells via the air. For this purpose, Cultex® Laboratories developed the CULTEX® Radial Flow System (RFS) for direct exposure. The cells are cultivated in special culture inserts allowing supply with nutrition from the underside through a semi-permeable membrane. The apical side of the cells is in contact with the atmosphere (air-lifted cultivation), enabling direct exposure to airborne substances. In the presented study, we evaluated the effect of cigarette mainstream smoke on cells using the CULTEX® RFS. A human bronchial epithelial cell line was exposed to cigarette mainstream smoke. The cell viability, the dose-response and increase in cytokine IL-8 were evaluated.

Experimental setup & results

For the experiments, the CULTEX® Radial Flow System (RFS) was used (Figure 1). It houses three culture inserts which can be separately supplied with medium; the temperature is regulated using a water bath. The test atmosphere enters the CULTEX® RFS module via a central inlet from which three radial tubes guide the atmosphere to the culture inserts, guaranteeing a homogeneous distribution on the cell surface.

Figure 1: The CULTEX® Radial Flow System (RFS)

Smoke generation

A smoking robot generated mainstream smoke according to ISO guidelines (35 mL puff volume, 2 s duration, 1 puff/min). The freshly generated smoke was diluted with synthetic air (1 L per minute) and sucked into the module at a rate of 5 mL/min/insert via a vacuum pump. The flow rates are controlled by mass flow controllers. The exhaust air was led back to the fume hood.

Cell exposure

The undifferentiated human bronchial epithelial cells were seeded one day prior to exposure into 12-well transwell inserts ($2.5 \cdot 10^5$ cells/insert; Corning Inc.). In case of differentiation of cells, they were seeded for 3 days in culture medium and then transferred to differentiation medium for 12 days before the exposure was started. Human bronchial epithelial cells were exposed at the air-liquid interface to mainstream smoke of increasing numbers of cigarettes (Kentucky research cigarette K3R4F) and analyzed with regard to their metabolic activity (WST-1 assay). Furthermore, a dose-response curve was calculated with the data obtained by the viability assay. The analysis of cytokines, which are involved in tissue remodeling and inflammation, gave a deeper insight into the mechanisms in the cells after exposure. They were cultivated in special culture inserts and supplied with nutrition from the underside through a semi-permeable membrane. The apical side of the cells was in contact with air, enabling their direct exposure at the air-liquid interface. The procedure was carried out using the CULTEX® RFS. To determine the decrease in cell viability after exposure, the values of the smoke-exposed cells were normalized to those of a clean air-exposed control. The other controls remaining in the incubator were not considered because their viability values are comparable to the clean air control (data not shown).

Viability assay

The viability of the cells was evaluated performing the WST-1 assay. After exposure, the cells were post-incubated air-lifted at 37 °C/5 % CO_2 for 24 hours. 500 µL growth medium and 50 µL WST-1 were added to each transwell; after one hour incubation (37 °C), the amount of reduced WST-1 was quantified by measuring the light absorbance at 450 nm. Figure 2 shows the results: The viability of the smoke-exposed undifferentiated cells decreased steadily, while the differentiated cells show no loss of vitality after exposure.

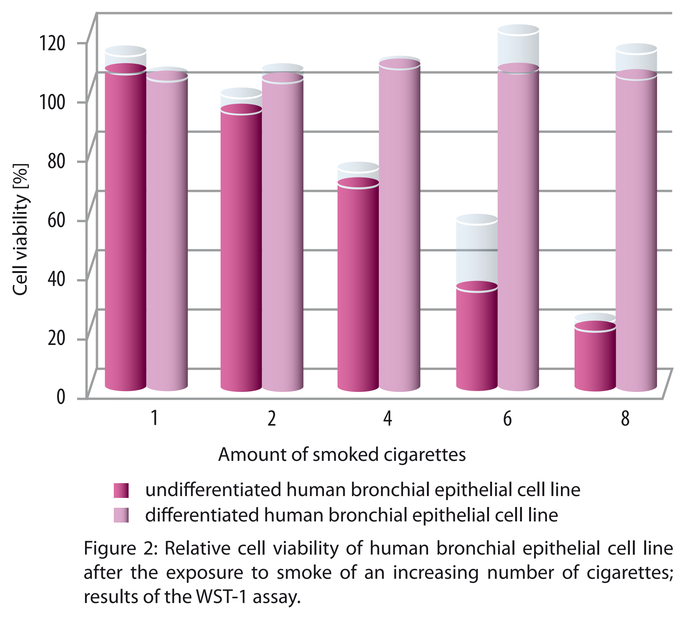

Figure 2: Relative cell viability of human bronchial epithelial cell line after the exposure to smoke of an increasing number of cigarettes; results of the WST-1 assay.

