A mechanistic study of cigarette smoke-induced COPD and cessation effects in C57BL/6 mice

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Introduction

Chronic obstructive pulmonary disease (COPD) is defined by the WHO as a lung disease that is characterized by chronic obstruction of lung airflow that interferes with normal breathing and is associated with narrowing of the small airways, chronic bronchitis, and the development of alveolar emphysema. In developed countries cigarette smoking is the main etiological factor in the pathogenesis of COPD. However, the underlying pathogenesis of the disease is not fully understood. Identification of a murine model is a prerequisite for the mechanistic study of smoking-induced COPD. Among various mouse models for the study of experimental emphysema/COPD, it has been shown that the C57BL/6 mouse is a useful model for cigarette smoking-induced COPD studies [1,2] .

This study analyzed the progression of emphysema over a 7-month exposure period (4 hours per day) to cigarette smoke (CS, 750 µg/l total particle matter (TPM) from 3R4F cigarettes) or to 2 months CS followed by 1, 3, or 5 months cessation (fresh air) in female C57BL/6 mice. A battery of markers of disease progression were investigated, focusing on lung inflammation (cell infiltration, lung cytokines), pulmonary function, and histopathological and morphometric measurement of emphysematous changes to the lung tissue.

Materials and Methods

Animals:

- Female (chosen because they have half the serum antitrypsin levels of males [3, 4]) C57BL/6 mice 8 – 10 weeks old weeks old.
- The animals were randomly allocated to the following 3 exposure groups just prior to the start of the exposure:

1) Sham – fresh air control group

2) 3R4F Reference cigarette, University of Kentucky (for specifications see University of Kentucky, http//www.ca.uky.edu/refcig) – Cigarette smoke

3) Cessation – 2 months cigarette smoke followed by up to 5 months fresh air

Exposure

- Whole-body exposure chambers
- 3R4F – Health Canada Intense Puffing Regime
- Target concentration – 750 µg/l TPM, 4 hours per day, 5 days per week
- This exposure regime was selected as similar exposure conditions have resulted in the progression of emphysematous changes to the lungs in C57Bl/6 mice [5]

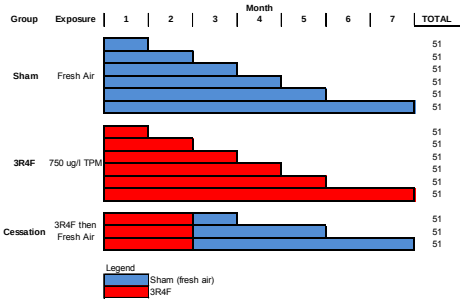


Figure 1. Study design with groups, and total animals per group, with 750 µg/l TPM exposure target concentration in the cigarette smoke groups, the total animals per exposure group in the 'total' column.

Results

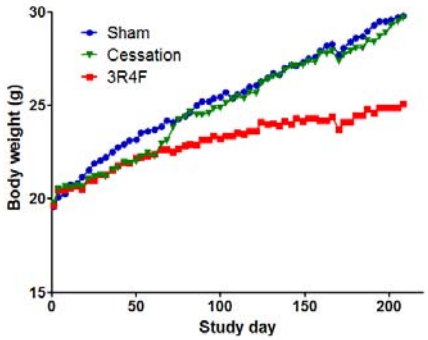


Figure 2. Body weight progression during the exposure period. CS exposure resulted in a lower rate of body weight increases relative to fresh air (sham)-exposed animals, with differences seen from 20 days after exposure start. Following a switch from cigarette smoke to fresh air (Cessation), the body weight gain was rapid, and reached the body weight of sham animals within approximately 2 weeks.

