Biochemical, immunohistochemical, and molecular biomarkers in rats following inhalation of cigarette mainstream smoke (MS) or a mixture of cigarette sidestream smoke (SS) and MS

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Objective

- Identify novel biomarkers responsive to cigarette smoke in a rat inhalation model for reduced exposure/risk research.

Study design

- Male Sprague-Dawley rats, 8 per group
- Smoke from the Reference Cigarette 2R1 under ISO conformity
  - Mainstream smoke (MS): 100 µg total particulate matter (TPM)/l and 250 µg TPM/l
  - Mixture of 89% sidestream smoke and 11% MS (SS/MS-mix): 100 µg TPM/l
- Exposure
  - Whole body, 2 x 3 h/day
  - Sham controls (fresh air)
  - 5 d/week, up to 4 weeks
  - *Gene expression: MS100, 1 x 3 h/day, 3 weeks*

Smoke composition

	N	Sham	'SS/MS mix'100	MS100	MS250
TPM (ug/l)	25	0.1	92.9 (8.0)	102.5 (8.8)	245.7 (15.3)
CO (ppm)	25	0.6	237.4 (14.6)	54.4 (7.4)	152.7 (9.7)
Nicotine (ug/l)	25	0.0	12.2 (2.6)	5.93 (0.7)	13.2 (1.8)
Formaldehyde (ug/l)	6	0.0	1.97 (0.47)	0.16 (0.06)	0.27 (0.04)
Acetaldehyde (ug/l)	6	0.0	9.71 (1.13)	4.18 (0.57)	9.33 (0.46)
Acrolein (ug/l)	6	0.0	2.06 (0.21)	0.50 (0.10)	1.09 (0.08)

mean (SE)

Biological endpoints

- Respiratory Tract

Neutrophils in BALF

Toxicity: LDH

Permeability: Albumin

Oxidative Stress: 8-OHdG

CYP1A1, CYP2B1, CYP2E1

DNA adducts

Gene expression profiling

- Systemic

Body weight

Hemoglobin adducts (4-ABP)

