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Short communication

Aerosol from a candidate modified risk tobacco product has reduced effects on chemotaxis and transendothelial migration compared to combustion of conventional cigarettes

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ABSTRACT

Reduction of harmful constituents by heating rather than combusting tobacco is a promising new approach to reduce harmful effects associated with cigarette smoking. We investigated the effect from a new candidate modified risk tobacco product, the tobacco heating system (THS) 2.2, on the migratory behavior of monocytes in comparison with combustible 3R4F reference cigarettes. The monocytic cell line (THP-1) and human coronary arterial endothelial cells (HCAECs) were used to analyze chemotaxis and transendothelial migration (TEM). To assess the influence of aerosol extract from THS2.2 and smoke extract from 3R4F on toxicity and inflammation, flow cytometry and ELISA assays were performed. The results show that treatment of THP-1 cells with extract from 3R4F or THS2.2 induced concentration-dependent increases in cytotoxicity and inflammation. The inhibitory effects of THS2.2 extract for chemotaxis and TEM were ~18 times less effective compared to 3R4F extract. Furthermore, extract from 3R4F or THS2.2 induced concentration-dependent decreases in the integrity of HCAEC monolayer. For all examined endpoints, the extract from 3R4F showed more than one order of magnitude stronger effects than that from THS2.2 extract. These data indicate the potential of a heat not burn tobacco product to reduce the risk for cardiovascular disease compared to combustible cigarettes.

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1. Introduction

Studies confirm that cigarette smoking increases the risk of developing atherosclerosis, the predominant underlying cause of cardiovascular disease (Go et al., 2014). However, the mechanisms by which the chemical compounds generated by tobacco combustion (Rodgman and Perfetti, 2013) drive the formation of atherosclerotic lesions are not completely understood. Monocytes play a key role in the pathogenesis of atherosclerosis by adhering to and crossing the endothelial cell layer to the subintimal space, where they take up large amounts of oxidized low-density lipoproteins and acquire a foam cell phenotype (Libby et al., 2011). Increases in extravasation of monocytes from the vasculature or reductions in the intravasation of these cells from vascular lesions contribute to both the formation and progression of atherosclerotic lesions (Randolph, 2008).

Recently, we postulated that a reduction in harmful constituents by heating rather than combusting tobacco would be a sound strategy to reduce the risk for cardiovascular disease associated with smoking combustible tobacco products. We demonstrated that the extract from heated tobacco is less cytotoxic and inflammatory, and exerts a lower effect on monocyte chemotaxis and transendothelial migration (TEM) than that from combustible tobacco (van der Toorn et al., 2015). In the present study, we confirmed that heating rather than combusting tobacco results in a smaller effect on endothelial functions and the migratory behavior of monocytes using a new candidate modified risk tobacco product (MRTP) (FDA, 2012). Compared with the prototype MRTP described

Abbreviations: 7-AAD, 7-aminoactinomycin D; BSA, bovine serum albumin; CIM, cell invasion/migration; CXCL12, C-X-C motif ligand 12; HCAECs, human coronary arterial endothelial cells; IL-8, Interleukin-8; MRTP, modified risk tobacco product; RTCA, real-time cell analyzer; TEM, transendothelial migration; THS, tobacco-heating system; TNF-α, tumor necrosis factor-α.

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in the earlier publication (van der Toorn et al., 2015), which uses a pressed carbon heat source, the tobacco heating system (THS)2.2 used in the present is a different innovation to heat rather than burn tobacco. THS2.2 is based on a battery-operated heating system that heats tobacco sticks in a controlled manner. The system consists of a heater blade that operates at a maximum temperature of 350 °C. The temperatures measured for the tobacco indicate that much of the tobacco in the plug experience much lower temperature. In contrast, during the combustion of conventional cigarettes, the temperature of the tobacco increases to more than 900 °C at the periphery of the burning zone (Baker, 1974).

In this study, we found that the extract of the aerosol from this new candidate MRTP exerted a lesser effect on the migratory behavior of monocytes compared to extract of smoke from combustible reference cigarettes.

2. Material and methods

2.1. Chemicals

Collagen A was obtained from Biochrom AG (Berlin, Germany). Human coronary artery endothelial cells (HCAECs) and Endothelial Growth Medium-2 were purchased from Promocell (Heidelberg, Germany). Human monocytic cell line (THP-1) was purchased from the American Type Culture Collection (Manassas, VA, USA). Interleukin-8 (IL-8), C-X-C motif ligand 12 (CXCL12), bovine serum albumin (BSA), and crystal violet were obtained from Sigma–Aldrich (Deisenhofen, Germany). 7-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

HCAECs were grown on 75 cm^2 collagen A-coated plates (VWR, Dietikon, Switzerland) in Endothelial Cell Growth Medium-2 until confluence. THP-1 cells were grown in RPMI 1640 supplemented with 2 mM L-glutamine, 10% heat inactivated fetal calf serum, and 1% **penicillin–streptomycin**. Eighteen hours before migration, cells were serum starved in RPMI 1640 supplemented with 2 mM L-glutamine, 1% **penicillin–streptomycin,** and 0.1% BSA.

