HIGH CONTENT SCREENING ASSESSMENT OF NICOTINE TOXICITY IN PRIMARY HUMAN BRONCHIAL EPITHELIAL CELLS

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Introduction and Objectives

Cigarette smoke (CS) is a complex mixture with more than 7000 chemicals [1]. Nicotine is a major CS constituent and the principal responsible for tobacco addiction. Several studies have investigated the genotoxic and cytotoxic effects of nicotine. However, the results are heavily dependent on the species and cellular model used, thus causing inconsistency. Furthermore, few *in vitro* data is available on primary human cells.

The aim of the study was to investigate the biological effects of nicotine exposure in primary normal human bronchial epithelial (NHBE) cells. For this purpose, we initially performed a real-time cellular analysis to determine nicotine impact on cell viability. In addition, thirteen multi-parametric indicators of cellular toxicity were measured, via high content screening (HCS), over a range of nicotine concentrations and at two different time points (4h and 24h).

Methods

Real-Time Cellular Analysis

High Content Screening

Real time cell analyzer:

NHBE cells (purchased from Lonza, Basel, Switzerland) were seeded in 96-well tissue culture plates and incubated in medium for 24h. Cells were then exposed in triplicate to increasing doses of nicotine for additional 24h. Impedance values were recorded every 15 minutes as a readout of cell viability. Water was used as vehicle. Carbonyl cyanide m-chlorophenyl hydrazine (CCCP) was used as a positive control.

High Content Screening:

NHBE cells were seeded in 96-well tissue culture plates and incubated in medium for 24h. Cells were then exposed in triplicate to increasing doses of nicotine for additional 4h or 24h. Water was used as vehicle. Appropriate positive controls were used for each endpoint. Thirteen multi-parametric toxicity endpoints, grouped into different assays were measured.

- *Cell count*
- *Nuclear size*
- *DNA structure*
- *P-H2AX (DNA damage)*
- *P-cJun (Stress kinase)*
- *P-H3 (Mitosis)*
- *Mitochondrial membrane potential*
- *Mitochondrial mass*
- *GSH content*
- *ROS formation*
- *Cell membrane permeability*
- *Cytochrome C release*
- *Caspase 3/7 activity*

Conclusions

➢ Exposure of NHBE cells to nicotine caused a dose-dependent decrease in GSH content, suggesting the presence of oxidative stress at concentrations above 1mM.

➢ An increase in caspase 3/7 activity, cytochrome C release, cell membrane permeability and mitochondrial health parameters was observed in NHBE cells after 24h of exposure to nicotine doses above 5mM, indicating the presence of cytotoxicity. These results are in agreement with the observed decrease in cell viability and cell number.

➢ Nicotine increased p-H2AX levels in NHBE cells only after 24h exposure to nicotine doses above 5mM. This observation is likely the consequence of elevated cytotoxicity.

References

1. Rodgman, A., and Perfetti, T.A. The Chemical Components of Tobacco and Tobacco Smoke. 2013.
2. GraphPad Prism Version 5.00 for Windows. GraphPad Software, San Diego, California, USA.

Results

Figure 1 (below). Cell Viability.

A) Representative real-time cell analysis experiment showing the effect of exposure to nicotine on NHBE cell viability. Impedance is used as a read-out of cell viability. Data was acquired every 15 minutes and represents the average of three replicate wells. Vertical dotted line indicates the beginning of the exposure period the 24h exposure period. CCCP was used as positive control (control +) B) Normalized impedance values after 4 h of exposure. C) Normalized impedance values after 24 h of exposure Data represents average ± SEM of 5 independent experiments and was fitted using GraphPad Prism® 5.0 [2]. D&E) Average number of cells per well, measured using a HCS platform after exposure to nicotine for 4h or 24h respectively. Values represent average ± SEM of at least three independent experiments. Horizontal dotted line indicates 50% cytotoxicity. R.U. means relative units