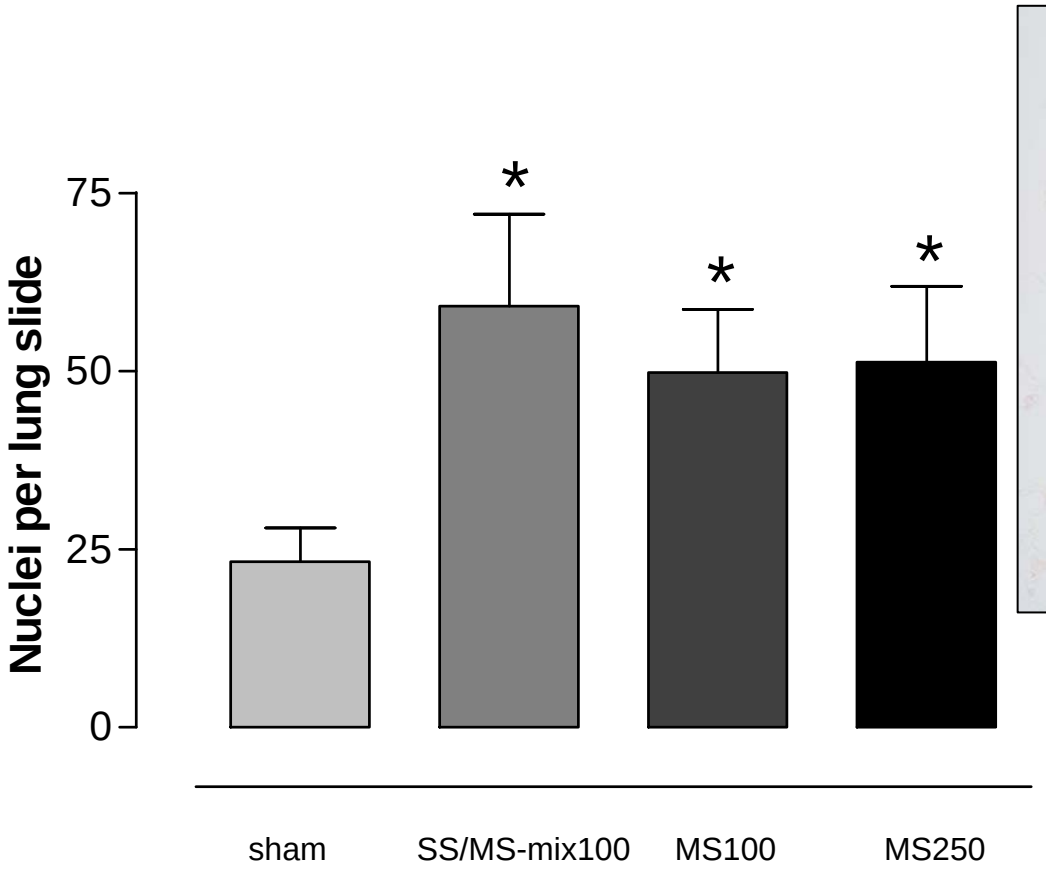
Proteomics: Apolipoprotein A1

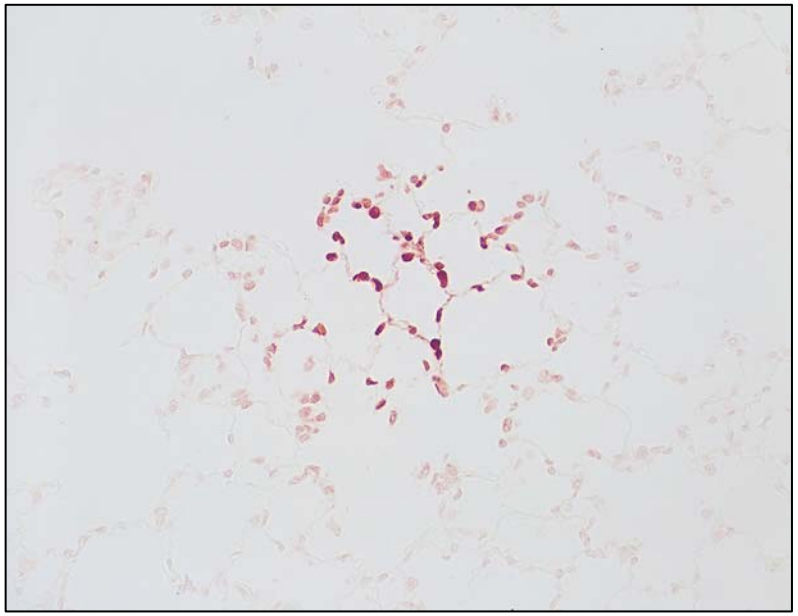
Micronucleus
(peripheral blood, bone marrow)

Comparison of aerosols at equal TPM concentrations (100 µg TPM/l).

Dose comparison for MS (100 and 250 µg TPM/l).

