Attenuation of lung inflammation and emphysema-like change following cigarette smoking cessation or switching to aerosol inhalation from a NTV

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Abstract

Mainstream cigarette smoke (MCS) is the one of the risk factors for the development and progression of respiratory diseases, such as chronic obstructive pulmonary disease (COPD). Novel tobacco vapor product (NTV) (Fig.1) , which is consisted of a battery, a cartridge with a heater and nicotine-free liquid, and a capsule filled with tobacco. Previous study[1] found that the vapor from NTV showed distinctively lower yields of potentially harmful constituents compared with MCS.

In this study, we used a murine model of COPD which we previously established[2], to investigate the effects of chronic exposure to vapor from NTV or MCS from 3R4F reference cigarettes (3R4F), and the effects of cessation and switching to NTV after 2 months of MCS exposure. The effects of chronic exposure (3R4F and NTV), cessation and switching to NTV were investigated for emphysema-related changes as well as standard toxicological endpoints.

MCS from 3R4F induced lung inflammation, proteolytic enzyme activities associated with alveolar destruction in bronchoalveolar lavage fluid (BALF), and emphysema-like changes (perturbation of lung function and alveolar destruction). Whereas exposure to vapor from NTV did not induce these effects. The effect of vapor from NTV on biological processes was very similar to that of filtered air (FA). Both cessation and switching to NTV attenuated the effect of 3R4F to levels similar to FA.

Materials and Methods

The study was conducted in a contract laboratory accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). General procedures for animal care and housing complied with the "Guide for Care and Use of Laboratory Animals and Public Health Service Policy on Humane Care and use of Laboratory Animals" (National Research Council, 1996). The protocol of the study was approved by the Committee for Ethics in Animal Studies of the test facility prior to commencing the study.

Female C57BL/6J mice were exposed to MCS from 3R4F (700 µg total particulate matter (TPM)/L), filtered air (FA) or vapor from NTV (700 µg TPM/L) for up to six months under whole-body exposure. In other groups, after two months of MCS exposure, mice were exposed to FA (cessation) or NTV (switching) for four months(Fig.2). Interim dissections were performed after two months of exposure in FA, 3R4F and NTV groups. Terminal dissections were performed at six months in all groups. After completion of each exposure, mice were allocated to groups for standard toxicological endpoints and emphysema-related endpoints; differential white blood cell count , proteolytic enzyme activity, multi-analyte assay (cytokines/chemokines) in BALF and lung function.

All groups underwent intermittent exposure (i.e., 4-hour daily exposure arranged as two 2-hour periods with a 30-minute break of FA exposure) (Fig.3). MCS from 3R4F and vapor from NTV were generated using our customized smoking machine, SG-1000 (Borgwaldt KC GmbH).

Fig.1. Novel Tobacco Vapor Product

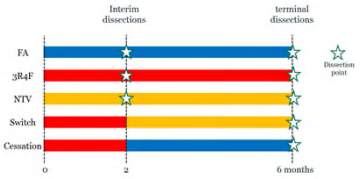

Fig. 2. Schematic overview of the study design. *FA, 3R4F and NTV groups were exposed to each test article up to six months. Switch group was exposed to vapor from NTV for four months after two months of MCS from 3R4F exposure. Cessation group was exposed to FA for four months after two months of MCS from 3R4F exposure.*

Fig. 3. Schematic overview of intermittent exposure. Four hour daily exposure arranged as two two-hours periods with a 30-minute break of FA exposure

Results

Parameter	FA	NTV	3R4F
TPM, [µg/L]	0.6 ± 0.7	689 ± 33	720 ± 20
Carbon monoxide, [ppm]	0.0 ± 0.0	0.1 ± 0.4	890 ± 15
MMAD, [µm] ± GSD	-	0.96 ± 1.60	0.95 ± 1.45
Nicotine, [µg/L]	N.D.	3.1 ± 0.5	42 ± 3.2
Plopylene Glycol, [µg/L]	N.D.	176 ± 19	N.D.
Glycol, [µg/L]	N.D.	347 ± 9.1	71 ± 2.7
Acrolein, [µg/L]	N.D.	N.D.	4.9 ± 0.1
Acetaldehyde, [µg/L]	N.D.	N.D.	49.3 ± 2.1

Table 1. Test atmosphere characterization. TPM(every exposure block) and CO(continuously) were monitored daily. Nicotine concentrations in the test atmosphere were measured once a week. Particle size distribution was measured every other week. Data are presented as mean ± SD.

MSC/NTV vapor were generated by a smoking machine (SG-1000) and delivered to a whole-body exposure chamber (H-1000). Each component of test atmosphere was stable throughout the study. Constituents (CO, nicotine, glycol and carbonyls) in the vapor from NTV were lower compared with that in MCS from 3R4F at the same TPM concentration.

