

Inflammation and Morphometric Changes in the Lungs of Spontaneously Hypertensive Rats Following Cigarette Smoke Exposure

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Introduction

Exposure to cigarette mainstream smoke (MS) induces lung inflammation, a key factor in the development of emphysema. Previously we have reported that acute and subchronic inhalation exposure of rats to diluted MS induces changes in inflammatory mediators and greater numbers of neutrophils in bronchoalveolar lavage fluid (BALF) (Vanscheeuwijck et al., 2004; Vanscheeuwijck et al., 2005; Friedrichs et al., in press). Here we report on inflammation and morphometric changes in the lungs of spontaneously hypertensive rats (SHR) following subchronic exposure to MS. The SHR strain is known to be susceptible to cigarette smoke and particle-induced cardiopulmonary alterations (Gilmour et al., 2004) and has been shown to have increased concentrations of MIP-2 and increased numbers of inflammatory cells in BALF following cigarette smoke exposure (Smith et al., 2002). We investigated pulmonary inflammation (cytokines, chemokines, and free lung cells in BALF) and morphometric changes in the lungs.

Objective

Investigate inflammatory and morphometric changes in the lungs from SHR following exposure to MS from the Kentucky Reference Cigarette 2R4F.

Materials and Methods

Smoke Generation

- MS from Kentucky Reference Cigarette 2R4F generated according to ISO protocol (35 ml/puff in 2 s, each cigarette puffed once every minute to a butt length of 35 mm, Vanscheeuwijck et al., 2002) and diluted to 450 µg total particulate matter (TPM/l) with conditioned fresh air (with a continuous flow of aerosol).

Test Atmosphere Characterization

- Concentrations of TPM, carbon monoxide (CO), nicotine, and selected aldehydes (formaldehyde, acetaldehyde, and acrolein) determined at the breathing zone of the animals.

Rats and Treatment

- Spontaneously hypertensive rats (SHR) from Charles River, Italy, 6 to 8 weeks old at start of study, 10 rats per group
- Nose-only exposure to fresh air (sham) or to MS from the 2R4F (450 µg TPM/l), 3 x 1 hour/day (with 30 min fresh air exposure after each hour), 5 days/week, 13 weeks

Statistics

- Results expressed as means ± SE.
- 2-tailed t-tests applied, p-values indicated in charts.

Sample Collection and Determination

- BALF
 - approximately 20 h after exposure
 - cannulation of isolated lungs via trachea
 - lavage with 5 consecutive cycles of flushing (15 cm water pressure) and emptying (-8 cm water pressure)
 - filling medium: phosphate-buffered saline (PBS, Mg²⁺- and Ca²⁺-free) for the first cycle; PBS + 0.3% bovine serum albumin (BSA) for cycles 2 to 5
 - determination of inflammatory mediators: in first cycle lavage fluid, after centrifugation (cell-free)
 - free lung cells: cycles 2 to 5 combined, adjusted to 20,000 cells/ml, fixation in 2% formalin
- Inflammatory mediators in BALF
 - aliquots of the samples sent to Rules Based Medicine (Austin, Texas, USA) for 'Multi Analyte Profile analysis' (high-density, quantitative immunoassay panels for mice and rats)
 - CINC-1 determined using ELISA kit from Assay Designs Inc.
- Free lung cell differential counting in BALF
 - staining: anti-granulocyte mAb-FITC (clone HIS48), anti-CD68-FITC (clone ED1), nucleic acid counterstaining using propidium iodide
 - flow cytometry using a Becton Dickinson FACS Vantage – 40,000 events/sample counted
- Morphometric analysis of lung tissue
 - determination of standard morphometric parameters—mean linear intercept (Lm) and mean chord length (Cm) (Escobar et al., 1994), number of bronchiolar attachments (Ba) (Saetta et al., 1995)—by TransMIT GmbH (Marburg, Germany), 1 HE-stained slide per animal

Results

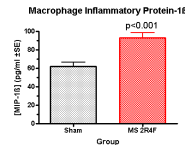
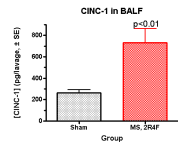
Test Atmosphere Characterization

Parameter	MS 2R4F
TPM (µg/l)	453 ± 14 (n = 71)
CO (ppm)	475 ± 24 (n = 71)
nicotine (µg/l)	35.1 ± 4.7 (n = 18)
formaldehyde (µg/l)	0.50 ± 0.05 (n = 13)
acetaldehyde (µg/l)	19.5 ± 0.6 (n = 13)
acrolein (µg/l)	1.59 ± 0.07 (n = 13)

Remarks: All parameters below the detection limit or not detected for sham.

Inflammatory Mediators in BALF

- Mediators related to neutrophil recruitment are higher in BALF from MS-exposed SHR.

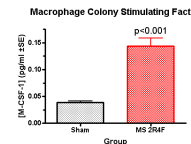
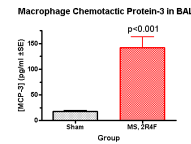
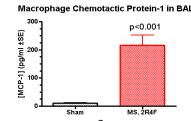


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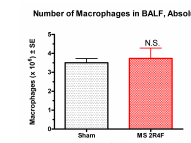
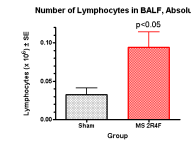
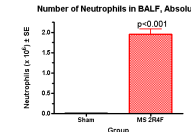
Inflammatory Mediators in BALF (cont.)

- Mediators related to macrophage recruitment/activation are greater in BALF from MS-exposed SHR.



Free Lung Cells in BALF

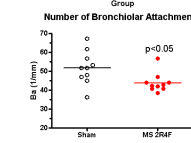
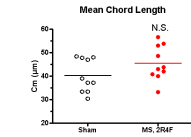
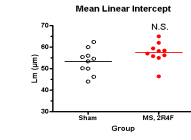
- Absolute numbers of neutrophils and lymphocytes are greater in MS-exposed SHR; no change in macrophages.
- The relative number of neutrophils among total free lung cells is 35% in MS-exposed SHR.



N.S.: not statistically significantly different

Morphometric Analysis

- Indications of mild emphysema in MS-exposed SHR
 - Lm and Cm are greater (not statistically significant).
 - Number of Ba is lower.
- Histopathological evaluation confirms the mild emphysema (results not shown).



Summary and Discussion

In MS-exposed SHR (compared to sham):

- Inflammatory mediators that recruit and/or activate macrophages and neutrophils are more abundant.
- Numbers of free lung cells, neutrophils, and lymphocytes are greater.
- Morphometric analysis of lung tissue indicates mild emphysema, the only statistically significant finding being the lower number of bronchiolar attachments.

Conclusion

- Exposure of spontaneously hypertensive rats to mainstream smoke from the Kentucky Reference Cigarette 2R4F induced both pronounced pulmonary inflammation and a mild form of emphysema.
- The SHR should be further investigated as a potential model for MS-induced pathological conditions associated with COPD.