Endpoints responsive to both SS/MS-mix and MS: 8-OHdG adducts in lung slides

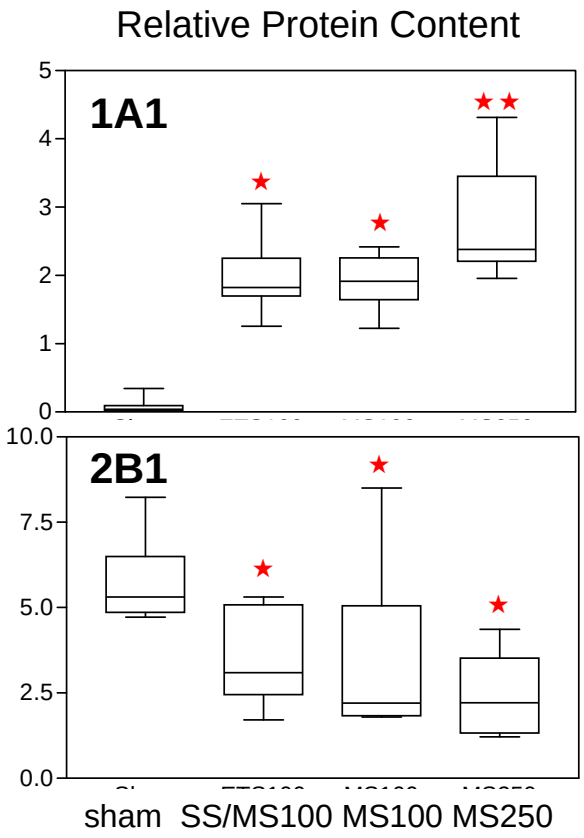


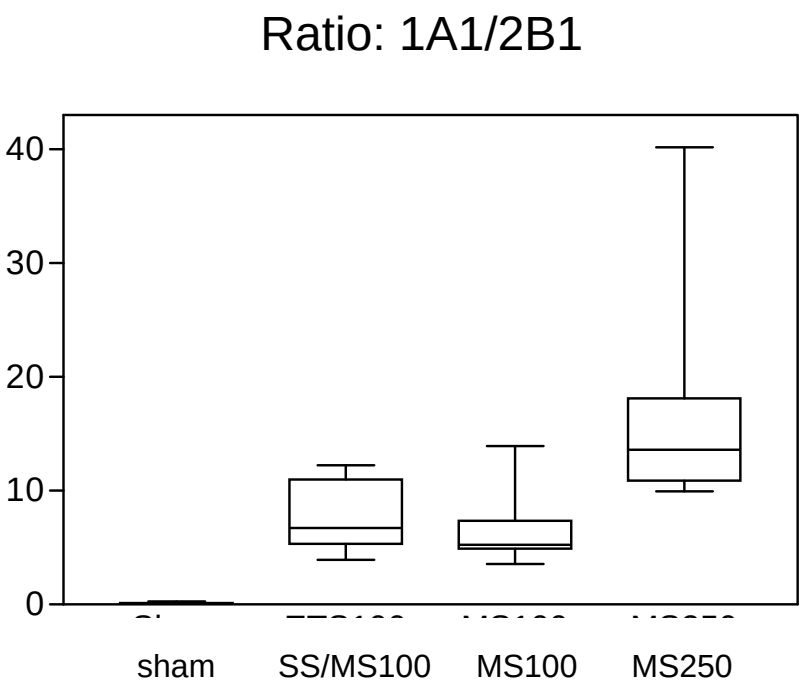


mean ± SE, N=8
p< 0.05 vs. sham

2-fold increase in 8-OHdG adducts compared to sham.

Endpoints responsive to both SS/MS-mix and MS: CYP 1A1 and 2B1, protein content and activity ratio in the lung



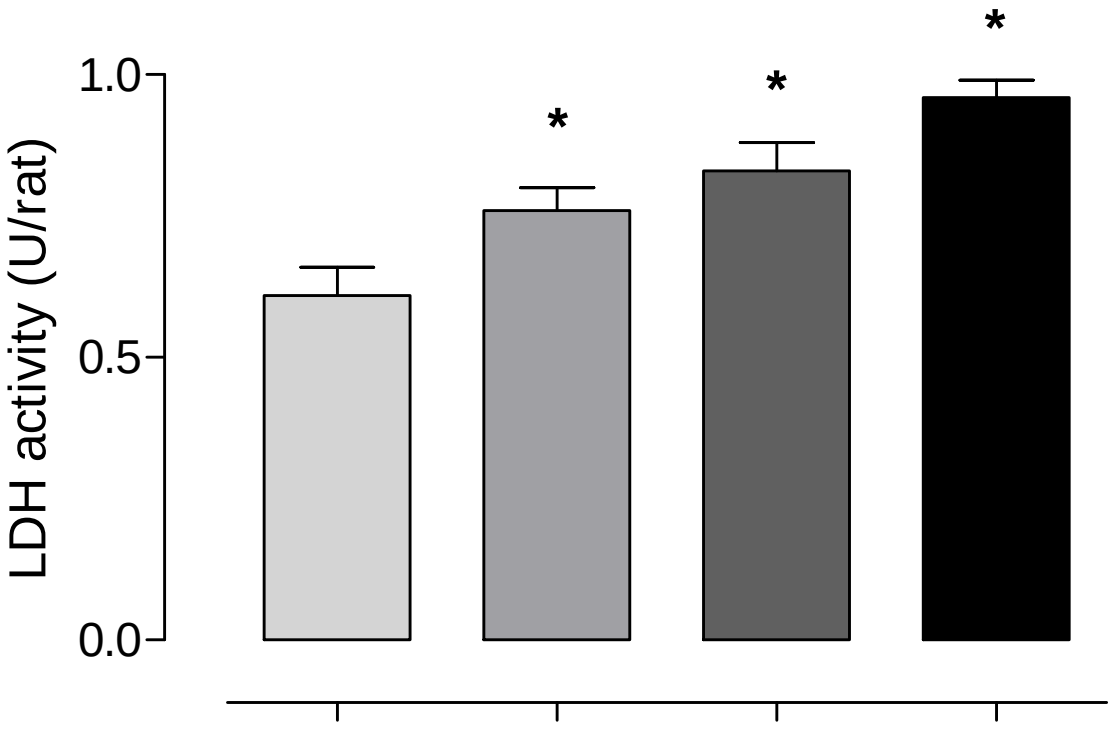


Relative protein content.