Results and Endpoints

Bronchoalveolar lavage fluid (BALF)

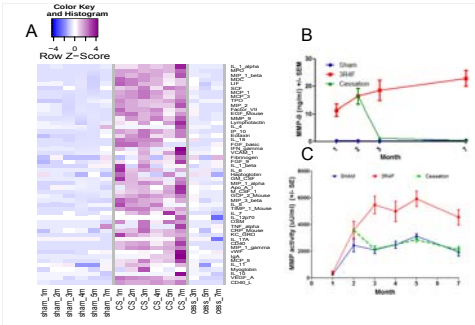


Figure 3. There was a general increase, to various degrees, of the levels of all mediators measured in the BALF obtained from animals after CS exposure relative to the sham-exposed mice (A). However, even after 1 month of cessation, the levels returned to a pattern resembling that of the Sham-exposed mice. This is highlighted by MMP-9 protein levels (B), which increased after CS exposure, but rapidly returned back to the levels of sham after cessation. The protein levels were mirrored by enzymatic proteolytic (MMP) activity levels (C).

Free lung cells

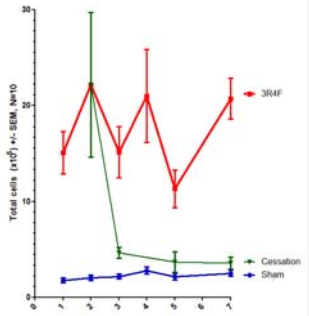
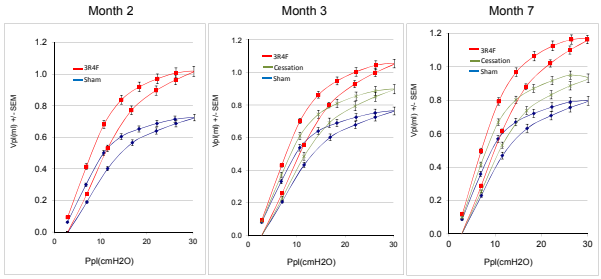


Figure 4. The total number of free lung cells increased after 1 month exposure to cigarette smoke, remaining high throughout the 7 month exposure period. The composition of cells was mainly neutrophils and lymphocytes. The total cell number rapidly decreased within 1 month following cessation from cigarette smoke, though still remained slightly elevated relative to the sham group throughout the exposure period.

Pulmonary function

Figure 5. Pulmonary function over time. The upward-shifting PV loops with cigarette smoke-exposure was clearly evident after 2 months, then remaining stable up until month 7. Cessation 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 for the cessation animals then remained stable between the sham and 3R4F even after a total of 5 months cessation.



Histopathology

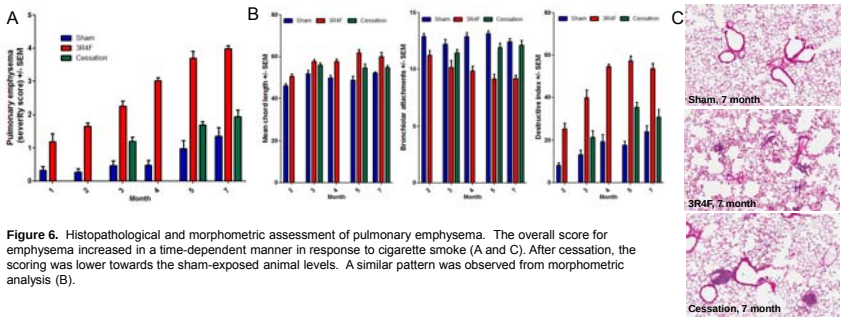


Figure 6. Histopathological and morphometric assessment of pulmonary emphysema. The overall score for emphysema increased in a time-dependent manner in response to cigarette smoke (A and C). After cessation, the scoring was lower towards the sham-exposed animal levels. A similar pattern was observed from morphometric analysis (B).

Conclusions

1. We have established a model of smoke-induced emphysema in C57BL/6 mice that mimics many of the characteristics of human COPD.
2. Cigarette smoke exposure resulted in increased infiltration of inflammatory cells and mediators into the lung which were quantified in the bronchoalveolar lavage fluid.
3. Pulmonary function was decreased by cigarette smoke and resulted in the leftward-shift of the PV loops following exposure.
4. Histopathological and morphometric assessment of lung tissue showed time-dependent progression of pulmonary emphysema in response to cigarette smoke exposure.
5. Cessation following 2 months of cigarette smoke exposure resulted in the amelioration of the above mentioned parameters to near sham levels as soon as within 1 month of cessation.
6. This model is an excellent platform for investigating the efficacy of smoking cessation as a benchmark for MRTP assessment.

References

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