Lung Function in A/J Mice Following Subchronic Cigarette Smoke Exposure

Patterns of Emphysematous and Fibrotic Changes

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Introduction and Objective

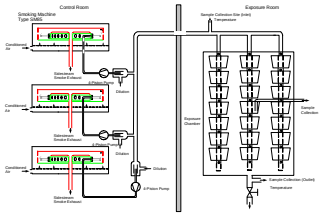
The defining feature of chronic obstructive pulmonary disease (COPD) is an irreversible airflow limitation caused by an increase in the resistance of small conducting airways, an increase in compliance due to emphysematous lung destruction, or both [1]. Cigarette smoke exposure is one of the major risk factors associated with the development of COPD. Characterising the functional consequences of cigarette mainstream smoke (MS) exposure in mice is therefore an essential step in developing a murine model of COPD.

The objective of this study was to develop a murine model of COPD that reflects inflammatory and functional features characteristic of the human disease.

Materials and Methods

Animals and Cigarette Smoke Exposure

Female A/J mice (24-25 weeks old at the start of the study) were exposed in whole body chambers to fresh air or to MS from the Reference Cigarette 2R4F at 750 µg total particulate matter (TPM)/l for 2, 3, or 4 hours/day, [corresponding to 1500, 2250 and 3000 µg TPM/(l x day), respectively], 5 days/week for 3 and 5 months.



Smoking Machine Type SM85 [2]

Determination of Respiratory Mechanics

Respiratory mechanics were determined at 3 and 5 months, 24 h after the last exposure. Each mouse was anaesthetised (pentobarbital 75 mg/kg, i.p.) and tracheostomised, and the trachea was cannulated. The cannula was connected to a computer-controlled small animal ventilator (Flexivent; Scireq, Montreal, PQ, Canada [3]) and regular quasisinusoidal ventilation was delivered at a frequency of 150 breaths/minute and a tidal volume of 10 ml/kg. Spontaneous respiration was abolished by administration of pancuronium bromide 0.8 mg/kg, i.p. and after stabilization, lung mechanics were measured at a positive end expiratory pressure of 3 cm H_2O. After the measurements were performed, the mice were euthanised.

Test Atmosphere Characterisation and Biomonitoring (HbCO)

- The test atmosphere was continuously monitored to ensure constant smoke generation, reproducibility, and correct dosing.
- HbCO was determined in all groups at the end of the exposure periods to confirm exposure.

Parameter	Unit	Sham	2R4F (2 h/day)	(3 h/day)	(4 h/day)
TPM conc.	µg/l	<26.8	726.4 ± 49.3	732.0 ± 44	737.8 ± 40.2
CO conc.	ppm	<4.5	793.7 ± 44.6	793.7 ± 44.6	793.7 ± 44.6
TPM (daily dose)	µg (*day)	-	1452.8 ± 98.6	2195.9 ± 132.9	2951.2 ± 160.9
nicotine (daily dose)	µg/l	<0.03	85.1 ± 8.9	127.7 ± 17.3	170.3 ± 17.8
formaldehyde (daily dose)	µg/l	-	0.97 ± 0.10	1.45 ± 0.15	1.94 ± 0.20
acetaldehyde (daily dose)	µg/l	-	98.19 ± 5.39	147.29 ± 8.08	196.39 ± 10.78
acrolein (daily dose)	µg/l	-	9.61 ± 0.47	14.42 ± 0.71	19.23 ± 0.95
Biomonitoring					
carboxyhemoglobin	%	0	62 ± 5	59 ± 2	66 ± 7

Data shown are the mean ± SD (mean ± SEM for HbCO).

Inflammatory Cells in Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) was performed at 3 and 5 months, 24 h after the last exposure. Free lung cell differentiation was performed using a Becton Dickinson FACSCanto flow cytometer and analysed using Becton Dickinson FACSDiva software.

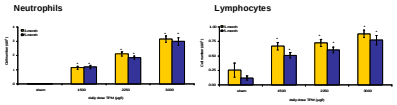
Statistical Analysis

For comparison of groups, the one-way ANOVA was followed by Dunnett's multiple comparison test (for inflammatory cells in BALF) or Newman-Keuls multiple comparison test.