mean ± SE, N=8
p< 0.05 vs. sham

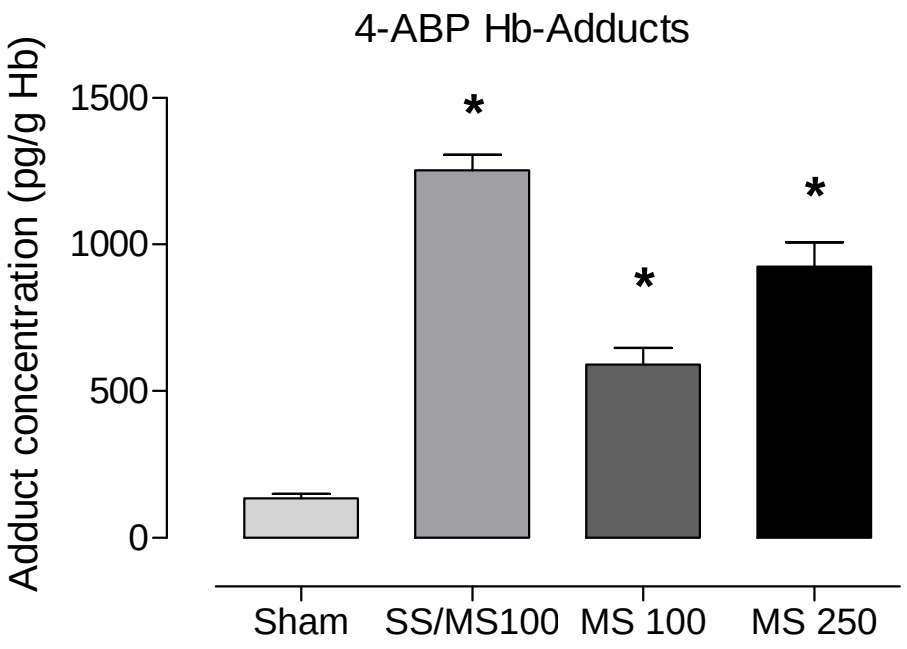
Increased CYP1A1/2B1 activity ratio.

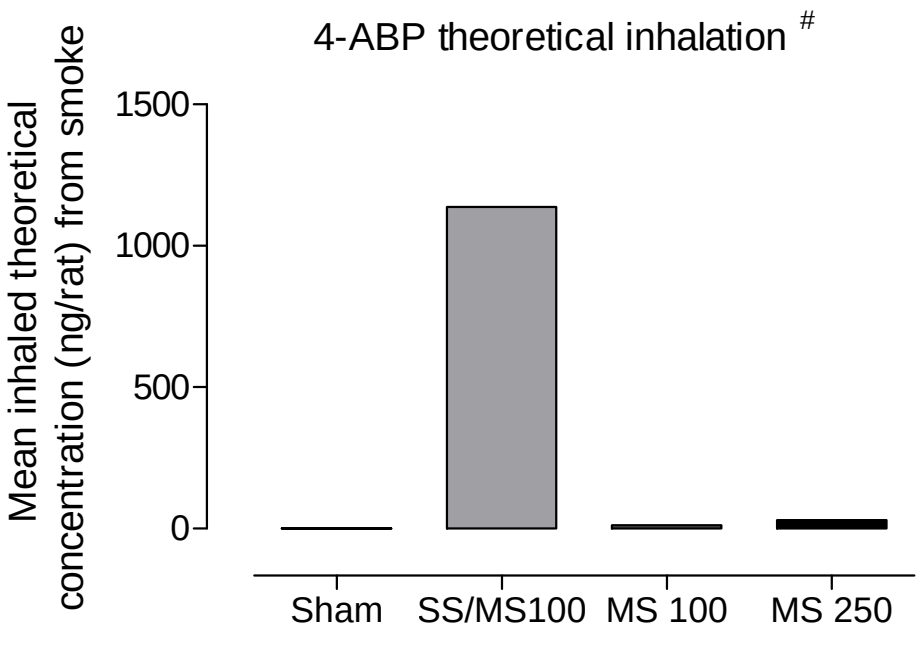
Endpoints responsive to both SS/MS-mix and MS: LDH in BALF



Increased LDH-activity in BALF in all groups compared to sham.

