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Evaluation of the Tobacco Heating System 2.2. Part 7: Systems toxicological assessment of a mentholated version revealed reduced cellular and molecular exposure effects compared with mentholated and non-mentholated cigarette smoke

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ABSTRACT

Modified risk tobacco products (MRTPs) are being developed with the aim of reducing smoking-related health risks. The Tobacco Heating System 2.2 (THS2.2) is a candidate MRTP that uses the *heat-not-burn* principle. Here, systems toxicology approaches were engaged to assess the respiratory effects of mentholated THS2.2 (THS2.2M) in a 90-day rat inhalation study (OECD test guideline 413). The standard endpoints were complemented by transcriptomics and quantitative proteomics analyses of respiratory nasal epithelium and lung tissue and by lipidomics analysis of lung tissue. The adaptive response of the respiratory nasal epithelium to conventional cigarette smoke (CS) included squamous cell metaplasia and an inflammatory response, with high correspondence between the molecular and histopathological results. In contrast to CS exposure, the adaptive tissue and molecular changes to THS2.2M aerosol exposure were much weaker and were limited mostly to the highest THS2.2M concentration in female rats. In the lung, CS exposure induced an inflammatory response, triggered cellular stress responses, and affected sphingolipid metabolism. These responses were not observed or were much lower after THS2.2M aerosol exposure. Overall, this system toxicology analysis complements and reconfirms the results from classical toxicological endpoints and further suggests potentially reduced health risks of THS2.2M.

1. Introduction

The U.S. Family Smoking Prevention and Tobacco Control Act (FSPTCA) defines a Modified Risk Tobacco Product (MRTP) as "any tobacco product that is sold or distributed for use to reduce harm or the risk of tobacco related disease associated with commercially marketed tobacco products" (Family Smoking Prevention and Tobacco Control Act). This publication is part of a series of nine publications describing the nonclinical and part of the clinical assessment of a candidate MRTP, THS2.2 regular and a mentholated version (THS2.2M). THS2.2 and THS2.2M are based on the concept that heating tobacco, rather than burning it, reduces or eliminates the formation of harmful and potentially harmful constituents in the inhaled aerosol. The series of publications provides part of the overall scientific program to assess the potential for THS2.2 to be a reduced risk product. The first publication in this series describes THS2.2 and the assessment program for MRTPs (Smith et al., submitted (this issue)). This is followed by six publications,

Abbreviations: THS2.2, Tobacco Heating System 2.2; THS2.2M, mentholated THS2.2; HPHC, harmful and potentially harmful constituent; CS, cigarette smoke; MRTP, modified risk tobacco product; FDR, false-discovery rate; iTRAQ, isobaric tag for relative and absolute quantitation; MRC, mentholated reference cigarette; LM, low menthol; HM, high menthol.

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including this one, that describe the nonclinical assessment of THS2.2 regular and THS2.2M (Kogel et al., submitted (this issue)); (Oviedo et al., submitted (this issue)); (Schaller et al., submitted (this issue)-a, submitted (this issue)-b); (Sewer et al., submitted (this issue); Wong et al., submitted (this issue)). The eighth publication in the series describes a clinical study to assess whether the reduced formation of Harmful and Potentially Harmful Constituents (HPHC) for THS2.2 regular also leads to reduced exposure to HPHCs when the product is used in a clinical setting (Haziza et al., submitted (this issue)). A final publication utilizes data gathered from the reduced exposure clinical study on THS2.2 regular to determine if a systems pharmacology approach can identify exposure response markers in peripheral blood of smokers switching to THS2.2 (Martin et al., submitted (this issue)).

In this part, the systems toxicology arm of a 90-day rat inhalation study for the assessment of THS2.2M compared with cigarette smoke (CS) from reference cigarettes is presented. The overall study was conducted according to OECD test guideline 413: the exposure characteristics, classical endpoints, and global molecular response profiles are summarized in our accompanying manuscript (Oviedo et al., submitted (this issue)).

A challenge with standard toxicological endpoints is that they are often detectable only after substantial damage has already occurred and therefore can lack sensitivity (Ellinger-Ziegelbauer et al., 2011). In addition, the limited mechanistic insights gained can hamper the refinement and eventual replacement of these traditional animal tests (OECD, 2013). Thus, (i) to increase sensitivity, (ii) to obtain additional insights into the molecular mechanisms of toxicity, and (iii) to gain knowledge that could eventually be applied to reduce the number of animal studies, we complement the standard endpoints in these studies with systems toxicology approaches – using transcriptomics, proteomics, and lipidomics measurements (Sauer et al., 2015; Sturla et al., 2014).