2.3. Reference cigarette 3R4F and THS2.2

Reference research filtered cigarettes 3R4F were purchased from the University of Kentucky (Lexington, KY, USA; http://www.ca.uky.edu/refcig/). THS2.2 sticks were provided by Philip Morris (Neuchâtel, Switzerland). The THS2.2 operates by inserting a stick into a tobacco stick holder that heats the tobacco plug to generate an aerosol containing water, glycerin, nicotine, and tobacco flavors with reduced concentrations of harmful constituents. The tobacco stick holder included a battery, electronics for control, a heating element, and stick extractor. Tobacco stick holders were provided by Philip Morris Products SA. Prior to use in the study, 3R4F and THS2.2 were conditioned according to ISO Standard 3402 (International Organization for Standardization, 2010) for 7–21 days.

2.4. Generation of extracts from 3R4F and THS2.2 for cell stimulation

Smoke from 3R4F cigarettes was generated on a 20-port Borgwaldt smoking machine (Hamburg, Germany) and aerosol from THS2.2 was generated on a 30-port SM2000/P1 smoking machine according to the Intense Health Canada protocol (55 mL puff volume; 2 s puff duration; 2 min^{-1} puff frequency; 100% blocking of filter ventilation holes) (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: six items/36 mL RPMI 1640; THS2.2: 10 items/40 mL RPMI 1640) on ice. Before testing, 0.1% BSA was added to the extracts. The 3R4F extract stock solution was further diluted in serum-free medium to obtain final concentrations ranging from 0.01 to 0.5 puffs/mL. The THS2.2 stock extract solution was diluted in serum-free medium to obtain final concentrations ranging from 0.01 to 3 puffs/mL. To monitor the concentration of nicotine and eight key aldehydes, 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 THS2.2. Then, the cells were washed with fresh medium and seeded in chemotaxis and TEM inserts as described below.

2.5. Selective chemical analysis of 3R4F and THS2.2 extracts

To monitor batch consistency of the extracts, the amounts of formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, methyl ethyl ketone, and butyraldehyde were determined by liquid chromatography-electrospray ionization tandem mass spectrometry according to the method described by van der Toorn et al. (2015). Nicotine concentration was analyzed using an AT1100 liquid chromatography system including a binary pump and a column oven (Agilent Technologies, Waldbronn, Germany) connected to an HTC Pal autosampler (CTC Analytics, Zwingen, Switzerland). A hybrid triple quadrupole mass spectrometer API 4000 equipped with a Turbo V ion source, operating in positive APCI mode, was used for detection (AB Sciex, Darmstadt, Germany). High purity nitrogen was produced by a nitrogen generator NGM 22-LC/MS (cmc Instruments, Eschborn, Germany). Chromatographic separation was achieved by isocratic elution with 20% ammonium acetate (0.1% in water) and 80% methanol using a Synergi MAX RP 80 Å 4 μm C12, 150 × 4.6 mm column (Phenomenex, Aschaffenburg, Germany). Flow rate was set to 1.0 mL/min. The retention time for nicotine was 2.3 min. The turbo ion source was operated in positive APCI mode with the following settings: Source gas 1 = 25 psi, heater temperature = 500 °C, nebulizer current = 3 μA, curtain gas = 25 psi. Mass transitions were monitored by multiple reaction monitoring (MRM), setting the target scan time to 250 ms. Quantifier transition in the MRM was m/z = 163.0 → 129.9. Qualifier transition was set to m/z = 163.0 → 117.1 and IS to 166.0 → 129.9. Collision energies of 29 V (Quan/IS) and 35 V (Qual), respectively, were applied. Quadrupoles Q1 and Q3 were working at unit resolution.

2.6. Identification of monocyte–macrophage differentiation

Concentration-dependent migration towards CXCL12 in the presence or absence of HCAECs was performed using cell invasion/migration (CIM) plates and a real-time cell analyzer (RTCA) xCelligence instrument (Bucher Biotec, Basel, Switzerland). Briefly, THP-1 cells were seeded at a density of 1 × 10^5 cells in the upper chamber of CIM plates in the absence or presence of a HCAEC monolayer. Serum-free medium with various concentrations of CXCL12 were added to the bottom chamber. Real-time chemotaxis and TEM were monitored for 4 h.