Dose-response

On the basis of the cell viability data, the dose-response curves were calculated for the status of both cell lines (software Curve Expert 1.4) by non-linear regression (Harris Model, Figure 3). Accordingly, values for the effective dose (ED) could be calculated (undifferentiated cells: ED_{10}: 2.48 cigarettes, ED_{50}: 4.63 cigarettes; differentiated cells: ED_{10}: 14.51 cigarettes, ED_{50}: 211.32 cigarettes). Regarding these values, the different cytotoxic potential of cigarettes on cells in different stages of differentiation became apparent.

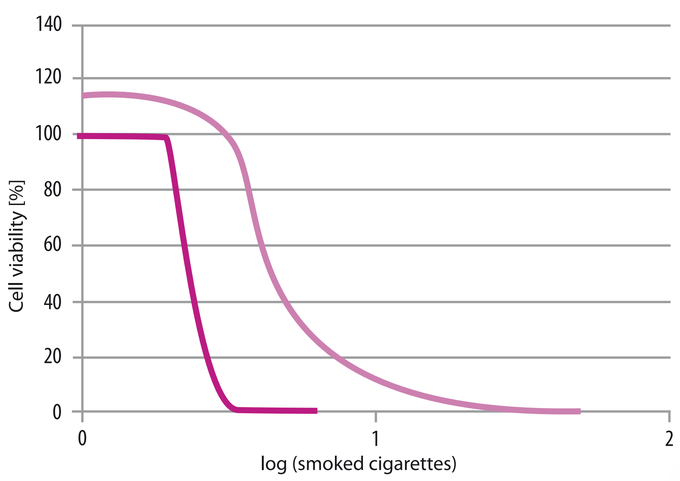

Figure 3: Dose-response curve for a human bronchial epithelial cell line after the exposure to mainstream smoke of increasing numbers of Kentucky research cigarettes (K3R4F) and followed 24 hours post-incubation. The curve was calculated by non-linear regression (Harris-Model) from the data achieved by WST-1 assay. The calculation was performed using the software Curve Expert 1.4.

Cytokines

To determine the presence of specific proteins in the cell culture medium after cigarette smoke exposure using the ELISA technique, the basal cell culture medium, which was used for post-incubation, was analyzed for the presence of the cytokine interleukin-8. The ELISA kit was used according to the manufacturer's instructions. For the IL-8 ELISA, the cell culture medium was diluted 1:200 in sample buffer (kit-specific). Due to the fact that exposure to the smoke of an increasing amount of cigarettes leads to a decrease in cell viability, we correlated the estimated values for the inflammatory marker IL-8 with those for cell viability (Figure 4). Apparently, the increase in the number of cigarettes smoked leads to an increase in IL-8 secretion independent of the different stages of differentiation of the cells.

Conclusions & Outlook

In conclusion, the dose-response relationship was calculated with Curve Expert 1.4 by measuring the mitochondrial activity (WST-1 assay) of the cells after smoke exposure with an increasing number of cigarettes. Also, a dose-dependent increase in the IL-8 secretion could be observed. Furthermore, a different cigarette smoke susceptibility of the cells is detected, depending on their cell status. The next step is to extend the biological endpoints by several molecular biology methods, to obtain a deeper insight into the changes concerning the cell metabolism after exposure.

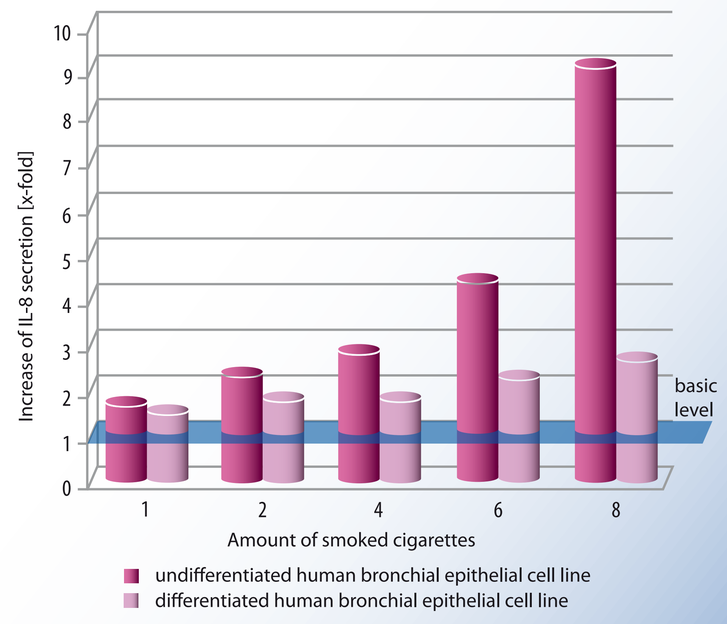

Figure 4: The increase in IL-8 secretion after smoke exposure. In this figure, the secretion level, under consideration of the cell viability, is shown. To consider the cell viability in the analysis, the level of IL-8 was calculated from the amount of secreted interleukin, which was set in relation to cell viability. The value 1 is the basic level, determined by the clean air exposure.

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