Pulmonary Neoplasia in Strain A Mice following Long-Term Tobacco Smoke Inhalation

Rosemarie B. Lichtner

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The A/J Mouse as a Lung Tumor Model

• Philip Morris International is committed to the development of Reduced-Risk Tobacco Products. This requires a state-of-the-art scientific approach to assess the disease risk of new products

• Animal models with enhanced lung tumor formation after exposure to cigarette smoke are required to substantiate a reduced risk

• The A/J mouse has been shown to respond to cigarette smoke exposure with enhanced lung tumor formation after a recovery period of several months (Witschi et al., 1997; D’Agostini et al., 2001; Stinn et al., 2005; Curtin et al., 2004)
Nodules in the A/J Mouse Lung

- Lung nodules
- Bronchioloalveolar adenoma
- Bronchioloalveolar adenocarcinoma
- Hyperplasia
Objectives of A/J Mouse Lung Cancer Study

Characterize the effects of chronic MS exposure on lung tumor response with respect to relevance for human tumors:
• Time course (5, 10, and 18 months exposure)
• Increasing MS concentrations (0, 150, and 300 mg total particulate matter [TPM]/m³)
• Different post-exposure periods (up to 13 months)

Endpoints:
• Classical histopathology of step-serial sections to differentiate and quantify proliferative lesions and bronchiolo-alveolar adenomas and adenocarcinomas
• Gene expression analysis of tumor nodules and normal lung tissue
• K-ras mutation analysis in cells from lung nodules
• Analysis of bronchoalveolar lavage fluid (BALF) (see poster #33)
### Exposure Regimens of A/J Mouse Study

**Exposure:** 6 hours/day, 5 days/week  
**Exposure mode:** whole-body  
**MS concentrations:** 150 and 300 mg TPM/m³ (MS-150 and MS-300)

<table>
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<th>5-month inhalation</th>
<th>mice per time point</th>
<th>MS exposure</th>
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<td>22 - 36</td>
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</table>

- **MS dose:** 97 and 187 g x h/m³
- **MS dose:** 196 and 377 g x h/m³
- **MS dose:** 365 and 692 g x h/m³

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Histopathological Evaluation of Lung Tumor Multiplicity: 5 mo Exposure + 13 mo Post-Exposure

Sham

MS-150

MS-300

Lung Tumor Multiplicity (Tumors/mouse)

Time (Months)

(mean ± SE)

Exposure Post-Exposure

Exposure Post-Exposure

Bronchioloalveolar Carcinoma

Bronchioloalveolar Adenoma

Nodular Hyperplasia

Sum Microscopy

Macroscopy
Histopathological Evaluation of Lung Tumor Multiplicity: 10 mo Exposure + 8 mo Post-Exposure

Sham

MS-150

MS-300

(mean ± SE)
Histopathological Evaluation of Lung Tumor Multiplicity: 18 mo Exposure

Exposure

(mean ± SE)

- Bronchioloalveolar Carcinoma
- Bronchioloalveolar Adenoma
- Nodular Hyperplasia
- Sum Microscopy
- Macroscopy
Lung Tumor Multiplicity: 18-Month Dissection

| 5 Months Exposure + 13 Months Post-Exposure | 10 Months Exposure + 8 Months Post-Exposure | 18 Months Exposure |

- **Sham**
  - Lung Tumor Multiplicity (Tumors/mouse): 1.0

- **MS-150**
  - Lung Tumor Multiplicity (Tumors/mouse): 2.7

- **MS-300**
  - Lung Tumor Multiplicity (Tumors/mouse): 2.6

**Bronchioloalveolar Carcinoma**
- **Sham**: 1.0
- **MS-150**: 1.0
- **MS-300**: 1.0

**Bronchioloalveolar Adenoma**
- **Sham**: 1.0
- **MS-150**: 1.0
- **MS-300**: 1.0

**Nodular Hyperplasia**
- **Sham**: 1.0
- **MS-150**: 1.0
- **MS-300**: 1.0

* * p ≤ 0.05 compared to sham
* X p ≤ 0.05 compared to low MS

(mean ± SE)
mRNA Expression Analysis of Normal Lung Tissue and Nodules

PROCEDURE

• Laser capture microdissection (LCM) of lung nodules and normal lung tissue
• mRNA analysis using Agilent technology

RESULTS

• Normal lung tissue: differential gene expression pattern was induced by MS exposure
Kinetics for Genes Coding for Antioxidant and Phase I/II Xenobiotic-Metabolizing Enzymes: Normal Lung Tissue

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Increase of 2-fold or more
## Kinetics for Genes Coding for Inflammatory Responses: Normal Lung Tissue

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### ≥ 2-fold increase
- chemokines
- acute-phase response
- macrophage marker
- matrix metallopeptidase
- tissue inhibitor of metalloproteinase 1
- secretory leukocyte protease inhibitor
- cathepsin K
- cathepsin S

### ≥ 2-fold decrease
mRNA Expression Analysis of Normal Lung Tissue and Nodules

PROCEDURE

• Laser capture microdissection (LCM) of lung nodules and normal lung tissue
• mRNA analysis using Agilent technology

RESULTS

• Normal lung tissue: differential gene expression pattern was induced by MS exposure

• Lung nodules: no differential gene expression pattern was induced by MS exposure (31 nodules, 14 normal lung tissues)

Possible explanations

Technical reasons: mainly ruled out

Biological reasons: High heterogeneity of nodules: e.g., independent transformation events, different tumor progression stages, and mixture of adenoma and carcinoma.
**K-ras Mutation analysis of Lung Nodules from MS-exposed A/J Mice**

**PROCEDURE**

- LCM of lung nodules from snap-frozen tissue and formalin-fixed, paraffin-embedded tissue

- Isolation of DNA, amplification with subsequent sequencing of the Exon 1 and Exon 2 fragments of the *K-ras* gene, mutation analysis of the hotspots: codons 12, 13, and 61

**RESULTS**

- No MS-specific pattern was observed
**K-ras Mutations in LCM-derived Lung Nodules: No MS-specific Pattern**

Totals for snap-frozen tissue and formalin-fixed, paraffin-embedded tissue combined.

<table>
<thead>
<tr>
<th>Group</th>
<th># of Tumors and K-ras Mutations</th>
<th>Incidence of K-ras mutations</th>
<th># of K-ras Mutations in Hotspot Codons</th>
<th># of Transversions</th>
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<td>86</td>
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Totals for snap-frozen tissue and formalin-fixed, paraffin-embedded tissue combined.
Summary

A/J mice exposed to cigarette smoke: major findings

• Significant, concentration-dependent enhancement of lung tumors, i.e., adenomas and adenocarcinomas

• No obvious shift in tumor spectrum (from adenoma to adenocarcinoma)

• Differential gene expression in normal lung tissue
  – 3 main classes: genes related to oxidative stress, xenobiotic metabolism, or inflammatory processes

• No differential gene expression in isolated lung nodules

• No MS-specific mutation pattern in exons 1 and 2 of the K-ras gene
Conclusion

• Chronic exposure (18 mo) of A/J mice to cigarette smoke results in increased lung tumor formation

• Dose-dependency and good reproducibility of cigarette-smoke-dependent increased lung tumor formation in A/J mice

• The relevance of the A/J mouse model for cigarette-smoke-induced lung tumors in humans requires further validation
Acknowledgement

Co-authors

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• Walter Stinn (Philip Morris Research Laboratories, Cologne, Germany)
• Frans van Overveld* (Philip Morris Research Laboratories, Leuven, Belgium)

*Current address: Kessel-Lo, Belgium

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