Transmigrated THP-1 cells were incubated for a further 72 h in the absence or presence of HCAECs. Then, cells in the lower chamber were collected, washed, and prepared for flow cytometric

analysis. Non-migrated THP-1 cells served as a negative control. Non-specific antibody-binding sites were blocked with 1% BSA in PBS for 15 min at 4 °C prior to antibody staining. To detect cell surface markers, samples were incubated with monoclonal mouse anti-human antibodies IgG1k CD18-BV421 and IgG1k CD11b-AF488 (BD Bioscience, Allschwil, Switzerland) or relevant isotypes controls for 15 min at 4 °C. After incubation, the cells were washed, resuspended in 1% BSA/PBS and analyzed using a FACS-Canto™ II and BD FACSDiva™ software (Becton Dickinson, Allschwil, Switzerland).

2.7. Cytotoxicity and inflammation assays

THP-1 cells (1×10^6) were stimulated for 18 h in 24-well culture plates with various concentrations of freshly prepared extracts from the reference cigarette 3R4F and THS2.2. Then, the cells were centrifuged at 400 *g* for 5 min and stained with 7-AAD according to the manufacturer's instructions. Cells were analyzed by flow cytometry using the FACSCanto™ II. THP-1 cell culture supernatants were collected and stored at −80 °C for cytokine analysis. IL-8 and tumor necrosis factor-α (TNF-α) levels in the supernatants were measured with enzyme-linked immunosorbent assay kits (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's instructions.

2.8. Analyses of monocyte chemotaxis using conventional Boyden chambers and TEM in an impedance-based system

Chemotaxis analysis using Boyden chambers (Corning, Amsterdam, the Netherlands) and TEM assays using CIM plates and a RTCA xCELLigence instrument were conducted as described by van der Toorn et al. (2015).

2.9. Real-time impedance-based analysis of endothelial monolayer integrity

The effects of extracts from the reference cigarette 3R4F and THS2.2 on the integrity of an endothelial monolayer were evaluated using the RTCA xCELLigence instrument and electrical impedance plates with methods described previously by van der Toorn et al. (2015).

2.10. Statistics

Data are expressed as the mean ± standard error of the mean (SEM) unless indicated otherwise. Two-way analysis of variance (ANOVA) was used to assess dose–response experiments. Comparisons between experimental groups were performed by one-way ANOVA. When the overall F-test of the ANOVA analysis was significant, multiple comparison tests (Dunnett's test) were applied to determine the sources of differences, which were considered to be statistically significant at $P < 0.05$. The t-test for paired observations was used for comparisons between the chemical analyses of extracts from 3R4F and THS2.2.

3. Results and discussion

3.1. Chemical analyses of 3R4F and THS2.2 extracts

In this study, we tested a new candidate MRTP, THS2.2, the principle of which is similar to an earlier prototype MRTP described in our previous study (van der Toorn et al., 2015). To monitor batch consistency of the extracts and compare selective chemical compositions, the concentration of nicotine and eight different carbonyls were determined in 3R4F and THS2.2 extracts. Nicotine levels in both extracts did not differ significantly. Carbonyl concentrations (μg/cigarette) in extract from THS2.2 were significantly lower than those in extract from 3R4F for all examined carbonyls (Table 1). 3R4F/THS2.2 ratios for all carbonyls ranged from 1.9 to 21. These results indicate that the THS2.2 aerosol contained substantially lower levels of harmful constituents than combustible cigarette smoke without modifying the amount of delivered nicotine.

3.2. CXCL12 and a functional monolayer of HCAECs induce an adhesive phenotype in monocytes

During the development of atherosclerosis, the interactions of endothelial cells and monocytes play an important role. Adhesion of circulating monocytes to the endothelium and migration of activated monocytes to the subendothelial space rely primarily on cell surface integrins (Carlos and Harlan, 1994; Lee et al., 2014). Monocytic cells have an intracellular pool of the integrin CD11b/CD18 [also known as membrane-activating complex 1 (MAC-1)]. Upon activation of these cells, MAC-1 translocates to the cell surface. Recently, Westhorpe et al. (2012) showed that endothelial cells and exogenous factors such as cytokines are required to activate and differentiate migrated monocytes. As shown in Fig. 1, we determined whether THP-1 cells would migrate through a monolayer of HCAECs in response to CXCL12. In the absence of HCAECs, concentration-dependent migration towards CXCL12 was induced (see images in Fig. 1A) without THP-1 cells adhering to the lower side of the CIM membrane as indicated by the lack of an impedance signal (Fig. 1A). In the presence of a HCAEC monolayer, THP-1 cells transmigrated through the monolayer, traversed the CIM membrane, and adhered to the lower side as revealed by increased impedance (Fig. 1B). Monocyte activation and differentiation are key events in the formation and progression of atherosclerotic lesions. Our findings indicate that the passage of monocytes across an endothelial monolayer was accompanied by significant changes in adhesion (Fig. 1B) and expression of MAC-1 (Fig. 1C). Monocyte migration in the absence of endothelial cells did not result in monocytes acquiring an adhesive phenotype, which was determined by the lack of the cell surface marker MAC-1.

