Lung sphingolipidome in a mouse model of cigarette smoke-induced emphysema

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Abstract

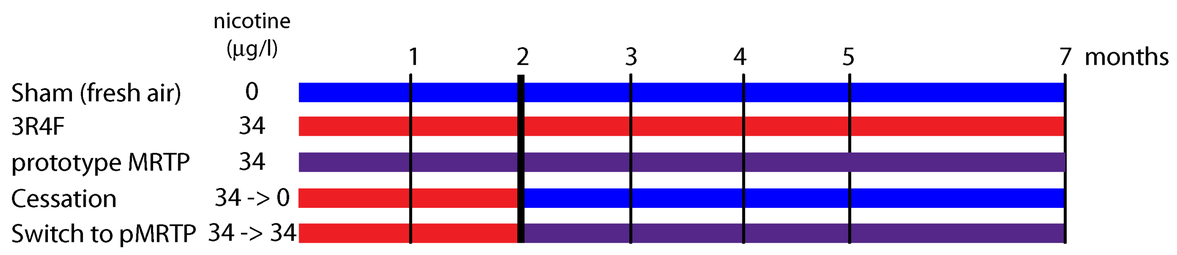
Background. Cigarette smoking is the primary cause of chronic obstructive pulmonary disease (COPD). Sphingolipids have been shown to play important roles in the pathobiology of lung diseases with the effects of ceramide on emphysematous changes amongst the best documented. In addition, sphingosine-1-phosphate (S1P) and S1P receptors have also been characterized as mediators in the pathobiology of fibrosis and acute lung injury.
Aim. Using state-of-the-art lipidomics supplemented with physiological and molecular endpoints, we assessed the biological responses in the lungs of C57BL/6 mice switching to a prototype Modified Risk Tobacco Product (pMRTP) compared to smoking cessation after an initial exposure to 3R4F reference cigarette smoke (CS).
Methods: Detailed lipidomics profiles were obtained from the lungs of C57BL/6 mice, a rodent model susceptible to emphysema. Mice were exposed to mainstream CS, pMRTP aerosol, or to fresh air (sham) for up to seven months, or to pMRTP (switch) or fresh air (cessation) for up to five months after an initial CS exposure period of two months. Molecular and histopathological parameters were also investigated to characterize the lung inflammatory response and emphysema at different time points.
Results. The findings of the present study link CS-induced pathophysiological changes to alterations in lung lipid metabolism, with major impact on the sphingolipidome. Long chain ceramides, gangliosides, and sphingosine were all affected by CS, with increasing effect over time. Levels of these lipids in mice switching to a pMRTP or to fresh air (cessation) rapidly returned to those of fresh air-exposed mice.
Conclusion. A switch to the pMRTP aerosol after 2 months of cigarette smoke exposure tracked closely with the cessation exposure group in all assessed parameters.

Study design

Animals:
- C57Bl/6 mice, 8 – 10 weeks old females randomly allocated to one of five exposure groups just prior to the exposure start.

Exposure

- Whole-body exposure chambers
- 3R4F reference cigarette, University of Kentucky, (http//www.ca.uky.edu/refcig)– Health Canada Intense Puffing Regime
- Target concentration (3R4F, and pMRTP) – 34.4 µg/l nicotine (3R4F equivalent to 750 µg/l TPM), 4 hours per day, 5 days per week, for up to 7 months total exposure time.
- Switching/cessation – After 2 months exposure to 3R4F reference cigarette smoke, the animals were subsequently exposed to fresh air (cessation) or to the aerosol from a pMRTP (switch) for up to 5 additional months.
- This exposure regime was selected as similar exposure conditions have resulted in the progression of emphysematous changes to the lungs in C57Bl/6 mice [(1)]



