Lung Inflammation in Rats after Acute Exposure to Cigarette Smoke

Introduction

Last year (SOT, 2004) we reported that acute (1 day) inhalation exposure of rats to diluted cigarette mainstream smoke induces changes in inflammatory mediators in bronchoalveolar lavage fluid (BALF) and serum. Others have shown that upon exposing rodents to cigarette mainstream whole smoke (Ofulue et al., 1998; Obot et al., 2004), the number of neutrophils is increased in BALF. What is not known, however, is the relative contribution of the particulate phase and the gas phase of the smoke to pulmonary inflammation. Here, we investigate the inflammatory potential of cigarette mainstream whole smoke (WS), gas-phase-depleted particulate phase (PP), and gas phase (GP) in an acute and subchronic rat inhalation model.

Materials and Methods

Smoke Generation

- Generation of mainstream smoke according to ISO protocol (35 ml/puff n=2, each cigarette puffed once every minute, 4 puff cycle 35 mm; Tazoe et al., 2003)
- WS collected from the exhalation
- PP generated by passing WS through a 220 µm activated charcoal filter to remove the majority of gas phase constituents
- GP generated by passing WS through an electrophoretic filter to remove particulate matter

Test Atmosphere Characterization

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

Rats and Treatment

Acute Inhalation Study

- Sprague-Dawley rats, 200 to 220 g at start of inhalation, 8 rats/group
- Nose-only exposure, 2 x 1 h with a 30-min break between the 2 h, 1 day only
- Exposure to fresh air (sham) or to mainstream smoke from the Kentucky Reference Cigarette 2R4F
- Exposure to fresh air (sham) or to mainstream smoke from the Kentucky Reference Cigarette 1R4F
- Respiratory physiology parameters (6 rats/group)
- Inflammatory mediators
- Free lung cell differentiation: cycles 2 to 5 combined, adjusted to 20,000 cells/ml, fixation with 2% formalin
- Determination of inflammatory mediators: in first cycle of lavage after centrifugation (cell-free)
- Filling medium: phosphate-buffered saline (PBS, Mg
- Lavage with 5 consecutive cycles of filling (15 cm water pressure) and emptying (8 cm water pressure)
- Respiratory minute volume in the rats exposed to PP.
- Respiratory minute volume in the rats exposed to PP.

Subchronic Inhalation Study

- Sprague-Dawley rats, 200 to 220 g at start of inhalation, 10 rats/group
- Nose-only exposure, 2 x 1 h with a 30-min break between the 2 h for 35 consecutive days
- Exposure to fresh air (sham) or to mainstream smoke from the Kentucky Reference Cigarette 1R4F at TPM concentrations of 500 (NS only) and 750 µg/l

Results

Acute Inhalation Study

Test Atmosphere Characterization

<table>
<thead>
<tr>
<th>Group</th>
<th>TPM (µg/l)</th>
<th>CO (ppm)</th>
<th>Nicotine (µg/l)</th>
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Subchronic Inhalation Study

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Inflammatory Mediators in BALF

- Cytokines and chemokines increased after exposure to WS
- Most pronounced effect after exposure to PP
- No effect after exposure to GP
- Similar response seen for CINC-1 and MCP-1 (data not shown)
- Neutrophils increased after exposure to PP

Neutrophils in BALF

- Neutrophils increased after exposure to WS
- Most pronounced effect after exposure to PP
- Neutrophils increased after exposure to WP
- No effect after exposure to GP

Neutrophils in BALF

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Summary and Discussion

- WS induces inflammatory changes in BALF from rats after acute and subchronic inhalation exposure.
- PP is responsible for the inflammatory changes in the rat lung, GP had no effect.
- PP induces qualitatively the same inflammatory changes as WS, but to a greater extent.
- This is most likely due to the higher uptake as indicated by the lack of depression of respiratory minute volume in the rats exposed to PP.
- Inflammatory changes in the rat lung after acute inhalation exposure parallel the changes observed after subchronic inhalation exposure.

Subchronic Inhalation Study

- Respiratory minute volume in the rats exposed to PP.
- No depression during exposure to PP

Neutrophils in BALF

- Neutrophils increased after exposure to WS
- Most pronounced effect after exposure to PP
- No effect after exposure to GP

Respiratory Minute Volume

- RMV depressed during exposure to WS and GP
- No depression during exposure to PP

Conclusion

- Mainstream whole smoke and gas-phase-depleted particulate phase cause similar inflammatory changes in the rat lung.
- Acute inhalation exposure may be a useful short-term in vivo assay for the evaluation of the inflammatory effects of cigarette smoke.

References


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EMKA Technologies
- Evaluation of respiratory minute volume (RMV) for each rat relative to pre-exposure value; data analysis IOX-software.
- Respiratory physiology parameters (6 rats/group)
- Neutrophils: anti-granulocyte mAB-FITC (clone HIS48), anti-CD68-FITC (clone ED1), nucleic acid counterstaining using 4,6-diamidino-2-phenylindole (DAPI; Pierce Biotechnology, Inc., Boston, MA)
- Inflammatory mediators: assessment of IL-1β, TNFα, IL-6, IL-10, IL-12, IFNγ, IL-13, IL-17, IL-23, IL-22
- Free lung cell differentiation: cycles 2 to 5 combined, adjusted to 20,000 cells/ml, fixation with 2% formalin
- Determination of inflammatory mediators: in first cycle of lavage after centrifugation (cell-free)
- Filling medium: phosphate-buffered saline (PBS, Mg
- Lavage with 5 consecutive cycles of filling (15 cm water pressure) and emptying (8 cm water pressure)
- Respiratory minute volume in the rats exposed to PP.
- No depression during exposure to PP.
- RMV depressed during exposure to WS and GP.
- No depression during exposure to PP.

*2N4F is the remade of the 1N4F.