Introduction

Smoking causes serious fatal diseases such as lung cancer and chronic obstructive pulmonary disease. The best way for smokers to reduce the adverse health effects is to quit smoking. However, there has been a growing interest in recent years in harm reduction, stimulated perhaps by the observations that in spite of the significant efforts directed towards tobacco control and communication of the risks of smoking, many smokers still have little interest and/or success in quitting smoking. A significant development in tobacco control in the US has been the enactment of the Family Smoking Prevention and Tobacco Control Act (FSPTCA), which empowers the US Food and Drug Administration (FDA) to evaluate and regulate Modified Risk Tobacco Products (MRTPs). This FSPTCA defines a MRTP as "any tobacco product that is sold or distributed for the purpose of reducing the harm or risk of tobacco-related disease associated with commercially marketed tobacco products." Although the causal relationship between smoking and several diseases is well established, there is still little understanding of the underlying mechanisms.

With the aim to demonstrate the applicability of a systems toxicology approach to contribute to the evaluation of the risk associated with a prototype MRTP aerosol and to get mechanistic insights in key cellular processes, we have investigated human bronchial epithelial cells (NHBE) cells to an aquatic cigarette smoke fraction (smoke-bubbled phosphate-buffered saline (sbPBS)) derived from the 3MRF reference cigarette and an unexposed control. In a static, headspace GC-MS method focusing on volatile and semi-volatile constituents was used to compare the amount of chemical constituents between 3MRF and sbPBS.

Methods & Study Design

NHBE cells were exposed to an aquatic cigarette smoke fraction (sbPBS) from the 3MRF reference cigarette at three non-toxic concentrations based on cell viability for either 4 or 24 hours. Exposure to pMRTP matched to the highest concentrations of 3MRF in terms of puff/s.

Cell viability was determined after 24 hours of exposure using a resazurin assay. RNA was isolated and further processed on GeneChip Human Genome U133 plus 2.0 arrays (Affymetrix). To place the gene expression data into the context of known biology, a novel computational-modeling approach based on tissue-specific causal biological networks was applied. The computable biological network models are specific to non-diseased pulmonary and cardiovascular cells/tissues and capture the molecular events that can be affected following exposure to environmental toxicants. The biological mechanisms covered by our networks encompass cell proliferation, cellular stress, lung inflammation, DNA damage, apoptosis, cellular death and senescence (ODACs). Each network is built in a modular way where each module (sub-network) describes a specific biological aspect of the entire network. Gene expression fold-changes were translated into differential values for each node within the network. The node differential values are then summarized into a quantitative score (Q-score)24. The model represents the mechanism of xenobiotic metabolism and illustrates the quantification approach24. The activation of the nodes in the network model were predicted from the gene expression levels calculated from contrast of between the control and exposed samples. The highly contributing nodes to the overall impact on xenobiotic metabolism (i.e., the leading nodes) are displayed as a heatmap. Diesel exhaust particles, the transpiration activity for the aryhydrocarbon receptor, the oxidation of CPVE and reactive oxidative species are predicted to have a major impact on the xenobiotic metabolism perturbation. Importantly, the sum of the overall impact is lower after pMRTP exposure than after 3MRF exposure.

Conclusion

In summary, we provide mechanistic insight into the biological impact on key cellular processes in NHBE cells upon sbPBS exposure, suggesting reduced biological network perturbation from exposure to pMRTP compared to 3MRF. The lower amount of chemicals detected in the pMRTP could be associated with the lower biological impact in NHBE. In addition, we demonstrated the applicability of a systems toxicology approach to contribute to the evaluation of the risk associated with pMRTPs.

References


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