3.3. Extracts from 3R4F are considerably more cytotoxic and inflammatory than those from THS2.2

Combustible cigarette smoke contains many reactive constituents that can easily pass through the epithelial lung barrier and enter systemic circulation where they induce oxidative stress and inflammation (van der Toorn et al., 2009). We therefore assessed the influence of extract generated from the aerosol of THS2.2 on the cytotoxicity and inflammatory behavior of monocytes. Exposure of THP-1 cells for 18 h to increasing concentrations of extracts from

Table 1
Selective chemical analysis of 3R4F and THS2.2 extracts.

	3R4F(μg/cig)	THS2.2(μg/stick)	3R4F/THS2.2
Nicotine	80.2 ± 7.3	76.8 ± 15.9	1
Formaldehyde	37.9 ± 8.6	3.0 ± 0.6*	12
Acetaldehyde	1089 ± 150	174 ± 44*	6.2
Acetone	408 ± 80	24.1 ± 4.8*	17
Acrolein	100 ± 13	5.5 ± 1.0*	18
Propionaldehyde	65 ± 10	11.5 ± 1.6*	5.6
Crotonaldehyde	48 ± 6	2.3 ± 0.4*	21
Methyl ethyl ketone	87 ± 21	4.6 ± 0.9*	19
Butyraldehyde	19 ± 3	9.9 ± 2.0*	1.9

Samples were applied to chemical analyses. Data are expressed as the mean ± SD and are referred to 5 to 8 independent analyses. *P < 0.0001 vs. 3R4F (reference cigarette) by the paired t-test. cig: cigarette; THS2.2: tobacco-heating system 2.2.

