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Systems toxicology-based assessment of the candidate modified risk tobacco product THS2.2 for the adhesion of monocytic cells to human coronary arterial endothelial cells

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ABSTRACT

Alterations of endothelial adhesive properties by cigarette smoke (CS) can progressively favor the development of atherosclerosis which may cause cardiovascular disorders. Modified risk tobacco products (MRTPs) are tobacco products developed to reduce smoking-related risks. A systems biology/toxicology approach combined with a functional in vitro adhesion assay was used to assess the impact of a candidate heat-not-burn technology-based MRTP, Tobacco Heating System (THS) 2.2, on the adhesion of monocytic cells to human coronary arterial endothelial cells (HCAECs) compared with a reference cigarette (3R4F). HCAECs were treated for 4 h with conditioned media of human monocytic Mono Mac 6 (MM6) 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 aerosol/smoke extracts (fresh direct treatment). Functional and molecular investigations revealed that aqueous 3R4F smoke extract promoted the adhesion of MM6 cells to HCAECs via distinct direct and indirect concentration-dependent mechanisms. Using the same approach, we identified significantly reduced effects of aqueous THS2.2 aerosol extract on MM6 cell-HCAEC adhesion, and reduced molecular changes in endothelial and monocytic cells. Ten- and 20-fold increased concentrations of aqueous THS2.2 aerosol extract were necessary to elicit similar effects to those measured with 3R4F in both fresh direct and indirect exposure modalities, respectively. Our systems toxicology study demonstrated reduced effects of an aqueous aerosol extract from the candidate MRTP, THS2.2, using the adhesion of monocytic cells to human coronary endothelial cells as a surrogate pathophysiologically relevant event in atherogenesis.

1. Introduction

Smoking is a major risk factor for the development of cardiovascular diseases (Messner and Bernhard 2014). Cigarette smoke (CS) contains constituents that cross the alveolar barrier into the blood stream, elicit systemic effects, and affect peripheral organs and tissues such as blood vessels and the heart distal from the lungs. Over time, smokers develop low grade inflammatory, (e.g., release of cytokines and eicosanoids) and pro-oxidative (e.g., oxidized low-density lipoprotein) features in the blood that can affect normal vascular regulatory functions including vasotone regulation, endothelial permeability, and coagulation. These changes favor and accelerate the appearance of atherosclerotic plaques (Favero et al., 2014; Messner and Bernhard 2014; Yanbaeva et al., 2007).

The development of innovative modified risk tobacco products (MRTPs) that reduce the incidence of cardiovascular and other smoking-related diseases (e.g., lung diseases), and subsequently decrease the morbidity and mortality of smoking is necessary for public health concerns (Administration, 2012b). CS contains thousands of toxic chemicals, including acrolein, benzene, and cadmium (Haussmann, 2012; Perfetti and Rodgman, 2011; Rodgman and Perfetti, 2013). However, by heating rather than burning tobacco it is possible to markedly decrease the amount of harmful constituents in the aerosol (e.g., including, but not limited to,

Abbreviations: CS, cigarette smoke; FDR, false discovery rate; HCAECs, human coronary artery endothelial cells; I, D, and FD, indirect, direct and fresh direct, respectively; ICAM-1, intercellular adhesion molecule 1; MM6, Mono Mac 6; MRTP, modified risk tobacco product; NPA, network perturbation amplitude; (R)BIF, (Relative) biological impact factor; SELE, E-selectin; SM, starvation medium; TACE, tumor necrosis factor-α-converting enzyme; THS2.2, Tobacco Heating System 2.2; TNFα, tumor necrosis factor-alpha; TRAG, tissue repair and angiogenesis network; VCAM-1, vascular cell adhesion molecule 1; V-IPN, vascular-inflammatory processes network; IPN, inflammatory processes.

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aldehydes and polycyclic aromatic hydrocarbons) (Schorp et al., 2012; Werley et al., 2008). Recent investigations into the biological impact of a heat-not-burn technology-based prototypic (p) MRTP have shown reduced exposure effects compared with those observed with a reference cigarette. For instance, a 7-month smoke inhalation study reported significant reductions of inflammation and emphysema in the lungs of mice exposed to pMRTP (carbon-heated tobacco technology) compared with a 3R4F reference cigarette at matching nicotine concentrations (Phillips et al., 2015). Diminished inflammatory and oxidative stress responses were also measured in nasal and bronchial epithelia, and lung parenchyma of rats exposed to pMRTP aerosol for 28 days (Kogel et al., 2014). Regarding the cardiovascular system, a recent in vitro study showed that the aqueous extract of a pMRTP (smoke-bubbled media) exhibited reduced effects on chemotaxis and the transendothelial migration of monocytes compared with the 3R4F reference cigarette (van der Toorn et al., 2015b).

CS has been shown to modify the adhesive properties of the endothelium, leading to higher rates of leukocyte–endothelial cell adhesion (Winkelmann et al., 2009). Mechanistic studies from our group and others have reported direct and indirect effects of CS on monocyte–endothelial cell adhesion promoted by the increased expression of adhesion proteins at the surface of endothelial cells (Kalra et al., 1994; Lehr 1993; Poussin et al., 2014; Wang et al., 2004; Zhang et al., 2002). The direct effect of CS was reported to be mediated by the direct influence of CS constituents on endothelial cells, while the indirect effect was described to be driven via the paracrine inflammatory effect of soluble mediators secreted by CS-treated monocytic cells (Poussin et al., 2014; Zhang et al., 2002).

