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A prototypic modified risk tobacco product exhibits reduced effects on chemotaxis and transendothelial migration of monocytes compared with a reference cigarette

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ABSTRACT

Monocyte adhesion and migration to the subendothelial space represent critical steps in atherogenesis. Here, we investigated whether extracts from the aerosol of a prototypic modified risk tobacco product (pMRTP), based on heating rather than combusting tobacco, exhibited differential effects on the migratory behavior of monocytes compared with that from the reference cigarette, 3R4F. THP-1 cells, a monocytic cell line, and human coronary arterial endothelial cells (HCAECs) were used to investigate chemotaxis and transendothelial migration (TEM) of monocytes in conventional and impedance-based systems. THP-1 cells migrated through a monolayer of HCAECs in response to C-X-C motif ligand 12 (CXCL12), a chemokine involved in diverse cellular functions including chemotaxis and survival of stem cells. Treatment of THP-1 cells with extracts from 3R4F or pMRTP induced concentration-dependent increases in cytotoxicity (7-aminoactinomycin D), and inflammation (IL-8 and TNF-α). CXCL12-mediated chemotaxis and TEM were decreased in extract-treated THP-1 cells. Extracts from 3R4F were ~21 times more potent than those from pMRTP in all examined endpoints. Extracts from 3R4F and pMRTP induced concentration-dependent responses in assays of inflammation, cytotoxicity, chemotaxis, and TEM. Furthermore, our findings indicate that extracts from a pMRTP are significantly less cytotoxic and induce less inflammation than those from the reference cigarette, 3R4F.

1. Introduction

Monocytes entering the subendothelial space of injured vessels play a key role in the pathogenesis of atherosclerosis. During this process, monocytes respond to chemotactic factors and begin adhering to and migrating through a layer of endothelial cells. Once in the subintimal space, monocytes may differentiate into macrophages. Uptake of oxidized low-density lipoproteins transforms macrophages into foam cells that accumulate and contribute to the development of atherosclerotic lesions (Libby et al., 2011). Chemokines are a large family of cytokines defined as small molecules that can trigger cell migration and arrest along concentration gradients during leukocyte extravasation (Doring et al., 2014; Sallusto and Baggiolini, 2008). A body of evidence defining the regulation of chemokine expression and function in atherosclerosis has accumulated in recent years (Zernecke et al., 2008). Neutrophils and monocytes accumulate in the adventitia layer of injured vessels; this perivascular inflammation is accompanied by the expression of cytokines and cell adhesion molecules in endothelial cells of the vasa vasorum (Okamoto et al., 2001). The chemokine C-X-C motif ligand 12 (CXCL12, also known as stromal cell-derived factor-1α, SDF-1α) and its receptors, CXCR4 and CXCR7, represent a remarkable biological example of functional versatility because they participate in critical functions including hematopoiesis, leukocyte traffic, and vascularization (Nagasawa et al., 1996; Sallusto and Baggiolini, 2008; Sierro et al., 2007; Zou et al., 1998). The expression of CXCL12 in human atherosclerotic plaques (Abi-Younes et al., 2000), the

Abbreviations: 7-AAD, 7-aminoactinomycin D; BSA, bovine serum albumin; CIM, cell invasion/migration; CO, carbon monoxide; CS, cigarette smoke; CXCL12, C-X-C motif ligand 12; DNPH, dinitrophenylhydrazine; E-plate, electrical impedance plate; fMLP, N-formylmethionyl-leucyl-phenylalanine; GM-CSF, granulocyte-macrophage colony-stimulating factor; HCAECs, human coronary arterial endothelial cells; HGF, hepatocyte growth factor; ICAM1, intercellular adhesion molecule-1; IL-8, interleukin-8; LC-MS, liquid chromatography-electrospray ionization tandem mass spectrometry; MCP-1, monocyte chemoattractant protein-1; NO, nitrogen monoxide; pMRTP, prototypic modified risk tobacco product; RTCA-DP, real-time cell analyzer dual-plate; SELE, E-selectin; TEM, transendothelial migration; THP-1, human monocytic cell line; TNF-α, tumor necrosis factor-α; VCAM1, vascular cell adhesion molecule.

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association between CXCL12 levels and coronary artery disease (Damas et al., 2002), and the potent chemotactic effects of CXCL12 on peripheral blood mononuclear cells (Liu et al., 2006) prompted us to investigate the effects of extracts from the reference cigarette 3R4F and a prototypic modified risk tobacco product (pMRTP) on CXCL12-dependent transendothelial migration (TEM) of THP-1 cells, a monocytic cell line that expresses CXCR4 (Tripathi et al., 2014).

