Lung Inflammation, Emphysema, and Lung Cancer Development in A/J mice in Response to Chronic Exposure to Aerosol from a Candidate Modified Risk Tobacco Product and Mainstream Cigarette Smoke

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Abstract

Objective: Chronic exposure to cigarette smoke (CS) is the leading cause of chronic obstructive pulmonary disease and lung cancer. The A/J mouse model was used to evaluate lung inflammation, emphysema, and lung tumor incidence and multiplicity upon lifetime exposure to CS from the 3R4F reference cigarette or to aerosol from the Tobacco Heating System (THS) 2.2, a candidate modified risk tobacco product, at three concentrations.

Methods: A/J mice were exposed for six hours per day for five days per week for up to 18 months. Quantification of pulmonary inflammation, lung function tests, lung morphometric assessments by stereological approach and histopathological evaluation of the lungs were performed at selected interim and terminal dissections.

Results: Exposure to CS resulted in pulmonary inflammation, altered lung function, enlargement of distal airspaces in the lungs, all indicative of emphysema. Only minimal effects on the lungs were observed following THS 2.2 aerosol exposure that were independent of THS 2.2 aerosol concentrations and exposure duration. Exposure to CS resulted in increased incidence and multiplicity of lung tumors (adenoma and carcinoma), but not in THS2.2 groups compared to the Sham group.

Conclusions: In summary, lung inflammation, emphysematous changes, and lung tumor incidence and multiplicity were significantly lower in mice exposed to aerosol from THS 2.2 as compared with CS exposure.

Study Design and Endpoints

Female A/J mice were exposed to filtered air (Sham), to three concentrations of THS2.2 aerosol (6.6, 13.4, 26.8 µg/L nicotine) and one concentration of 3R4F CS (13.4ug/L nicotine; Figure 1). Additional male mice were exposed to Sham and THS2.2 aerosol (26.8 µg/L nicotine). Care and use of the mice was in accordance with the National Advisory Committee for Laboratory Animal Research Guideline 2004 . All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC). Interim dissections were performed after 1, 5 and 10 months of exposure for female mice. Terminal dissections were performed at months 15 and 18 for the male and female mice respectively. At selected time points, animals were allocated for the following endpoints: bronchoalveolar lavage fluid (BALF) analysis by FACS, and multi-analyte (cytokines/chemokines) profiling; histopathological evaluation of the lungs; lung function tests; lung morphometry; lung tumor analysis and an extensive molecular high-throughput analysis (transcriptomics, proteomics).

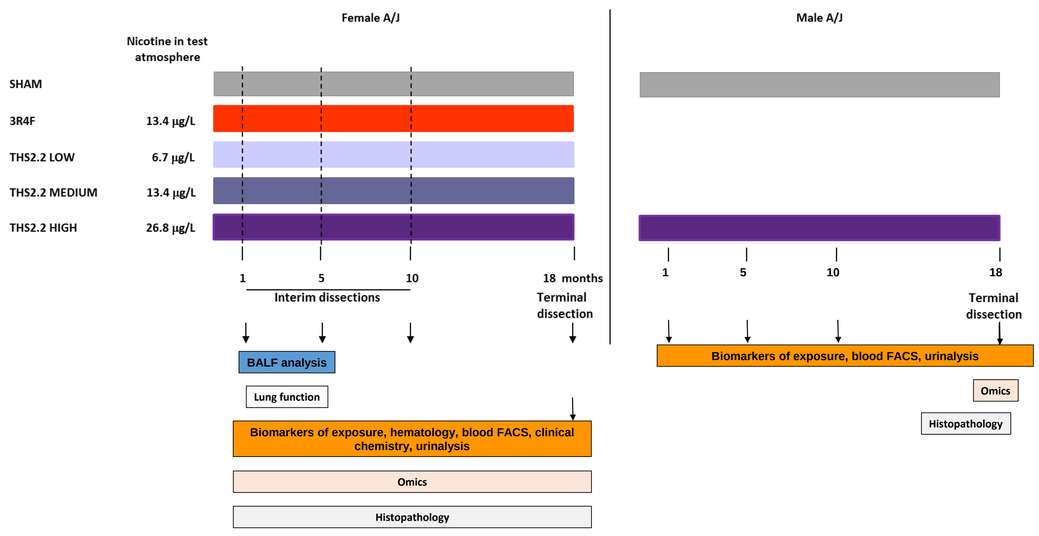


Figure 1. Schematic overview of study design, dissection time points and study endpoints.