Current literature findings diverge with regards to direct and indirect effects of CS on monocyte-endothelium adhesion, which may reflect the heterogeneity of experimental set ups (e.g., exposure time, types of CS fractions and cellular systems) and may capture the aspects of different mechanisms. To mechanistically investigate the direct and indirect effects of aqueous 3R4F smoke extracts on the adhesion of monocytes to endothelial cells, we previously established an in vitro adhesion assay combined with a comprehensive computational transcriptomics data analysis approach (Poussin et al., 2015). We identified that, at low 3R4F smoke extract concentrations, monocyte–endothelial cell adhesion was promoted indirectly by an inflammatory response in human coronary arterial endothelial cells (HCAECs) induced by soluble mediators secreted by Mono Mac 6 (MM6) cells preexposed to aqueous 3R4F smoke extract (indirect treatment, I). We discovered that tumor necrosis factor (TNF)α, one of the main drivers of the MM6 cell-HCAEC adhesion was enzymatically shed from the MM6 cell surface by TNFα-converting enzyme (TACE) activated by a subset of unstable CS constituents (possibly reactive oxygen species, ROS) present in the medium. When HCAECs were exposed to a high concentration of fresh aqueous 3R4F smoke fraction (fresh direct treatment, FD), MM6 cell-HCAEC adhesion occurred directly via a different mechanism associated with cytotoxic effects induced by another subset of CS constituents that were unstable upon aging and freeze/thaw cycle (Poussin et al., 2015). Although the FD condition may be less physiologically relevant than the I one, it remains important to expose the system to conditions that induce toxicological effects and gain mechanistic insights in vitro. Unstable CS constituents, present in whole smoke, may interact and be metabolized in lung cells such as bronchial epithelial cells and alveolar macrophages before entering the circulation with modified functional properties. Despite some limitations, the indirect condition mimics exposure of the endothelial cell monolayer to inflammatory mediators released by other cells exposed to CS as well as low concentrations of stable CS-derived constituents or metabolites. In vivo, chronic exposure to low concentrations of inflammatory molecules concomitant with oxidants present in circulating blood plasma modifies endothelial functions, and favors the formation of atherosclerosis (Favero et al., 2014; Messner and Bernhard, 2014; Yanbaeva et al., 2007). Leveraging a previously established experimental and computational framework (Poussin et al., 2015), the present study aimed to compare the biological impact of aqueous extracts from a candidate MRTP, Tobacco Heating System (THS) 2.2 (electrically-heated tobacco technology), and the 3R4F reference cigarette on monocytic cell-HCAEC adhesion combining functional measurements from an in vitro adhesion assay with transcriptomics and inflammatory protein marker data to investigate changes at the molecular level.

2. Material and methods

2.1. Cell culture

HCAECs (Vitaris, Baar, Switzerland) were cultured in collagen A-coated flasks with endothelial cell growth medium MV2 containing 5% fetal calf serum (FCS). MM6 cells (DSMZ, Braunschweig, Germany) were maintained in RPMI 1640 medium (PAA, #E15-0.39, Pashing, Austria) containing 10% FCS, L-glutamine (0.86%), non-essential amino acids (0.86%), oxaloacetate pyruvate insulin media supplement (0.86%, Sigma, #O5003, Buchs, Switzerland) and penicillin/streptomycin (1.7%; Ruwag, # P11-010, Bettlach, Switzerland).

2.2. Preparation of aqueous extracts from THS2.2 aerosol or 3R4F smoke

Mainstream smoke from reference cigarette 3R4F (University of Kentucky) was generated on a 20-port rotary Borgwaldt smoking machine according to the Health Canada standard protocol and bubbled through ice cold phosphate buffered saline (PBS) (6 cigarettes/36 ml PBS, stock solution concentration: ~1.8 puffs/ml; ~10.7 puffs per cigarette in average) at room temperature as described previously (Poussin et al., 2015). The aqueous extract from 3R4F smoke is also often termed as 3R4F smoke-bubbled PBS (sbPBS) (Poussin et al., 2014, 2015).

Mainstream aerosol from the candidate MRTP, THS2.2 developed by Philip Morris international R&D was produced using a predefined puff count of 12 puffs per stick on a 30-port rotary aerosol generator (type SM 2000 P1) according to the Health Canada protocol. The aerosol was bubbled into ice cold PBS to trap the water soluble fraction (10 heatsticks/40 ml, stock solution concentration: 3 puffs/ml).

Final concentrations of 3R4F aqueous extract ranged from 0.06 to 0.225 puffs/ml. Previously, Su et al. (1998) calculated that 2.5% (2.5 cigarettes/l) to 10% (10 cigarettes/l) final concentrations of a 100% cigarette smoke extract PBS stock solution of 100 cigarettes/L (3 cigarettes smoked in 30 ml) corresponded approximately to exposures associated with smoking less than ~0.5–2 packs per day. Therefore, our range of concentrations would correspond to less than 1–4 packs of cigarettes smoked per day. For each experiment, freshly prepared THS2.2 and 3R4F aqueous extract stock solutions were diluted in RPMI 1640 containing 0.5% FCS (starvation medium (SM)), to obtain final aqueous extract concentrations. Vehicle controls corresponded to 15% or 75% (v/v) PBS diluted in starvation medium (Table 1).

2.3. Chemical analysis of aqueous 3R4F smoke and THS2.2 aerosol extracts

2.3.1. Carbonyl concentration determination.

The concentration of eight carbonyls was determined in aqueous extracts by liquid chromatography–electrospray