Mainstream cigarette smoke (CS) contains constituents that enter the bloodstream, trigger systemic effects, and affect organs distal from the lungs, including the heart and coronary vessels (van der Toorn et al., 2009). Human and animal studies have demonstrated that cigarette smoking is associated with pathological effects on endothelial cells and adversely interferes with all stages of plaque development (Csordas and Bernhard, 2013; Messner and Bernhard, 2014). However, the precise identity and mechanisms of action of the more than 8000 chemical compounds generated by the combustion of tobacco (Rodgman and Perfetti, 2013) driving the CS-induced formation of atherosclerotic lesions is not completely understood. Some studies have shown that CS constituents induce endothelial gene expression of cell adhesion molecules, such as vascular cell adhesion molecule, intercellular adhesion molecule-1, and E-selectin, which promote monocyte adhesion through a proinflammatory paracrine effect (Lehr et al., 1994; Poussin et al., 2014; Shen et al., 1996).

It is widely recognized that the use of combustible tobacco products increases the risk of developing cardiovascular disease (Centers for Disease Control and Prevention 2014; Go et al., 2014). A promising approach to decrease such risk is the reduction of the concentrations of combustion-derived constituents in CS without modifying the amount of delivered nicotine. Heating rather than combusting tobacco has been shown to substantially reduce the amounts of constituents in CS (Patskan and Reininghaus, 2003). In a 28-day rat inhalation model, we previously showed that exposure to aerosol from a carbon-heated tobacco product significantly reduces its effects on the respiratory tract of rats compared with those of burning tobacco from the reference cigarette, 3R4F (Kogel et al., 2014). In the present study, the combined use of conventional Boyden chamber (Fig. 1A) and impedance-based cell migration systems (Fig. 1B) enabled us to investigate whether the aqueous phase of aerosol from a pMRTP exhibited differential effects on the migratory behavior of monocytes compared with the aqueous phase of CS from the reference cigarette 3R4F.

2. Material and methods

2.1. Chemicals

Collagen A was obtained from Biochrom AG (Berlin, Germany). Interleukin (IL)-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), hepatocyte growth factor (HGF), monocyte chemoattractant protein-1 (MCP-1), CXCL12, bovine serum albumin (BSA), and crystal violet were obtained from Sigma-Aldrich (Deisenhofen, Germany). Seven-aminoactinomycin D (7-AAD) was purchased from BD Pharmingen (Allschwil, Switzerland). RPMI 1640 and L-glutamine were purchased from PAA Laboratories (Pasching, Austria). Fetal calf serum and penicillin-streptomycin were obtained from GE Healthcare (Zürich, Switzerland). Trypsin/EDTA solution was obtained from Lonza (Basel, Switzerland).

2.2. Cell culture

Human endothelial coronary cells (HCAECs) and growth medium-2 were purchased from Promocell (Heidelberg, Germany). HCAECs were grown on collagen A-coated plates (VWR, Dietikon, Switzerland) in endothelial cell growth medium-2 containing a supplement mix until confluency. The human monocytic cell line THP-1 was purchased from the American Type Culture Collection (Manassas, VA, USA). THP-1 cells were grown in RPMI 1640 supplemented with 2 mM L-glutamine, 10% heat inactivated fetal calf serum, and 1% penicillin–streptomycin solution. Before the migration assays, THP-1 cells were cultured for 18 h in serum-free RPMI 1640 supplemented with 2 mM L-glutamine, 1% penicillin-streptomycin, and 0.1% BSA.

2.3. Reference cigarette 3R4F and the pMRTP

Reference research filtered cigarettes 3R4F were purchased from the University of Kentucky (Lexington, KY, USA; http://www.ca.uky.edu/refcig/). The pMRTP was provided by Philip Morris Products S.A. (Neuchâtel, Switzerland) and it is based on a design in which a carbon tip serves as a fast-lighting heat source to generate an aerosol containing water, glycerin, nicotine, and tobacco flavors with reduced concentrations of tobacco products as a result of heating. Prior to use in the study, the 3R4F and pMRTPs were conditioned according to ISO Standard 3402 (International Organization for Standardization, 2010) for 7–21 days.