Results: Cigarette smoke-induced emphysema

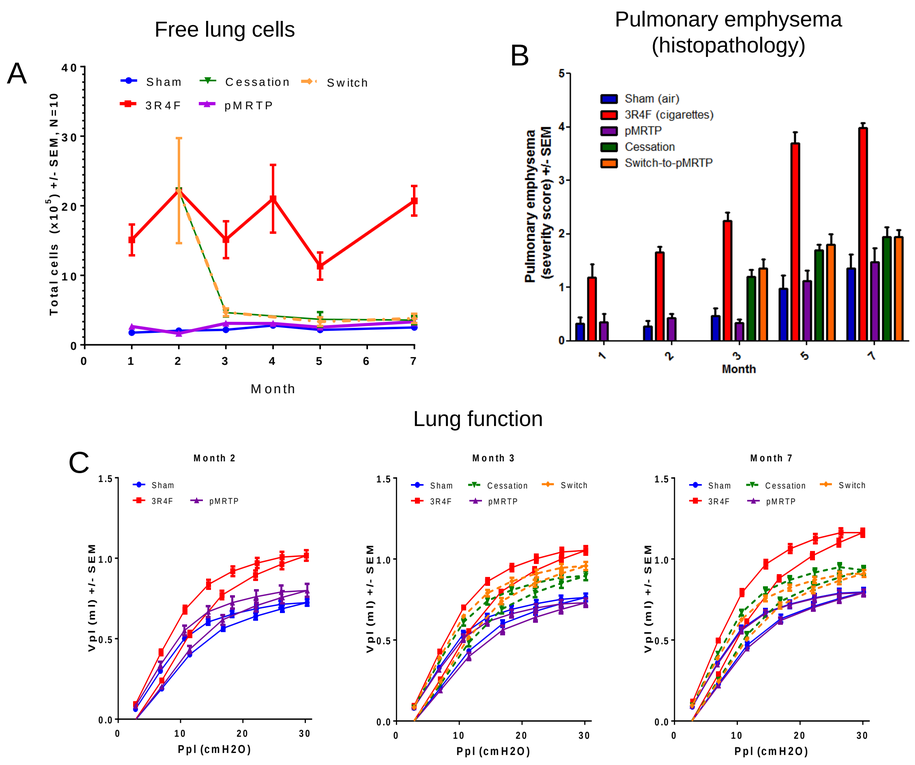


Figure 1. Cigarette smoke exposure resulted in the following biological changes consistent with the development of emphysema:
A) Infiltration of inflammatory cells in the lungs. After 1 month exposure, the total free lung cell count was significantly increased in cigarette-exposed groups. These levels reverted to the sham levels within 3 months of either cessation of switching.
B) Histopathological assessment and determination of emphysema. The overall score for emphysema increased in a time-dependent manner in response to cigarette smoke, compared to sham- or MRTP-exposed animals (A and C). After cessation or switching to an pMRTP aerosol, the emphysema score was lower towards the sham- or pMRTP-exposed animal levels.
C) Pulmonary function. The upward-shifting PV loops with cigarette smoke-exposure was clearly evident after 2 months, then remaining stable up to month 7. Exposure with pMRTP resulted in PV loops approaching those of the sham-exposed animals. Cessation or switch to a pMRTP aerosol resulted in a rapid change within 1 month, to a profile falling between the cigarette smoke and sham group PV loop profile. The PV loops of both the cessation of switching groups then remained stable between the sham and 3R4F even after 5 months post-CS exposure.

Results: Sphingolipid profiles in response to chronic cigarette or pMRTP aerosol exposure, cessation or switching-to-pMRTP

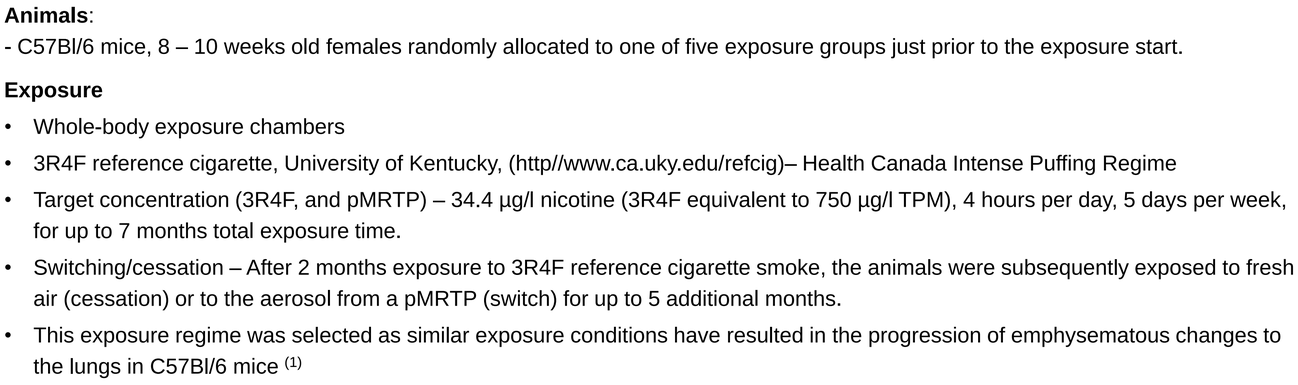


Figure 2. Changes in the sphingolipid profile in the right lungs of mice exposed to 3R4F and pMRTP aerosols. For each lipid class, the data depict concentration changes compared with sham in lung samples after 2, 3, or 7 months of exposure. Exposure to 3R4F resulted in clearly distinguishable lung lipid profiles compared with sham exposure. These changes were alleviated by smoking cessation or switching to pMRTP within one month post-3R4F exposure, and reached control levels at the 7-month time point. Exposure to 3R4F increased the levels of several sphingolipids, including ceramides, cerebrosides, sphingosines, and sphinganines.