Aerosol Exposure

The daily monitoring of aerosol components indicated that the aerosol/CS was generated and delivered to the inhalation chambers in a consistent manner, with mean nicotine test atmosphere concentrations very close to the target concentrations. Aerosol generated from THS2.2 sticks contained lower concentration of TPM, CO, and carbonyls compared to 3R4F CS at the same nicotine concentration.

Chamber name	Sham_F	3R4F_F	THS2.2 Low_F	THS2.2 Med_F	THS2.2 High_F	Sham_M	THS2.2 High_M
Chamber no.	1	2	3	4	5	6	7
Target nicotine concentration (µg/L)	0	13.4	6.7	13.4	26.8	0	26.8
Achieved nicotine concentration (µg/L)	0.0 ± 0.0 (92)	13.0 ± 1.4 (397)	6.7 ± 0.8 (397)	13.0 ± 1.3 (397)	26.7 ± 2.0 (397)	0.0 ± 0.0 (82)	26.1 ± 2.8 (332)
Achieved TPM concentration (µg/L)	−2.3 ± 1.7 (397)	295.7 ± 19.0 (397)	61.4 ± 6.4 (397)	138.7 ± 16.8 (397)	272.4 ± 27.2 (397)	−2.3 ± 2.0 (335)	291.0 ± 41.2 (334)
Achieved CO concentration (ppm)	0.2 ± 0.5 (391)	389.3 ± 26.3 (394)	3.6 ± 0.5 (390)	6.4 ± 1.2 (392)	11.0 ± 1.4 (373)	0.5 ± 0.3 (330)	12.9 ± 1.7 (332)
Achieved formaldehyde concentration (µg/L)	NA	0.40 ± 0.06 (80)	0.04 ± 0.01 (80)	0.06 ± 0.02 (79)	0.10 ± 0.02 (80)	NA	0.09 ± 0.02 (67)

Table 1: Test atmosphere characterization. *Data are presented as mean ± SD. The number of daily average measurements are shown in parentheses. F, female; M, male; TPM, total particulate matter; CO, carbon monoxide.*

Results

Biomarkers of Exposure

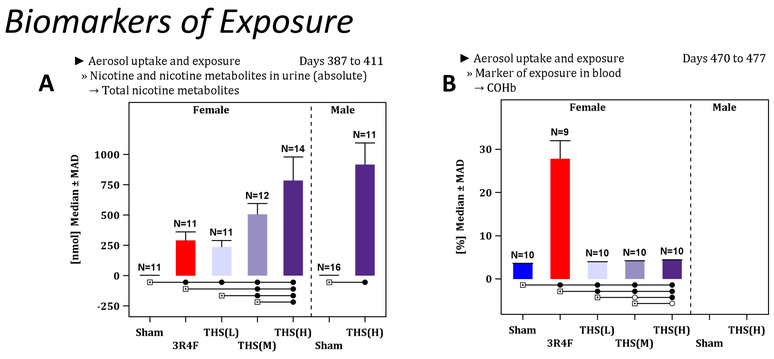
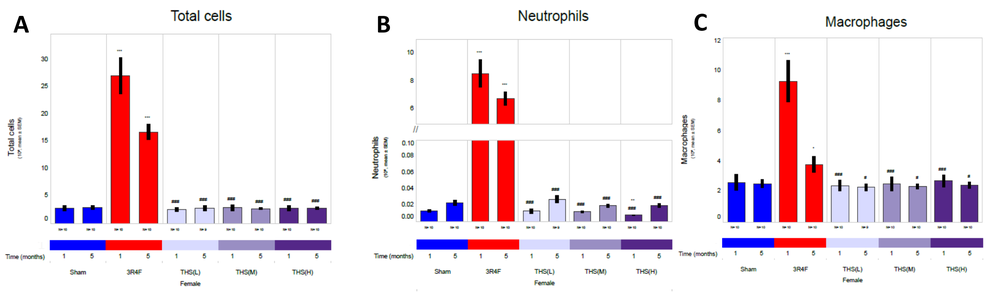


