Enhanced Lung Tumorigenicity in the A/J Mouse After Inhalation of Cigarette Mainstream Smoke and Gas Phase-Depleted Particle Phase

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Introduction

Several reports have described the causality between tobacco smoking and lung cancer (e.g., IARC 2004). Cigarette smoke-induced lung cancer models are needed for the evaluation of (a) biomarkers for early detection of disease, (b) chemoprevention, and (c) potential risk-reduced products (IOM 2001). Despite this, no reproducible and validated models have been established for this disease. One possible model under investigation is the A/J mouse.

A/J mouse studies have shown:

- Reproducible enhancement of lung tumors after exposure to an environmental tobacco smoke surrogate (ETSS) for 5 months followed by 4 months without further exposure (Witschi et al., 2004; Stinn et al., 2005 accepted).
- No production or promotion of lung cancer after exposure to mainstream (MS) concentrations that were considered "maximally tolerable" for 6 months followed by only 5 weeks without further exposure (Finch et al., 1996).
- No induction of lung tumors after whole-body exposure to MS for 5 months followed by 4 months without further exposure (D'Agostini et al., 2001).
- Increased lung tumor multiplicity after exposure to MS for 5 months followed by 4 months without further exposure, but statistically significant only after serial step lung section analysis (Curtin et al., 2004).

Additional research may help to establish and understand the A/J mouse as a model for lung tumors.

Objective

Investigate lung tumor incidence and multiplicity in the A/J mouse after chronic inhalation exposure to cigarette mainstream whole smoke (WS), to gas phase-depleted particle phase (PP), and to the gas phase (GP) as part of an overall effort to establish animal models for lung tumorigenicity.

Materials and Methods

- Male A/J mice bred under SPF conditions, animals from Jackson Laboratories (Maine, U.S.A.)
- Whole-body exposure in 2.3-m³ Hazleton chambers
  - 6 hours/day; 5 days/week; 9 months
    - 5 months exposure, 4 months post-exposure
- Mainstream smoke from the Reference Cigarette 2R4F (TPM yield: 11.6 mg/cig.) generated under ISO conditions
  - WS at TPM concentrations of 120 mg/m³ and 240 mg/m³
  - PP generated by passing WS through an activated charcoal filter; TPM concentration of 240 mg/m³
  - GP generated by passing WS through an electrostatic filter; carbon monoxide (CO) concentration equivalent to 240 mg TPM/m³)
- End points
  - body weight (weekly)
  - carboxyhemoglobin (HbCO) in blood
    - 6 mice/group; 5 times during exposure period
    - gas chromatography of methane after reduction of the CO released from hemoglobin
  - corticosterone in plasma (stress marker)
    - 8 mice/group; 2 times during exposure period
    - radioimmunoassay
  - inflammation
    - 8 mice/group; 5 and 9 months
    - differentiation of free lung cells (FLC) in bronchoalveolar lavage fluid (BALF) on May-Grünwald-Giemsa-stained cytosmears in BALF
  - macroscopic lung tumor multiplicity and incidence
    - 100 mice/group and time point; 5 and 9 months
    - macroscopic counting of nodules after immersion in Tellyesniczky's fixative for 1 day followed by fixation in ethanol
  - microscopy of lung sections
    - 65 to 97 mice/group; 9 months
    - histopathology of hematoxylin/eosin (HE)-stained step serial lung slides (distance: 100 µm)
    - lung tumor classification according to WHO guidelines (2001)
- Statistical tests
  - One-way ANOVA followed by Dunnett: plasma corticosterone and FLC in BALF
  - Chi-square test: lung tumor incidence
  - Kruskal-Wallis followed by Dunnett: lung tumor multiplicity

  Biological data represent means ± SE
  * in tables and figures: statistically significant at $p < 0.05$ compared to fresh air control
- The study was conducted in accordance with the
  - OECD principles of Good Laboratory Practice (1997)
  - American Association for Laboratory Animal Science Policy on the Humane Care and Use of Laboratory Animals (1991)

Results

Test Atmospheres

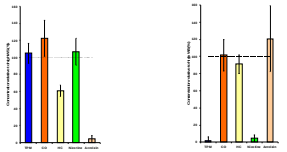
Concentrations Relative to High Whole Smoke

Particle Phase

- CO 20% higher
- Hydrocarbons in gas phase (HC) reduced by ~40%
- Acrolein reduced by >95%

Gas Phase

- Particles and nicotine reduced by more than 94%
- CO and HC similar to high whole smoke



Chemical Analysis

- Target concentrations were met.
- The size of particles indicates that they were respirable for the mice.

