Reduced effects of aqueous aerosol extract from THS2.2, a candidate Modified Risk Tobacco Product on the adhesion of monocytes to human coronary arterial endothelial cells

Carine Poussin, Alexandra Laurent, Manuel C Peitsch, Julia Hoeng, Hector De Leon
Philip Morris International R&D, Quai Jeanrenaud 5, CH 2000, Neuchâtel, Switzerland

ABSTRACT

Smoking is a major risk factor for the development of cardiovascular diseases (1,2). Modified risk tobacco products (MRTP) are designed to reduce smoking-related health risks. The present study was aimed to evaluate the impact of THS2.2, a candidate heat-not-burn technology-based MRTP, compared with a reference cigarette (3R4F), on the adhesion of monocytes to human coronary arterial endothelial cells (HCAECs). A critical stage in atheroma development, using a functional in vitro adhesion assay combined with systems toxicology, HCAECs were treated for 4 h with conditioned media of human monocyte mono-mac 6 (MM) cells preincubated with low or high concentrations of aqueous extracts from THS2.2 aerosol or 3R4F smoke for 2 h (indirect treatment), unconditioned media (direct treatment), or fresh aqueous extract (fresh direct treatment). Previous results showed that aqueous 3R4F smoke extract induced adhesion of MM cells to HCAECs via direct and indirect mechanisms (3). Leveraging the same experimental and computational framework, significant reduced effects of aqueous THS2.2 aerosol extract on MM cell-HCAEC adhesion were also observed, supported by molecular networks and highly differential gene expressions that such changes in expression both endothelial and monocyte cells. A shift towards 10 and 20 times higher concentrations of aqueous THS2.2 aerosol extract was required to observe similar effects as the ones measured with 3R4F in both fresh direct and indirect exposure modes, respectively. In conclusion, our in vitro systems toxicology investigations revealed reduced effects of THS2.2, a candidate MRTP, on monocyte cell-endothelial cell adhesion compared with a reference cigarette.

MATERIALS & METHODS

1. Cell exposure to 3R4F or THS2.2 s/aPBS (aqueous smoke/aerosol extract)

- Conditioned-and unconditioned-media preparation
- Indirect (s/aPBS) treatment: 24-hour-stocked (10% FBS) media were replaced with conditioned media and were preincubated for 3R4F or THS2.2 s/aPBS for 2 h.

2. Adhesion Assay

- Unstained MM and 4h-treated HCAECs were hemed-stained and incubated for 45 min. After cell fixing (formaldehyde, 4%) and washing, remaining adherent MM and HCAECs were counterstained with a Calcein-AmOxy-instrument (5). The adhesion rate was calculated as follow: Adhesion=(MM cell count/HCAEC cell count) X 100.

3. Other endpoints

- Cell viability: MM and HCAECs viability was determined using a resazurin assay (Sigma-Aldrich).
- Inflammation markers: A panel of 4 BioMark was measured in conditioned-media (MM supernatants) by Myriad-RBM Human Inflammation panel (1.0 kit).
- Transcripts: mRNA extracted from MM and HCAEC cell lysates was analysed in our transcriptional laboratory using Affymetrix GeneChip Human Genome U133 Plus 2.0 Array.

4. Computational analysis

- Transcripts data were processed using GCRMA R package. Painless differential gene expression (systems response profile, SPR) analysis comparing 3R4F or THS2.2 s/aPBS to PBS for each exposure-condition type (s/aPBS) was computed with limma R package. Relative biological impact factor (RBF) analysis was conducted for each SPR using two-layer causal network models representing different biological processes. The approach aims to identify significantly perturbed biological networks and to quantify their relative contribution to the overall biological impact of the treatment on cells.

RESULTS

1. Cell exposure to 3R4F or THS2.2 s/aPBS (aqueous smoke/aerosol extract)

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CONCLUSIONS

- In indirect: the effect is observed at a low concentration (0.06 puff/mL) and is mediated through the activation of an inflammatory mediator, which increases the expression of adhesion molecule(s) mediated by a paracrine effect of 3R4F-treated membrane-derived soluble mediators (e.g. TNF) present in conditioned-media.
- In fresh direct: the effect is observed at a high concentration (0.225 puff/mL) that also induces some toxicity and is mediated through an yet unknown mechanism promoted in HCAECs by unstable CS compounds still present in freshly generated s/aPBS, however, decayed in unconditioned-media obtained with the direct protocol.

At the same concentrations, significant adhesion effects of MM cells to HCAECs, little changes in HCAEC and MM gene expression and inflammatory marker release by s/aPBS-treated MM cells are observed with THS2.2.

The concentrations of THS2.2 required to be increased by 10 and 20 times to observe similar effects at functional and molecular levels to the ones observed with 3R4F s/aPBS using fresh direct and indirect exposure protocols, respectively.

REFERENCES

(4) Thomsen et al. (2004), Toxicol Appl Pharmacol. 204:373-385.