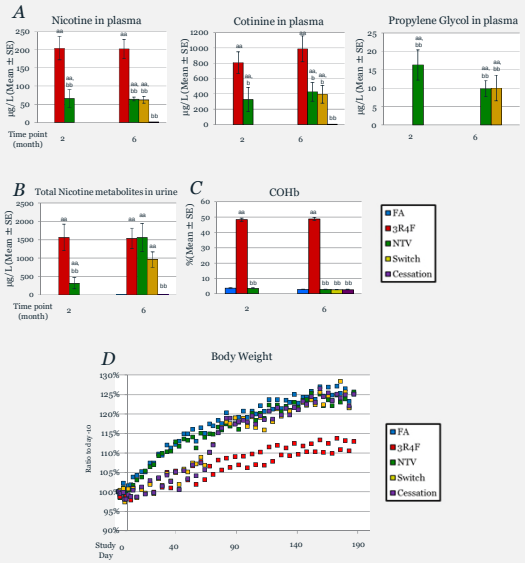

Fig. 4. Biomonitoring data. *(A) Nicotine, cotinine and propylene glycol concentrations in the plasma, and (B) total nicotine metabolites in urine were measured by LC-MS/MS. (C) COHb was measured with a gas auto-analyzer. Data are presented as mean ±SE, n=10 animals in each group. [a]p<0.05, [aa]p<0.01 vs. FA, [b]p<0.05, [bb]p<0.01 vs. 3R4F (D) Body weight was measured twice weekly. Data are presented as mean, percent of body weight at the start of acclimatizing exposure date (day -10).*

Changes of exposure markers (Nicotine, cotinine, propylene glycol and COHb in blood, and total nicotine metabolites in urine) indicated that mice inhaled MCS from 3R4F and vapor from NTV (A - C). Body weight was increased in all groups throughout the study. However, decreased weight gain was observed for 3R4F compared with FA. However, following cessation or switching to NTV, body weight was rapidly gained to reach a level similar to that of FA or NTV groups (D).

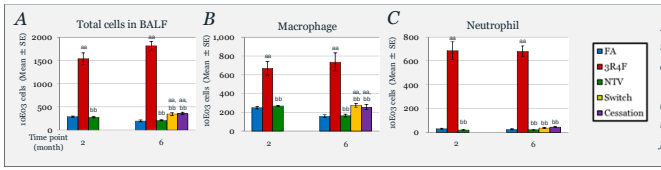

Fig. 5 .Cell differentiation in BALF. *Free lung cell differentiation in BALF was measured by XT-2000i (Sysmex) . Data are presented as mean ±SE, n=10 animals in each group. [a]p<0.05, [aa]p<0.01 vs. FA, [b]p<0.05, [bb]p<0.01 vs. 3R4F .*

MCS from 3R4F exposure recruited free lung cells into BALF (A), such as macrophages (B), neutrophils (C) and lymphocytes (data not shown). Cell recruitment was attenuated to the level of FA group following cessation or switching to NTV. In contrast to MCS from 3R4F exposure, vapor from NTV had no effect on free lung cells.

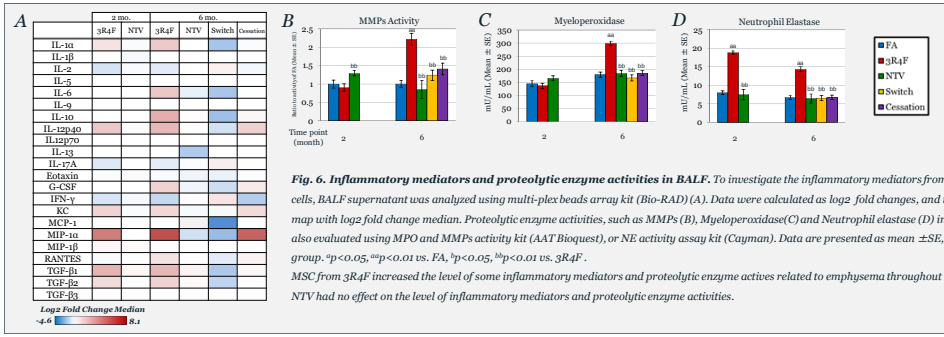

Fig. 6. Inflammatory mediators and proteolytic enzyme activities in BALF. *To investigate the inflammatory mediators from lung tissue and immune cells, BALF supernatant was analyzed using multi-plex beads array kit (Bio-RAD) (A). Data were calculated as log2 fold changes, and then presented as a heat map with log2 fold change median. Proteolytic enzyme activities, such as MMPs (B), Myeloperoxidase(C) and Neutrophil elastase (D) in BALF supernatant were also evaluated using MPO and MMPs activity kit (AAT Bioquest), or NE activity assay kit (Cayman). Data are presented as mean ±SE, n=10 animals in each group. [a]p<0.05, [aa]p<0.01 vs. FA, [b]p<0.05, [bb]p<0.01 vs. 3R4F .*

MSC from 3R4F increased the level of some inflammatory mediators and proteolytic enzyme actives related to emphysema throughout the study. Vapor from NTV had no effect on the level of inflammatory mediators and proteolytic enzyme activities.