Figure 2. (A) Total urinary nicotine metabolites in 24-hour urine following 13 months of exposure. (B) Blood carboxyhemoglobin levels following 16 months (female mice) of exposure. in 24-hour urine following 13 months of exposure. *Data are presented as median ± MAD. Lines indicate statistical group comparison (t-test) where p-values fell below 0.05 (not adjusted).*

Lung Inflammation



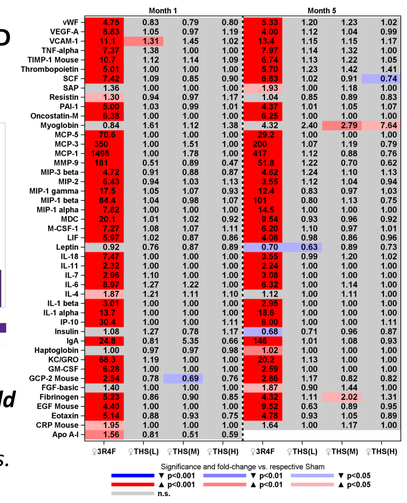


Figure 3. (A) Total BALF free lung cells, (B) Neutrophils in BALF, (C) macrophages in BALF, (D) Fold changes in the levels of BALF inflammatory mediators following 1 and 5 months of exposure. *Data presented as means ± SEM or fold changes. Statistical significant differences: ***:p<0.001 vs. Sham, #: p<0.05, ##: p<0.01, and ###:p<0.001 vs. 3R4F (t-tests).*

Pulmonary emphysema

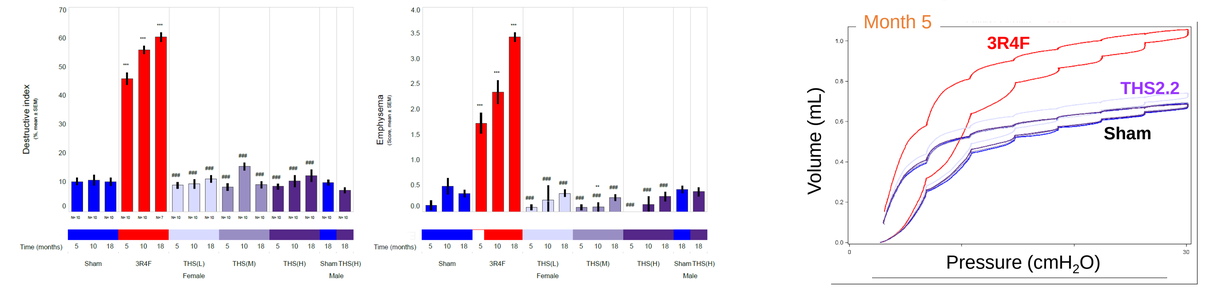


Figure 4: Morphometry, histopathology and physiologic parameters of emphysema. (A) Destructive index. (B) histopathology scores for lung emphysema. (C) Pressure-volume curves from lung function measurements at months 5. *Data are presented as mean ± SEM. Statistical significant differences: *:p<0.05, **: p<0.01, and ***:p<0.001 vs. Sham, #: p<0.05, ##: p<0.01, and ###:p<0.001 vs. 3R4F*

