INTRODUCTION AND OBJECTIVES

Introduction. Cigarette smoke (CS) has been reported to increase predisposition to oral cancer and recognized as a risk factor for many conditions including periodontal diseases, gingivitis and benign mucosal disorders [1]. Tobacco harm reduction through the development of Modified Risk Tobacco Products (MRTP) provides a promising opportunity for adult smokers, who would otherwise continue cigarette smoking. MRTPs are defined by the US FDA as "any tobacco product that is sold or distributed for use to reduce harm or the risk of tobacco-related disease associated with commercially marketed tobacco products" [2]. A candidate MRTP, the Carbon Heat and Light Tobacco Product (CHTP1.2), is a novel patented tobacco product, which uses a carbon source to heat a tobacco plug in a specially-designed stick to produce an aerosol which contains nicotine and tobacco flavor.

Objective. The objective of this study was to assess and compare the effects of repeated exposures (28-32 days) to the aerosols of CHTP1.2 with CS generated from reference cigarettes (3R4F) on human gingival organotypic epithelial cultures. We employed a systems toxicology approach based on the measurement of cytotoxicity, histopathological modifications, pro-inflammatory mediator secretion and the modelling of computational network biology to investigate the impact of exposure on the gingival epithelial transcriptome.

RESULTS

3R4F CS-exposed cultures exhibited an increased cytotoxicity compared with air controls (Figure 2A, 48 h post-exposure). Significant cytotoxicity was not observed following CHTP1.2 aerosol exposure. Exposure to 3R4F CS caused marked signs of damage (Figure 2B), leading to increased cell alterations, atrophy, apoptosis, hypergranulosis, parakeratosis, suprabasal splitting and epithelial splitting compared with the air controls. CHTP1.2 aerosol-exposed cultures exhibited changes of the same findings but much less marked, even following exposure to the highest concentration (100.0 mg/L).

The highest BIF, seen in the cultures exposed to 3R4F (39.7 mg/L), 48 h post-exposure, is represented in the heatmap as the highest scores of perturbation in the majority of networks (NPA), among all contrasts. The networks describing the gene expression, necroptosis, apoptosis, MAPK, Jak Stat, hedgeshog growth factor, cell cycle, xenobiotic metabolism response, oxidative stress, osmotic stress, NFE2L2 signaling, tissue damage, and epithelial innate immune activation were most affected by 3R4F CS, while CHTP1.2 aerosol did not induce comparable alterations at any of the concentrations tested.

CONCLUSIONS

A systems toxicology approach was applied for the biological impact assessment of CHTP1.2 aerosol compared with 3R4F CS on human organotypic gingival epithelial cultures. Multiple endpoints were combined toward a comprehensive assessment of the exposure effects

• Morphological alterations were observed after 3R4F CS exposure, and increase of cytotoxicity level was detected (maximum ~13%). CHTP1.2 aerosol caused less morphological alterations compared to 3R4F CS and no significant cytotoxicity levels

• The transcriptomics analysis indicated significant perturbations by 3R4F CS exposure in various network models, the stress responses following CHTP1.2 aerosol exposure were markedly lower than following 3R4F CS exposure

• 3R4F CS had an overall higher impact on the release of pro-inflammatory mediators in gingival organotypic cultures than CHTP1.2 aerosol at comparable concentrations

• Overall, repeated CHTP1.2 aerosol exposures exerted a significant lower impact than 3R4F CS on human gingival organotypic epithelial cultures