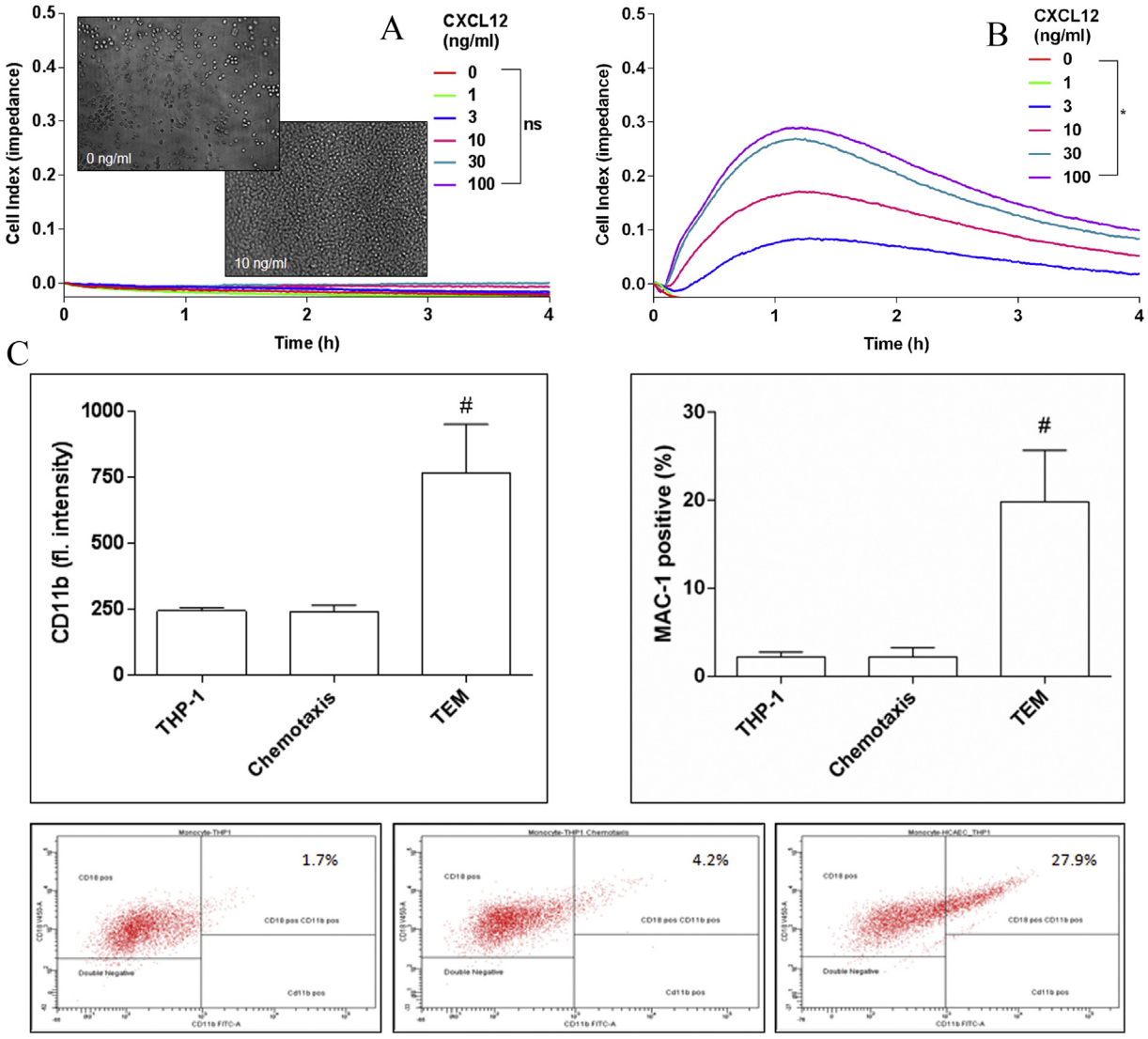


Fig. 1. TEM promotes acquisition of an adhesive phenotype in monocytes. A. Induction of concentration-dependent migration towards CXCL12 in the absence of HCAECs. THP-1 cell adherence to the lower side of the membrane did not occur as evidenced by the lack of changes in impedance (cell index). B. Increased impedance demonstrated concentration-dependent migration towards CXCL12 and THP-1 cell adherence to the lower side of the porous membrane in the presence of HCAECs. C. Acquisition of an adhesive phenotype by monocytes in the absence or presence of HCAECs. Acquisition of an adhesive phenotype was defined as positivity for CD11b or MAC-1. Representative flow cytometric data from non-migrated (lower left), migrated (lower middle), and transendothelial migrated (lower right) THP-1 cells towards CXCL12 are shown. Data are expressed as the mean ± standard deviation (SD) of three experiments. $^{*}P < 0.0001$ for the total increase of all CXCL12 concentrations used (repeated-measures two-way ANOVA). $^{\#}P < 0.05$ vs THP-1 control by paired t-test. ns: not significant. CXCL12: chemoattractant C-X-C motif ligand 12. MAC-1: membrane-activating complex 1. TEM: transendothelial migration.

3R4F and THS2.2 resulted in significant concentration-dependent increases in the number of cells positive for 7-AAD staining (Fig. 2A: 3R4F; IC_{50} = 0.085 puff/ml versus THS2.2; IC_{50} = 2.237 puff/ml). However, the effective concentration of THS2.2-induced cytotoxicity was more than ~26 times higher than that required for the 3R4F extract to induce cell death. Similarly, the 3R4F extract was ~10 times more potent than the THS2.2 extract in the induction of IL-8 and TNF-α secretion by THP-1 cells (Fig. 2B and C). These inflammatory responses increased rapidly, reached a maximum, and then attenuated at the highest doses of extracts from 3R4F and THS2.2, which were most likely due to cytotoxicity. These findings are in line with the data from our previous report, indicating that pretreatment of monocytes with an extract generated from a combustible cigarette product triggers a more potent cytotoxic and inflammatory response than extract from a carbon-heated tobacco product (van der Toorn et al., 2015).

3.4. Extracts from 3R4F more potently inhibit chemotaxis and TEM than those from THS2.2

Cigarette smoking influences the extravasation of monocytes to atherosclerotic lesions. Here, we used two *in vitro* approaches to examine these effects. In the first approach, we quantified the migration of 3R4F- and THS2.2-treated monocytes towards a potent chemoattractant, CXCL12. In the second approach, real-time chemotaxis of treated monocytes across a monolayer of endothelial cells was quantified using an impedance-based system. Fig. 3 shows that pretreatment of THP-1 cells with extract from either 3R4F or THS2.2 resulted in significant concentration-dependent inhibition of CXCL12-stimulated chemotaxis (Fig. 3A: 3R4F; IC_{50} = 0.016 puff/ml and Fig. 3B: THS2.2; IC_{50} = 0.860 puff/ml). We next investigated the effect of 3R4F and THS2.2 extracts on the TEM rate of THP-1 cells through a monolayer of HCAECs using real-time