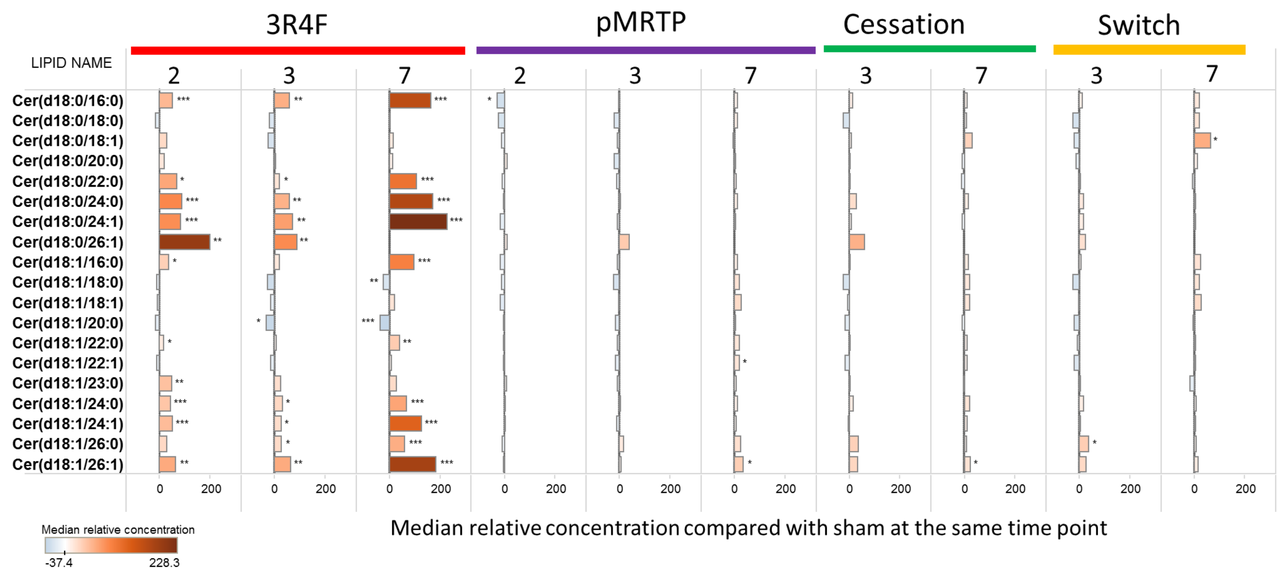


Figure 3. Changes in ceramide species profile in the right lungs of mice exposed to 3R4F and pMRTP aerosols. The specific upregulation of long-chain ceramides (C22-26) as well as palmitic acid (16:0) containing molecular sphingolipid species was observed after just 2 months of 3R4F exposure. pMRTP exposure resulted only in minor changes in the lung lipid profile. Similarly, cessation or switch-to-pMRTP resulted to a return to near sham exposure ceramide levels within 1 month post 3R4F exposure.

References

1. March et al., *Modulators of cigarette smoke-induced pulmonary emphysema in A/J mice*. Toxicological Sciences, 2006. 92(2): p. 545-559.

Conclusions

1. We quantified the infiltration of inflammatory cells into the lung, and assessed functional and histopathological signs of emphysema in a model of smoke-induced emphysema in C57Bl/6 mice. The sphingolipidome of the lungs of the mice was analysed in the context of this model.
2. Cigarette smoke, but not pMRTP exposure, resulted in changes in the levels of multiple sphingolipids in the lung.
3. Following two months of 3R4F cigarette smoke exposure, both cessation and switch-to-pMRTP resulted in the rapid return (within 1 month) toward the lipid profile seen following sham exposure.
4. When exposed solely to a pMRTP aerosol, the lungs had a very similar sphingolipid profile to that seen following sham exposure.
5. This model may be an excellent platform to examine efficacy of smoking cessation as a benchmark for pMRTP exposure.

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