Parameter	Fresh Air Control	Low WS (120 µg TPM/l)	High WS (240 µg TPM/l)	PP	GP
TPM (mg/m³)	0 ± 1	127 ± 11	227 ± 22	235 ± 30	4 ± 11
particle size (µm)	-	0.38 ± .3	0.56 ± .3	0.52 ± .3	-
carbon monoxide (ml/m³)	0 ± 1	201 ± 24	320 ± 41	384 ± 56	320 ± 38
hydrocarbon in gas phase (mg CH_4/m³)	-	-	239 ± 24	145 ± 16	219 ± 26
nicotine (mg/m³)	0.1 ± 0.1	2.7 ± 0.6	5.8 ± 0.9	6.2 ± 0.8	0.3 ± 0.2
formaldehyde (mg/m³)	0.02 ± 0.01	0.08 ± 0.01	0.11 ± 0.00	0.10 ± 0.02	0.16 ± 0.05
acetaldehyde (mg/m³)	0.1 ± 0.0	13.5 ± 1.0	22.9 ± 1.6	12.1 ± 3.5	23.3 ± 3.3
acrolein (mg/m³)	bdl	0.61 ± 0.14	0.86 ± 0.20	0.03 ± 0.03	1.00 ± 0.29

Remarks: values represent mean ± SD
bdl : below detection limit

Quality Parameters

HbCO

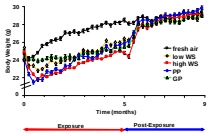
- Low WS group: between 23 and 28%
- High WS, PP, and GP groups: between 32 and 42%

Mortality Rate

- End of exposure period: <2% in all groups
- End of post-exposure period: <6% in all groups

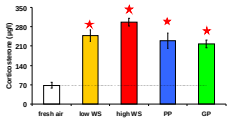
Body Weight

- Dose-dependent decrease in WS groups during exposure up to a body weight gain reduction of 12%
- First half of exposure period
  - PP similar to high WS
  - GP less pronounced compared to low WS
- Second half of exposure period
  - PP and GP similar to low WS



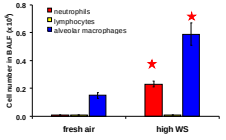
Plasma Corticosterone Level

- Dose-dependent increase of WS
- Similar stress effect of PP and GP



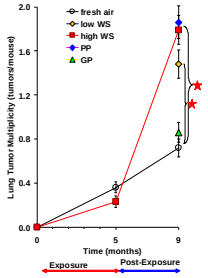
Inflammation

- A distinct inflammation was indicated in the high WS group by a severe increase in neutrophils (29% of FLC in BALF) and macrophages in BALF at the end of the exposure period.
- Both cell types returned to control values at the end of the post-exposure period.



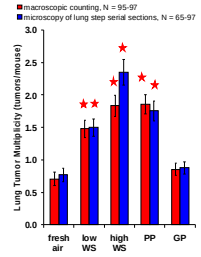
Lung Tumor Multiplicity

Macroscopic Results



Macroscopic vs Microscopic Results

- Step-serial lung section analysis confirmed the macroscopic results.



Lung Tumor Incidence

5-month exposure period (WS high group compared to control)

- No difference in lung tumor incidence and multiplicity

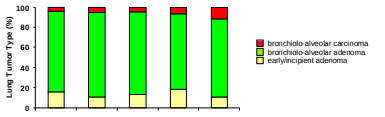
4-month post-exposure period (all groups)

- WS enhanced lung tumor incidence and multiplicity in a dose-dependent manner: high WS 3-fold over control.
- The lung tumor response was similar for high WS and PP groups.
- GP failed to enhance lung tumor multiplicity above control level. This was unexpected because in previous studies with mice exposed to ETSS, lung tumor multiplicity and incidence were the same for whole smoke and gas phase (Witschi, 2005).

Group	5-Month Exposure N	5-Month Exposure Incidence	4-Month Post-Exposure N	4-Month Post-Exposure Incidence
Fresh air control	98	32 = 33%	95	52 = 55%
low WS	-	-	96	71 = 74% *
high WS	100	19 = 19%	96	82 = 85% *
PP	-	-	97	78 = 80% *
GP	-	-	97	56 = 58%

Spectrum of Lung Tumors

- The lung tumor spectrum was the same in all groups.
- Bronchiolo-alveolar adenoma was the most prominent lung tumor type.
  - In humans carcinomas are the most prominent.



Summary of Lung Tumor Results

- Chronic mainstream smoke exposure caused a dose-dependent increase in lung tumor incidence and multiplicity.
- Lung tumor response was similar for high whole smoke and particle phase.
- The gas phase, at the concentrations used, failed to enhance lung tumor multiplicity above control levels.

Conclusion

- **While the results of these investigations suggest that the A/J mouse may be a possible model for cigarette smoke-related lung tumorigenicity, there are still several issues that need to be resolved with regard to its relevance for the human disease. These issues include**
  - **the time course of lung tumor development,**
  - **the lack of a shift in lung tumor spectrum, and**
  - **the role of the gas phase.**

Further research is needed to establish the A/J mouse as a model for cigarette smoke-related lung tumorigenicity.

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