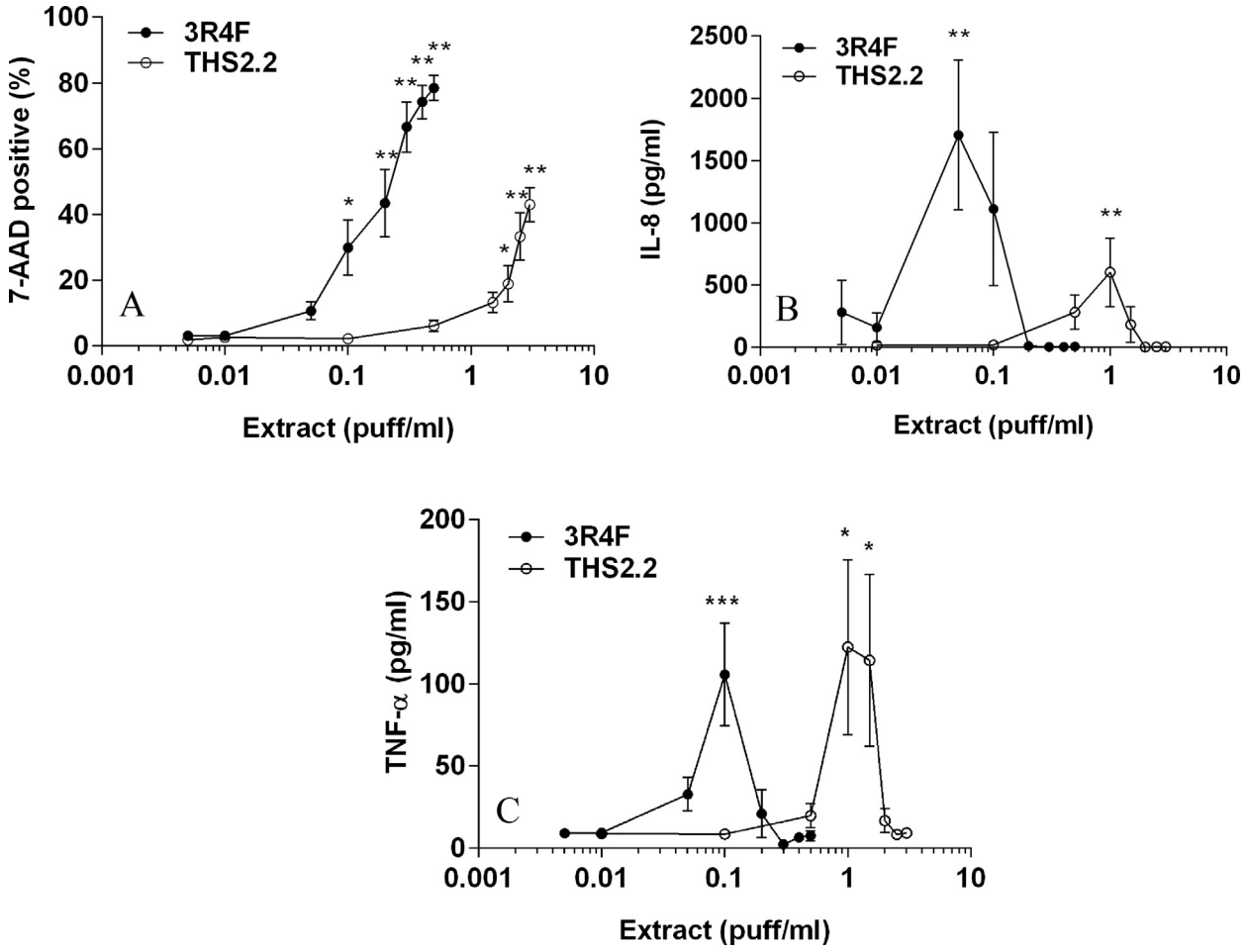


Fig. 2. Effects of 3R4F and THS2.2 extracts on THP-1 cell cytotoxicity and inflammation. A. Percentage of THP-1 cells positive for 7-AAD determined by flow cytometric analysis after stimulation with 3R4F (filled circles) or THS2.2 (hollow circles) extract. B and C. Mean concentrations of IL-8 and TNF-α in the culture supernatants of THP-1 cells treated with 3R4F (filled circles) or THS2.2 (hollow circles) extract. Data are expressed as the mean ± SEM of four independent experiments. *P < 0.05 vs control; **P < 0.01 and ***P < 0.001 vs control by Dunnett's multiple comparison test. THS2.2: tobacco heating system 2.2.