The comparative analytical specifications of pMRTP aerosol and 3R4F CS yields are presented in Supplementary Table S1, which shows the quantification of 78 harmful or potentially harmful constituents plus water and glycerol (as humectant) for smoke from 3R4F and the aerosol from the pMRTP, generated according to the Health Canada intense smoking regimen (Health Canada, 1999). The type of pMRTP used throughout this study was slightly different from that used in a previously published 28-day rat inhalation study (Kogel et al., 2014). From the 78 analytes, 47 were reduced to less than 10% or to undetectable amounts in the pMRTP aerosol compared with CS from 3R4F expressed on an equal nicotine basis. Tobacco-specific N-nitrosamines were reduced to 11–17%, and acrolein, nitrogen monoxide (NO) carbon monoxide (CO), catechol, and acetamide, were reduced to 14–29% of the amount measured in 3R4F, whereas total particulate matter increased to 160%, water to 397%, and glycerol to 373% in pMRTP. While many of the polycyclic aromatic hydrocarbons were markedly decreased, increases were observed for benzo(b)fluoranthene (174%) and pyrene (146%).

2.4. Generation of extracts from 3R4F and pMRTP for cell stimulation

CS from 3R4F cigarettes was generated on a 20-port Borgwaldt smoking machine (Hamburg, Germany) and aerosol from the pMRTP was generated on a 30-port SM2000 smoking machine, respectively, according to the Health Canada standard (55 mL puff drawn every 30 s) (Health Canada, 1999). Extracts were generated by bubbling aerosol or smoke through RPMI 1640 cell culture medium containing 2 mM L-glutamine and 1% penicillin–streptomycin (3R4F; 6 items/36 mL RPMI 1640, pMRTP; 10 items/25 mL RPMI 1640) on ice. Extracts were supplemented with 0.1% BSA before testing. The 3R4F stock extract solution was further diluted in serum-free medium to obtain final concentrations ranging from 0.031 to 0.5 puffs/mL. The pMRTP stock extract solution was diluted in serum-free medium to obtain final concentrations ranging from 0.003 to 4.8 puffs/mL. To monitor variability in the concentration of key constituents, chemical analyses were conducted directly after extract generation. Serum-starved THP-1 cells and HCAECs were stimulated for 4 h with increasing concentrations of freshly prepared extracts from 3R4F and pMRTP. Then, the cells were washed with fresh medium and seeded in chemotaxis and TEM inserts as described below.

2.5. Chemical analysis of 3R4F and pMRTP extracts

Determination of eight carbonyls was conducted in extracts by liquid chromatography-electrospray ionization tandem mass spectrometry (LC-MS) to monitor for batch consistency. Carbonyl compounds were trapped during CS exposure in the glass bottles containing RPMI 1640 (see Section 2.4 above). After exposure, an aliquot of 400 µL exposed medium was incubated with 600 µL of a dinitrophenylhydrazine (DNPH) solution (15 mM DNPH and 25 mM perchloric acid in acetonitrile) for 30 min at room temperature. Chemical derivatization was then quenched by addition of 50 µL pyridine. A 500 µL aerosol-derivatized sample was introduced in a LC-MS glass vial with 485 µL acetonitrile and 15 µL internal standard working solution containing acetone-d6-DNPH and methyl-ethyl-ketone-d5-DNPH (24 µg/mL each). Formaldehyde-DNPH, acetaldehyde-DNPH, acetone-DNPH, crotonaldehyde-DNPH, propionaldehyde-DNPH, acrolein-DNPH, methyl-ethyl-ketone-DNPH, and butyraldehyde-DNPH were analyzed by liquid chromatography (Agilent 1200, Agilent Technologies, Santa Clara, CA, USA) coupled to a negative electrospray ionization tandem mass spectrometer (5500 QqQ, AB Sciex. Framingham, MA, USA). Separation of the aldehydes was performed in the isocratic mode on a Chromolith Speedrod RP-18e HPLC column (Merck KGaA, Darmstadt, Germany) using water, acetonitrile, tetrahydrofuran, and isopropanol (59:30:10:1, v/v/v/v) at a flow rate of 2.5 mL/min (column set at 40 °C and equipped with a post-column splitter 1:6 before entrance into the LC-MS). LC-MS detection was performed in the multiple reaction monitoring mode. The carbonyl compound concentration (expressed as µg/cigarette) was calculated using an external calibration curve.

2.6. Monocyte chemotaxis and TEM assays using conventional Boyden chambers

Chemotaxis assays using Boyden chambers (Corning, Amsterdam, The Netherlands) were conducted in the absence or presence of a monolayer of HCAECs. THP-1 cells were serum-starved for 18 h and then seeded in 100 µL of serum-free medium on the insert of a Boyden chamber with a polycarbonate membrane pore size of 5 µm. The cells were seeded in the upper chamber at a density of 1×10^5 cells per insert.