Lung tumors

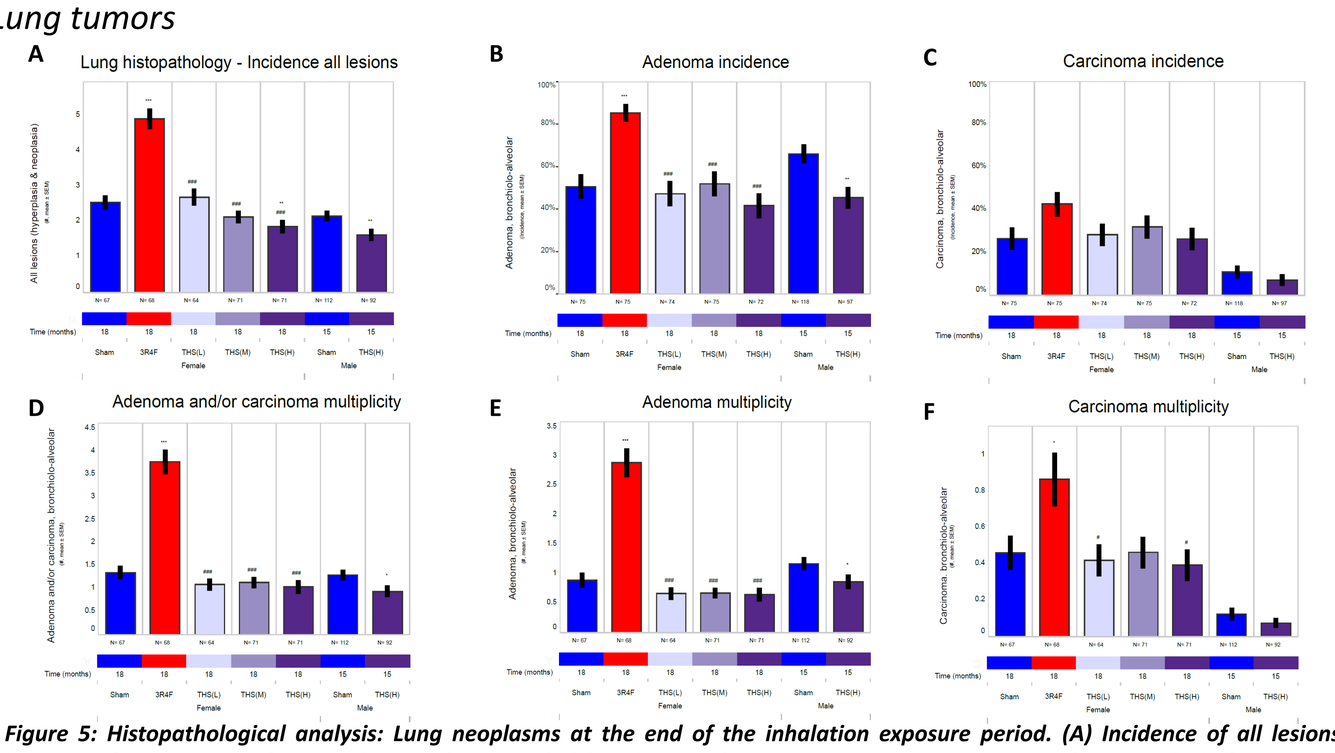


Figure 5: Histopathological analysis: Lung neoplasms at the end of the inhalation exposure period. (A) Incidence of all lesions (hyperplasia and neoplasia) and (D) combined multiplicity of bronchiolo-alveolar adenomas and carcinomas. (B) Incidence and multiplicity (E) of bronchiolo-alveolar adenomas. (C) Incidence and (F) multiplicity of bronchiolo-alveolar carcinomas. *Statistical significant differences: *:p<0.05, **: p<0.01, and ***:p<0.001 vs. Sham, #: p<0.05, ##: p<0.01, and ###:p<0.001 vs. 3R4F (t-tests, mortality adjusted). N=64-75 (female), N=92-118 (male)*

Summary and Conclusions

- The characterization of the **aerosol components** as well as **biomarkers of aerosol uptake** indicated that the delivery and uptake of 3R4F CS and THS2.2 aerosol are in-line with expectations.
- Exposure to CS resulted in increased **pulmonary inflammation**, altered **lung function** and histopathological changes in the lungs that are suggestive of **emphysema**. No significant lung effects were observed following THS2.2 aerosol exposure.
- CS exposure resulted in an increased incidence and multiplicity of lung **pre-neoplastic and neoplastic lesions**. Exposure to THS2.2 did not result in increased pre-neoplastic and neoplastic lesions.
- **THS2.2 aerosol does not cause lung inflammation, emphysema, and neoplastic lesions**, even at twice the test atmosphere nicotine concentration

PMI SCIENCE PHILIP MORRIS INTERNATIONAL **Declaration of interest: Philip Morris International is the sole source of funding and sponsor of this project.** **X Congresso Franco Brasileiro de Oncologia 2018, Rio de Janeiro. 19-22 Sep. 2018**