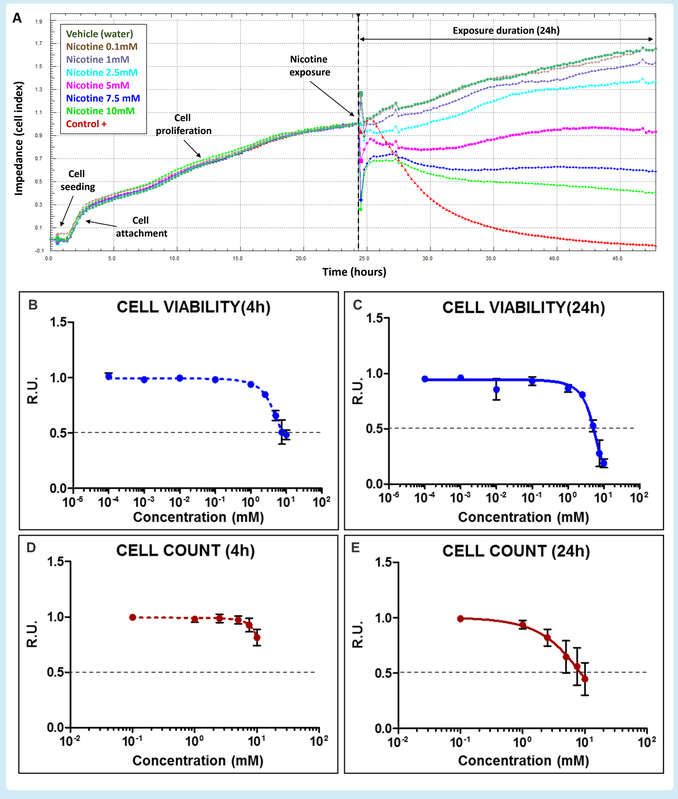
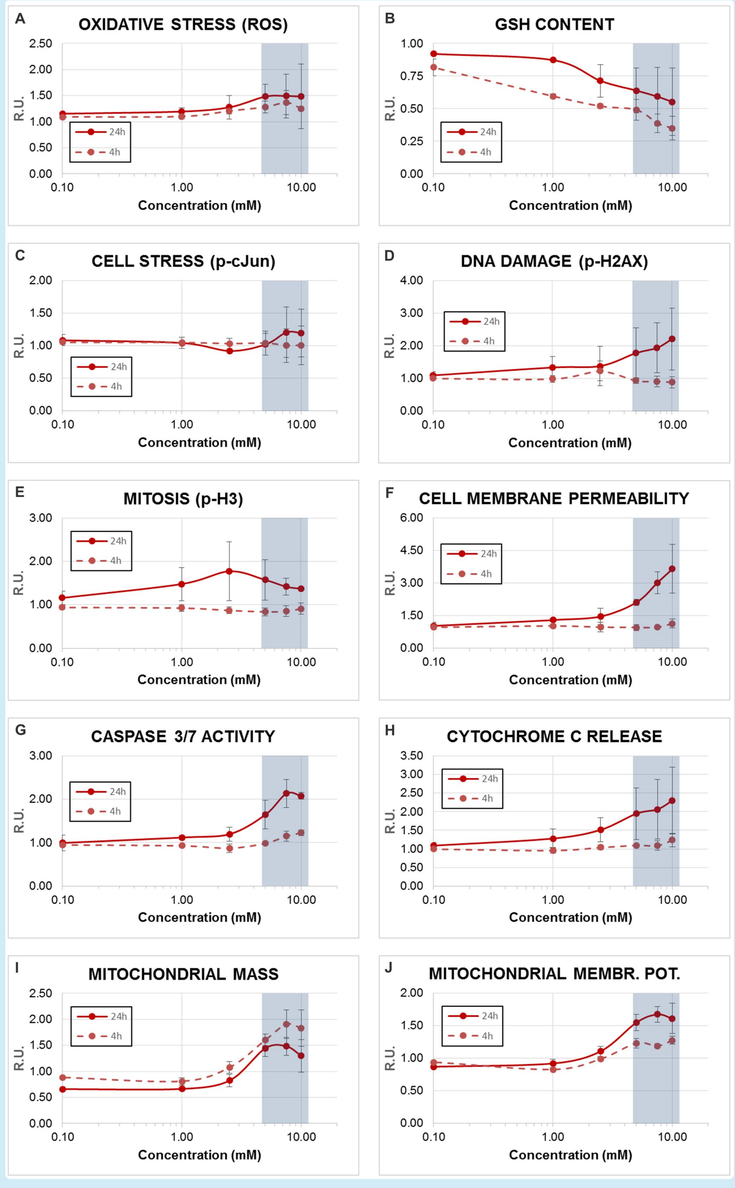


Figure 2 (right). High-Content Screening.

Only endpoints for which a positive response was observed are shown. A) Oxidative stress (measured as formation of reactive oxygen species, ROS). B) GSH content. C) Activation of the stress signaling pathway (measured as phospho-cJun). D) DNA damage (measured as phospho-histone H2AX, p-H2AX). E) Mitosis (measured as phospho-histone H3). F) Cellular Membrane permeability. G) Caspase 3/7 activity. H) Cytochrome C release. I) Mitochondrial mass. J) Mitochondrial membrane potential. Data represents average ± SEM of 2-3 independent experiments. Blue square indicates nicotine doses resulting in >50% cell death after 24h exposure. R.U. means relative units.



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