impedance-based TEM. Pretreatment of THP-1 cells with extract from either 3R4F or THS2.2 led to significant and concentration-dependent decreases in the TEM rate of THP-1 cells (Fig. 3C: 3R4F; IC_{50} = 0.049 puff/ml and Fig. 3D: THS2.2; IC_{50} = 0.871 puff/ml). However, the inhibitory effect of THS2.2 extract for chemotaxis was ~50 times and for TEM ~18 times less effective than that of 3R4F extract. In line with this finding, a study by Giunzioni et al. demonstrated that pre-treatment of monocytes with cigarette smoke extract resulted in reduced chemotaxis (Giunzioni et al., 2014). However, when monocytes and endothelial cells where exposed at the same time to cigarette smoke extract, increased transmigration was observed. This may be due to a disturbance of the integrity of the endothelial monolayer in which we also observed when endothelial cells were treated with smoke/aerosol extracts (Section 3.5; Fig. 4). In contrast to our observation, Shen et al. was able to show increased TEM of monocytes after pre-treatment with cigarette smoke extract (Shen et al., 1996). However they used differentiated monocytes which are normally found in the tissue and not in the blood circulation. In terms of the potential mechanism of action, a decrease in monocyte migration, as observed after exposure to cigarette smoke extract, will reduce their capacity to leave the vasculature and enter the subintimal space. However, this notion suggests a decrease in the risk of smokers developing atherosclerosis. Therefore, the mechanism by which monocytes in smokers contribute to increased formation of atherosclerotic lesions might be different because it may involve a decrease in the clearance of monocytes/macrophages from atherosclerotic lesions as a result of their reduced capacity to intravasate back into the vasculature. Suppressed intravasation has been shown to drive the monocyte/macrophage burden in favor of atherosclerotic plaque formation (Potteaux et al., 2011). An MRTP that reduces extravasation or activates intravasation may reduce the impact on health by allowing monocytes to be more readily cleared from atherosclerotic plaques compared with conventional cigarettes that have a more deleterious effect on monocyte migration.

3.5. Extracts from 3R4F more strongly decrease the integrity of an endothelial monolayer than those from THS2.2

The endothelium is a key regulator of vascular homeostasis. Endothelial barrier dysfunction can be an initiating factor that contributes to lesion development and clinical complications (Deanfield et al., 2007). Here, we challenged the barrier function of a confluent monolayer of HCAECs with increasing concentrations of extract from 3R4F or THS2.2 using electronic plates with micro-electrode sensors in the bottom of the wells. As a result, we observed rapid concentration-dependent disruption of endothelial barrier integrity as indicated by lower impedance values immediately after extract application (Fig. 4). The 3R4F extract was ~16