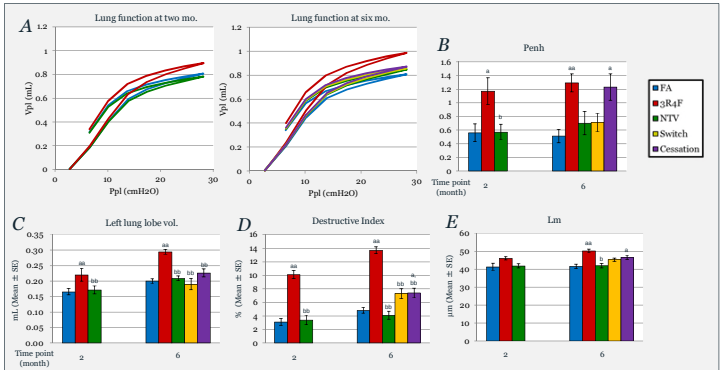

Fig. 7. Emphysema-related endpoints. *Lung function was measured using the flexiVent system. Pressure-volume loops data at two and six months are shown (A). In-life respiratory physiological data were measured using whole-body plethysmograph at the next day of exposure (without MSC from 3R4F and vapor from NTV) (B). Lung volume was measured according to the fluid displacement method (C) and the Cavalieri principle (data not shown). Destructive Index (D) and chord length of alveoli (Lm) (E) were quantitated and calculated using Stereo Investigator under a stereological approach. Data are presented as mean ±SE, n=10 animals in each group. [a]p<0.05, [aa]p<0.01 vs. FA, [b]p<0.05, [bb]p<0.01 vs. 3R4F . Emphysematous changes were observed by MCS from 3R4F exposure, and these were attenuated to levels similar to FA or vapor from NTV following cessation or switching to NTV .*

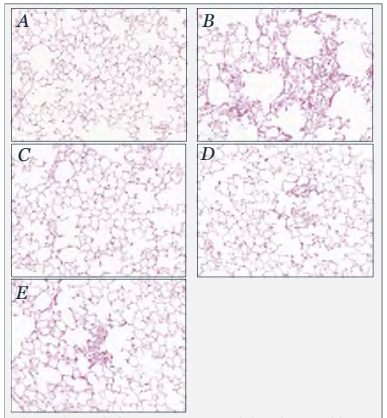

Fig. 8. Histopathology. *Representative pathological images of the FA group (A), 3R4F group (B), NTV group(C), Switch group (D) and Cessation group (E). The left lung lobe was fixed by EGAFS solution and embedded with paraffin. All paraffin blocks were sectioned to 5µm thickness, and stained with hematoxylin and eosin.*

Summary and Conclusion

- ✓ Concentrations of test atmosphere constituents in each whole-body exposure chamber and exposure markers in the plasma or urine indicated that MSC from 3R4F and vapor from NTV were well delivered by the whole-body exposure system.
- ✓ Exposure to MCS from 3R4F recruited inflammatory cells to BALF, increased proteolytic enzyme activities in BALF, and altered lung functions related to emphysematous changes, whereas exposure to vapor from NTV did not induce these effects at any time points examined. The effect of vapor from NTV on the biological processes was very similar to those of FA.
- ✓ Both cessation and switching to NTV attenuated the effect of MCS from 3R4F to levels similar to those for FA.

These data clearly show that vapor from NTV has little impact on the biological processes (inflammatory responses, emphysema-like changes, and impaired alveolar damage), compared with MCS from 3R4F in a murine model of COPD we have established. Furthermore, it suggests that the effects of switching to NTV on these processes might be similar to those of cessation of cigarette smoking.

References

[1] Takahashi Y et al. (2018) Chemical analysis and in vitro toxicological evaluation of aerosol from a novel tobacco vapor product: A comparison with cigarette smoke. Regul Toxicol Pharmacol. 92:94-103.
[2] Suzuki H et al. (2018) 6-months inhalation study to investigate the cigarette smoke induced emphysema-like changes and the effect of smoking cessation in mice. in poster, *SOT*