A mechanistic study of cigarette smoke-induced COPD in C57Bl/6 mice and the effects of cessation or switching to a pMRTP aerosol

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Introduction

Chronic obstructive pulmonary disease (COPD) is defined by the WHO as a lung disease 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 either cigarette smoke (CS, 750 µg/l total particle matter; 34.4 µg/l nicotine) from 3R4F cigarettes, to the aerosol from a potential Modified Risk Tobacco Product (pMRTP, nicotine concentration-matched), or to 2 months CS followed by up to 1, 3, or 5 months cessation (fresh air) or switch-to-pMRTP up to 1, 3, or 5 months 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 emphysematous changes to the lung tissue.

Materials and Methods

Animals:

- C57Bl/6 mice, 8 – 10 weeks old weeks old females (gender chosen because they have half the serum antitrypsin levels of males [3,4] .
- The animals were randomly allocated to the following exposure groups just prior to the exposure start:

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) pMRTP – aerosol

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

5) Switch-to-pMRTP – 2 months cigarette smoke, followed by up to 5 months MRTP aerosol

Exposure

- Whole-body exposure chambers
- 3R4F – Health Canada Intense Puffing Regime
- Target concentration – 34.4 µg/l nicotine (3R4F equivalent to 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]

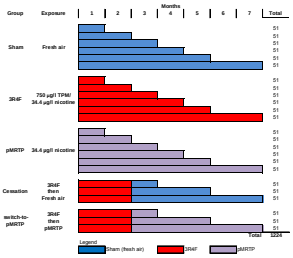


Figure 1. Study design with groups, and total Animals per group

Results – Transcriptomics

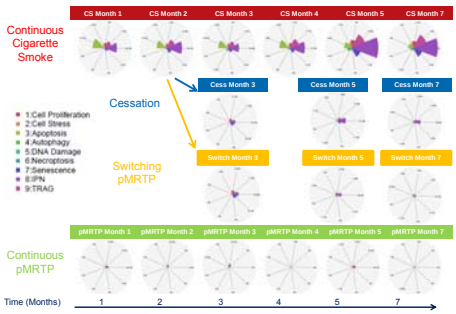


Figure 2. Network Perturbation Amplitudes (NPA) and Biological Impact Factor (BIF). Gene expression data (Affymetrix microarrays) was used to compute the **Network Perturbation Amplitude (NPA)** for each of our Biological Networks describing processes that are involved in smoke-induced diseases. The slices within each circular diagram are proportional to the NPA for each mechanism. The sum of all colored "slices" within each diagram is the **Biological Impact Factor**.
→The larger the colored slice for a network/mechanism the higher the NPA. The more colored surface in a circle the higher the BIF and vice versa.
The star plots show the biological processes that most contribute to the overall BIF phenotype at each time point in the COPD mouse model. As expected, the inflammatory processes (IPN Inflammation Processes Networks) increased in time and generally contributed the most to the BIF score with the exception of the early time points, when apoptosis seemed predominant.

Results and endpoints

Bronchoalveolar lavage fluid (BALF)

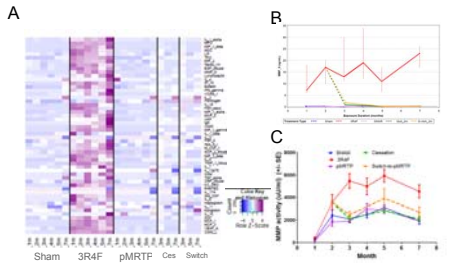


Figure 3. There was a general increase, to various degrees, of the levels of all measured mediators in the BALF obtained from animals after MS-cigarette exposure relative to the sham- or pMRTP-exposed mice (A). However, even after 1 month of cessation, or switching to the pMRTP aerosol, the levels returned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

Free lung cells

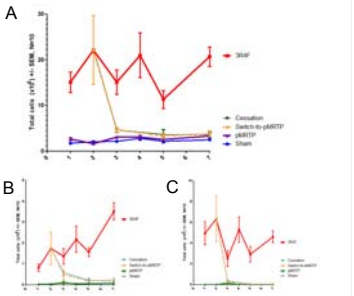
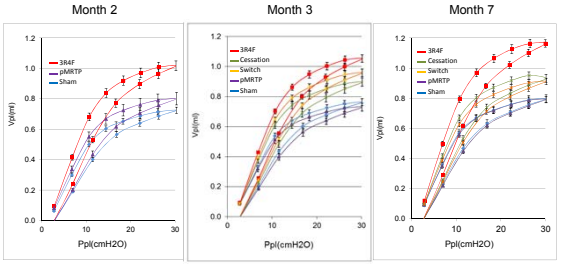


Figure 4. The total number of free lung cells (A) increased after 1 month exposure to cigarette smoke relative to sham or MRTP-exposed animals, remaining high throughout the 7 month exposure period. The composition of cells was mainly neutrophils and lymphocytes (B and C). The total cell number rapidly decreased within 1 month following either cessation from cigarette smoke or a switch to the pMRTP aerosol, approaching the sham or pMRTP-exposed group levels. Exposure to an MRTP aerosol did not cause any lung inflammatory infiltration relative to the sham group.

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 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.



Histopathology

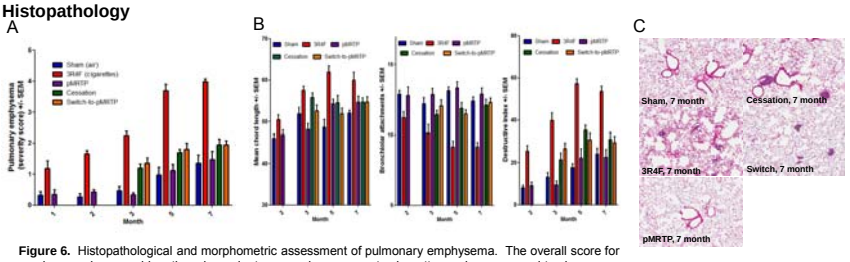


Figure 6. Histopathological and morphometric assessment of pulmonary emphysema. The overall score for emphysema increased in a time-dependent manner in response to cigarette smoke, compared to sham- or MRTP-exposed animals (A and C). After cessation or switching to an pMRTP aerosol, the emphysema score was lower towards the sham- or pMRTP-exposed animal levels. A similar pattern was observed following 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 results in increased infiltration of inflammatory cells and mediators into the lung, which were quantified in the bronchoalveolar lavage fluid.
3. Pulmonary function is decreased by cigarette smoke and resulted in the leftward-shift of the PV loops following exposure.
4. Histopathological assessment of the lung tissue showed time-dependent progression of pulmonary emphysema in response to CS exposure.
5. Cessation following 2 months of cigarette smoke exposure results in the amelioration of the above mentioned parameters to near sham levels as soon as within 1 month of cessation.
6. Switch-to-pMRTP aerosol after 2 months cigarette smoke exposure tracked closely with the cessation exposure group in all assessed parameters.
7. Exposure solely to an pMRTP aerosol gave results very similar to Sham exposure.
8. This model is an excellent platform to examine efficacy of smoking cessation as a benchmark for pMRTP assessment.

References

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