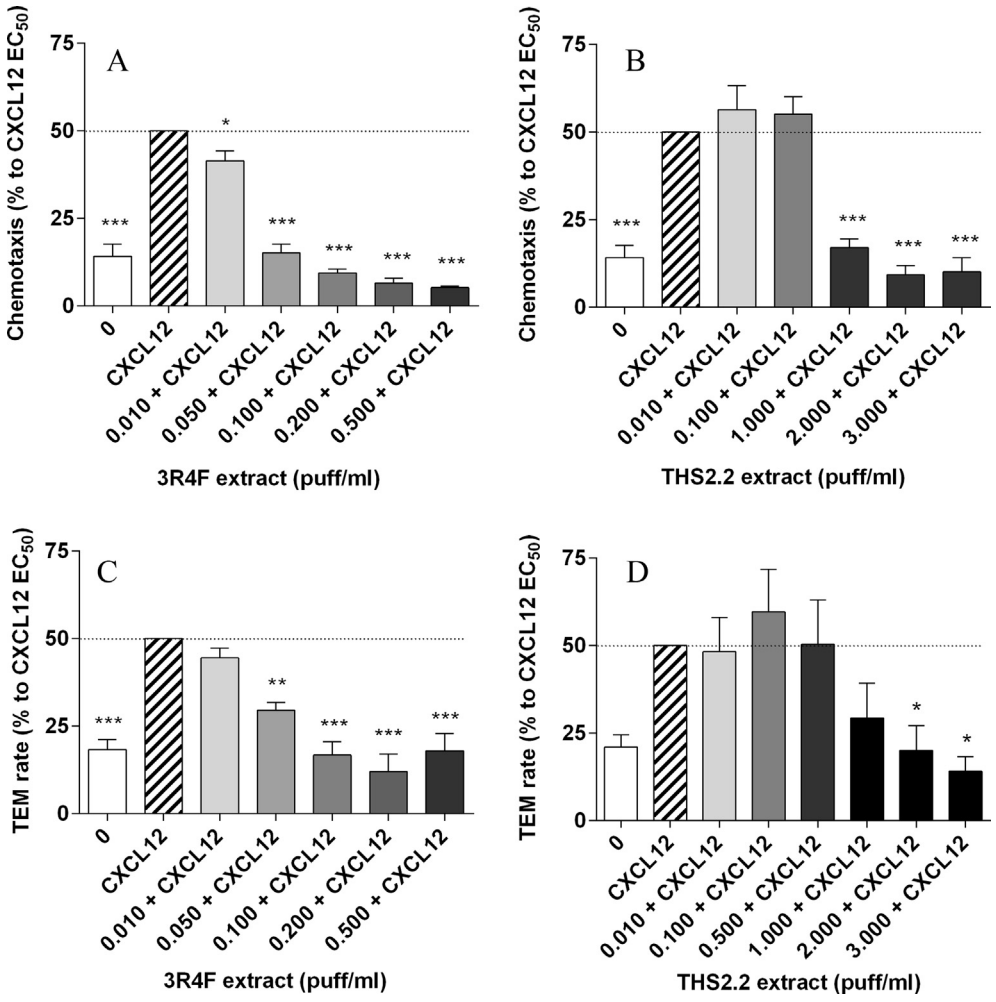


Fig. 3. Effect of extracts from 3R4F and THS2.2 on monocyte migration and real-time impedance-based TEM. A and B. Migration of THP-1 cells exposed to increasing concentrations of extract from 3R4F (A) or THS2.2 (B) in conventional Boyden chambers. C and D. Real-time impedance-based TEM of THP-1 cells exposed to increasing concentrations of extract from 3R4F (C) or THS2.2 (D) in CIM chambers. The CXCL12 concentration that induced 50% migration was used in these assays (EC_{50}). Data are expressed as the mean ± SEM of four independent experiments. *P < 0.05 vs control; **P < 0.01 vs control; ***P < 0.001 vs control by Dunnett's multiple comparison test. CIM: cell invasion/migration, THS2.2: tobacco-heating system 2.2, TEM: transendothelial migration.

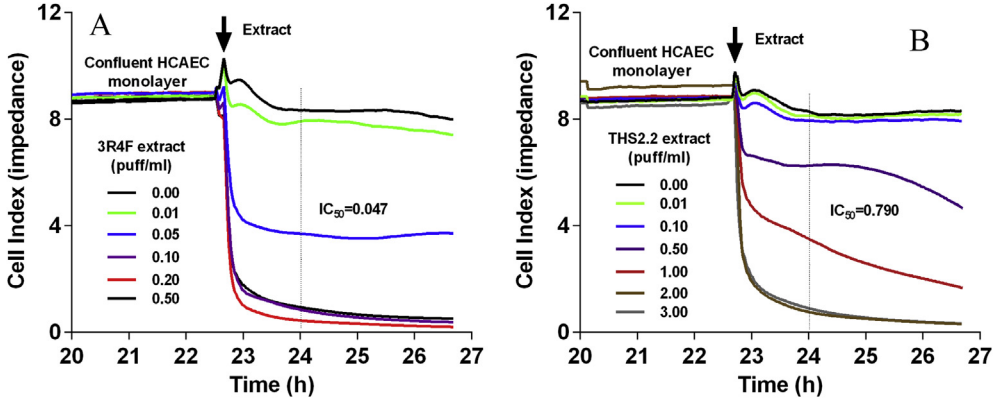


Fig. 4. Effects of extracts from 3R4F and THS2.2 on the integrity of a HCAEC monolayer. HCAECs seeded in electrical impedance plates were grown to confluency for ~24 h and then stimulated with increasing concentrations of extract from 3R4F (A) or THS2.2 (B). The concentrations required to inhibit transendothelial integrity by 50% (IC_{50}) were determined at 1 h after addition of the extracts. Representative recordings from three independent experiments are shown. *P < 0.0001 vs control by Dunnett's multiple comparison test. THS2.2: tobacco-heating system 2.2.

times more potent than the THS2.2 extract in the reduction of impedance (Fig 4A: 3R4F; IC_{50} = 0.047 and Fig 4B: THS2.2; IC_{50} = 0.790 puff/mL). These results are in line with the report by Schweitzer et al. (2011) who demonstrated that soluble constituents in extract from cigarette smoke induced dose- and time-dependent disruption of the lung endothelial barrier via a

mechanism dependent on oxidative stress. Because endothelial cells regulate vessel permeability, endothelial dysfunction may generate an additional cue to retain monocytes at inflamed/damaged tissues, thereby enhancing atherosclerotic plaque formation. More detailed studies are required to further elucidate the role of intra- and extravasation of monocytes in tobacco smoke-induced formation of atherosclerotic plaques.

4. Conclusions

Smoking cigarettes is a major risk factor for the development of cardiovascular diseases (Ambrose and Barua, 2004; Prevention, 2014). A reduction in the exposure to harmful constituents using heated rather than combusted tobacco could be a promising new approach to reduce the harmful effects associated with cigarette smoking. In this study, we demonstrated that the extract generated from a new candidate MRTP aerosol was less cytotoxic, induced less inflammation, and exerted lesser effects on chemotaxis compared with an extract from the smoke of combustible 3R4F reference cigarettes. These data indicate the potential of a heat not burn tobacco product to reduce the risk for cardiovascular diseases compared to combustible cigarettes.

Conflicts of interest

This research was funded by Philip Morris International. The authors declare that there are no conflicts of interest.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2015.09.016.

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