Briefly, in this study, groups of male and female rats were exposed for 90 days to three different concentrations of THS2.2M aerosol: 15 μg nicotine/L (low, THS2.2M(L)), 23 μg nicotine/L (medium, THS2.2M(M)), and 50 μg nicotine/L (high, THS2.2M(H)) (Fig. 1). THS2.2M aerosol exposure was compared with CS from three different reference cigarettes: the 3R4F standard reference cigarette, and corresponding low (LM) and high (HM) mentholated reference cigarettes (MRCs). Importantly, the nicotine concentration in the CS of these reference cigarettes (23 μg/L) was matched to the nicotine concentration in the aerosol of the medium THS2.2M (THS2.2M(M)). In addition, for female subsets from the exposure groups Sham, 3R4F, MRC(HM), and THS2.2M(H), the recovery of the exposure-related effects after a subsequent fresh-air exposure for 42 days was assessed (Fig. 1). For the systems toxicology analyses, respiratory nasal epithelium and lung tissue were collected and the molecular exposure responses of the transcriptome and proteome were measured. In addition, exposure effects on lung lipids were measured using lipidomics. These data were analyzed bioinformatically (Martin et al., 2014; Titz et al., 2014, 2015a) and evaluated in the context of the overall study, for example, in relationship to the histopathology results.

Altogether, our systems toxicology approach demonstrates the added benefit of these mechanistic details (e.g., on the decreased involvement of specific cell types and molecular response programs) and further supports the reduction in biological effects caused by THS2.2M aerosol exposure compared with CS from the reference cigarettes.

2. Materials and methods

2.1. Experimental design

The study was conducted to characterize potential adverse effects caused by subchronic exposure to an aerosol from the mentholated tobacco heating system THS2.2M, a *heat-not-burn* tobacco product, and to compare them with the effects induced by CS generated from three reference cigarettes (Fig. 1) (Oviedo et al., submitted (this issue)). In addition to the recommendations in OECD TG413 for study designs, additional rats were included for the purpose of obtaining 'omics' endpoints. These rats were subjected to the same exposure conditions and handling times as the rats in the OECD study. As required, three dose levels of the THS2.2M aerosols were tested. Respiratory nasal epithelium and lung tissue were collected from the exposed rats and after a recovery period of 42 days, and used for the generation of transcriptomic, proteomics, and lipidomics data.

2.2. Reference cigarettes and THS2.2M

Standard reference 3R4F cigarettes were purchased from the University of Kentucky (Lexington, KY, USA, http://www.ca.uky.edu/refcig). In addition, two MRCs were produced by Philip Morris International, Neuchatel. The MRCs were designed so that the nicotine, total particulate matter, and CO levels in the smoke matched the levels of the 3R4F reference cigarette. The menthol yield in the smoke from the MRCs was 2.04 mg/cig (MRC(LM)) and 2.58 mg/cig (MRC(HM)) when smoked according to the ISO 3308

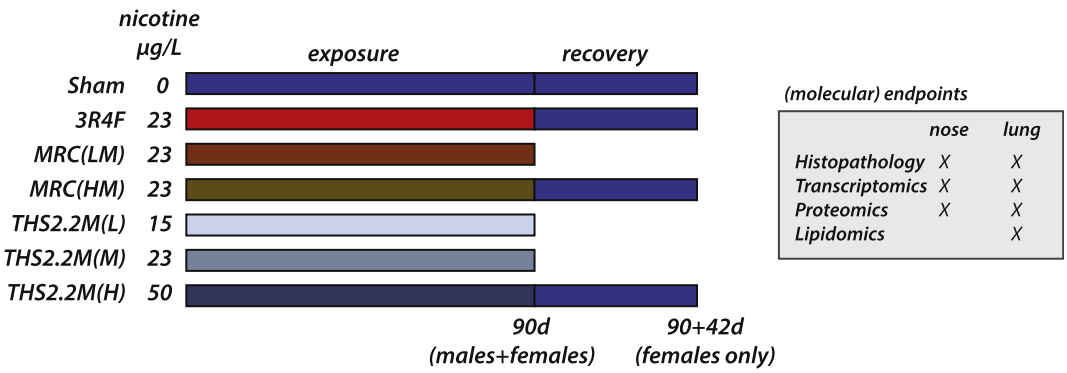

Fig. 1. Design of the 90-day systems toxicology study to assess effects of CS and THS2.2M exposures on rat respiratory organs. Groups of six male and six female rats were exposed for 90 days to fresh air (Sham) or cigarette smoke of three reference cigarettes: a standard reference cigarette (3R4F), and a low menthol (MRC(LM)) and high menthol (MRC(HM)) reference cigarette (all at 23 μg nicotine/L). In addition, groups of rats were exposed to aerosol from THS2.2M (15, 23, and 50 μg/L nicotine). The 90-day exposure period was followed by a 42-day recovery period with rats exposed to fresh air (indicated groups and female rats only). Rats were exposed for 6 h per day, for 5 days per week. Respiratory nasal epithelium and lung tissue samples were collected for proteomics, transcriptomics, and histopathology. Lipidomics analysis was conducted for the lung tissue samples.

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