Results

Inflammatory Cells in Bronchoalveolar Lavage

A dose-dependent increase in neutrophils and lymphocytes was found in BALF after 3 and 5 months of MS exposure.



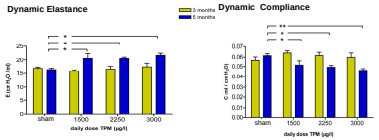
Data shown are the mean ± SEM of 13 to 15 animals (= p <0.05).*

Respiratory Mechanics

Dynamic Elastance and Compliance

After 5 months of MS exposure (compared to sham):
- higher dynamic elastance
- lower dynamic compliance

→ increased rigidity of the lung

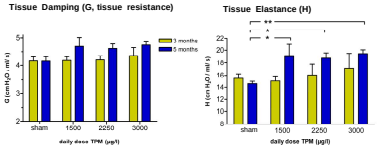


Measured by a "standardised breath" signal.
Data shown are the mean ± SEM of 7 to 9 animals (= p <0.05, ** = p <0.01).*

Tissue Damping and Tissue Elastance

After 5 months of MS exposure (compared to sham):
- no change in tissue resistance
- higher tissue elastance

→ increased rigidity of the lung

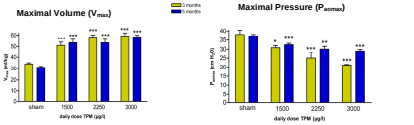


Data shown are the mean ± SEM of 7 to 9 animals (= p <0.05, ** = p <0.01).*

Maximal Pressure and Volume

After 3 and 5 months of MS exposure:
- higher maximal volume at an airway pressure of 30 cm H_2O
- lower maximal pressure at total lung capacity

→ loss of elastic recoil of the lung

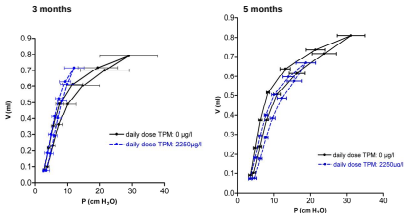


Data shown are the mean ± SEM of 7 to 9 animals (= p <0.05, ** = p <0.01, *** = p <0.001).*

Quasi-Static Pressure Volume Loops

After 3 months of MS exposure:
- leftward shift, suggesting emphysematous changes

After 5 months of MS exposure:
- rightward shift of the lower part of the PV loop suggesting the presence of a more rigid lung at lower volumes
- tendency towards a leftward shift in the upper part, suggesting emphysematous changes at higher volumes



Quasi-static pressure volume loops in mice following a daily dose of 2250 µg/l TPM.
Data shown are the mean ± SD of 7 to 9 animals.

Summary

Subchronic exposure of A/J mice to cigarette mainstream smoke resulted in a dose-dependent increase in neutrophils and lymphocytes in BALF and time- and dose-dependent changes in lung function parameters. The changes are similar to those previously observed in bleomycin (fibrosis)- and elastase (emphysema)-treated mice [4] and include:

- increased rigidity of the lung (at 5 months)
  - decreased dynamic lung compliance
  - increased tissue elastance
  - rightward shift of the lower part of the PV loop
  - smaller volume increase during stepwise inflation in the PV manoeuvre.

- loss of elastic recoil (at 3 and 5 months)
  - decreased maximal pressure measured at total lung capacity
  - increased maximal volume at an airway pressure of 30 cm H_2O
  - leftward shift of the PV loop at 3 months
  - tendency towards a leftward shift of the upper part of the PV loop at 5 months

Conclusion

Lung function parameters measured in A/J mice subchronically exposed to cigarette mainstream smoke suggest a combined pathology of a decreased elastic recoil and an increased rigidity of the lung.

References

1. Hogg, J.C. 2004, Lancet, **364**: 709-721
2. Vanscheeuwijck et al. 2002, Food Chem. Toxicol., **40**: 113-131
3. Schuessler, T.F. 1995, IEEEE. Trans. Biomed. Eng. **42**: 860-866
4. Pype et al. 2005, Proceedings of the British Pharmacological Society at http://www.pa2online.org/Vol1Issue1abst001P.html

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