Introduction and Objectives

Cigarette smoke (CS) is causally linked to the development of cardiovascular disease (CVD) through different pathophysiological pathways, which include endothelial injury and dysfunction, oxidative stress, a proinflammatory status, inflammation, and an abnormal lipid profile, all contributing to the development of atherosclerosis.

Thickened coronary atherosclerosis will be demonstrated as an end result. PHRTHS is a novel tobacco product that heats tobacco instead of burning it, never allowing the temperature to exceed 350°C, thereby preventing the combustion process from taking place and producing substantially lower levels of toxicants (on average more than 90%) compared with CS. In particular, the levels of eight cardiovascular toxicants (acrylonitride, benzene, butylmethylether, hydrogen cyanide, lead, phenol, propionaldehyde) are reduced by >90% in THS aerosol versus CS.

Methods

Adhesion of Monocytes to Human Coronary Artery Endothelial Cells (HCAEC), a Critical Stage in Atherosclerosis — THS 2.2 vs. CS (In Vitro Adhesion Assay)

Cell exposure to 3R4F reference cigarette or THS 2.2 aqueous smoke/air/aerosol extract (smoke/air/aerosol-bubbled phosphate-buffered saline [PBS]) (pEPPS/pabPBS).

Conditioned and Unconditioned Media Preparation
Monocytes (MM6) cells were starved in medium (two hours) and then exposed to 3R4F reference cigarette or THS 2.2 spPBS (or PBS) for two hours. Both media were frozen.

Treatment of HCAECs
Indirect and direct treatments: 24-hour-starved HCAECs were treated with thawed conditioned and unconditioned media for four hours. Fresh direct treatment: 24-hour-starved HCAECs were exposed to freshly generated 3R4F or THS 2.2 spPBS (or PBS) for four hours. HCAECs and MM6 lysates were collected and stored at −80°C for RNA extraction.

Adhesion Assay
Untreated MM6 cells and four-hour-treated HCAECs were nuclei stained for 15 minutes and then incubated together for 45 minutes. After cell fixing and washing, remaining adherent MM6 and HCAEC cells were counted, and adhesion rate was calculated.

In Vivo Study to Investigate Atherosclerotic Plaque of the Aortic Arch

This study examined the development of the hallmark of CVD in ApoE−/− mice chronically exposed to 3R4F. THS 2.2 aerosol (matched to the nicotine concentration in 3R4F [30 μg/mL]), or bubbled for three hours per day, five days per week, for up to eight months (approximately 40% of lifetime).

After two months of exposure to 3R4F, mice were switched to THS 2.2 aerosol (switching), bubbled air (cessation), or continued exposure to 3R4F. The exposure dose corresponded to ~30 cigarettes per day in human comparison.

Results

In Vitro Model: Adhesion Assay

- 3R4F aqueous cigarette smoke extract promoted adherence of MM6 cells to HCAECs in indirect and fresh direct exposure conditions.
- At the same concentrations, no significant adhesion of MM6 cells to HCAEC was observed following THS 2.2 treatment.
- To observe similar effects as 3R4F, concentrations of THS 2.2 required a concentration increase of ~10 to 20 times.

In Vivo Model: Atherosclerotic Plaque in the Aortic Arch Data from Micro Computed Tomography (μCT) at Month 7

- THS 2.2 reduced plaque volume (mm3) and plaque surface area (mm2) compared to 3R4F and CS treated groups.

Conclusions and Discussion

The results of the THS 2 assessment program demonstrate that:

- THS 2.2 aerosol contains no CBNPs. Additionally, cardiovascular toxicants are reduced by >90%.
- The adhesion of monocytes to HCAECs in vitro is significantly reduced following THS 2.2 treatment.
- Switching to THS 2.2 halted the progression of CVD-induced atherosclerotic changes in vivo.
- In humans, all co-primary endpoints representative of different pathophysiological pathways leading to atherosclerosis shifted favorably in the same direction as the smoking cessation effect observed in the literature, after six months of switching from cigarettes to THS 2.2.

PMI has completed 17 non-clinical studies and nine clinical studies presented here. The evidence available to date indicates that switching to THS has the potential to reduce the risk of smoking-related diseases, such as CVD.

As a next step, PMI will complement its THS assessment program with cardiovascular outcome studies intended to demonstrate the clinical benefits of switching to THS (e.g., reduction in the risk of cardiovascular death, myocardial infarction, and stroke) as compared with continued smoking and help to improve primary and secondary CVD prevention in clinical practice.

References


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