Endpoints responsive to both SS/MS-mix and MS: 4-ABP Hb-adducts

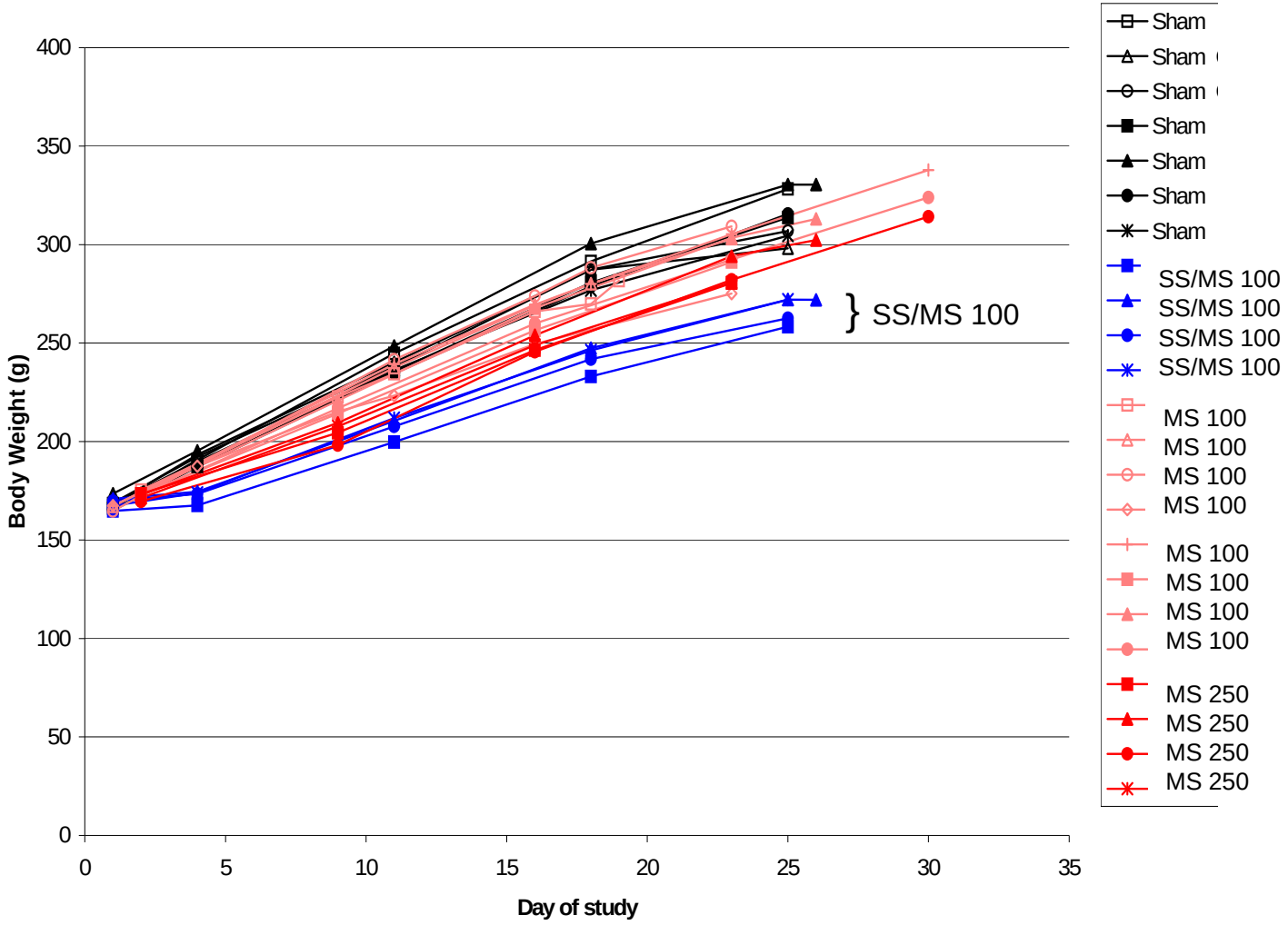




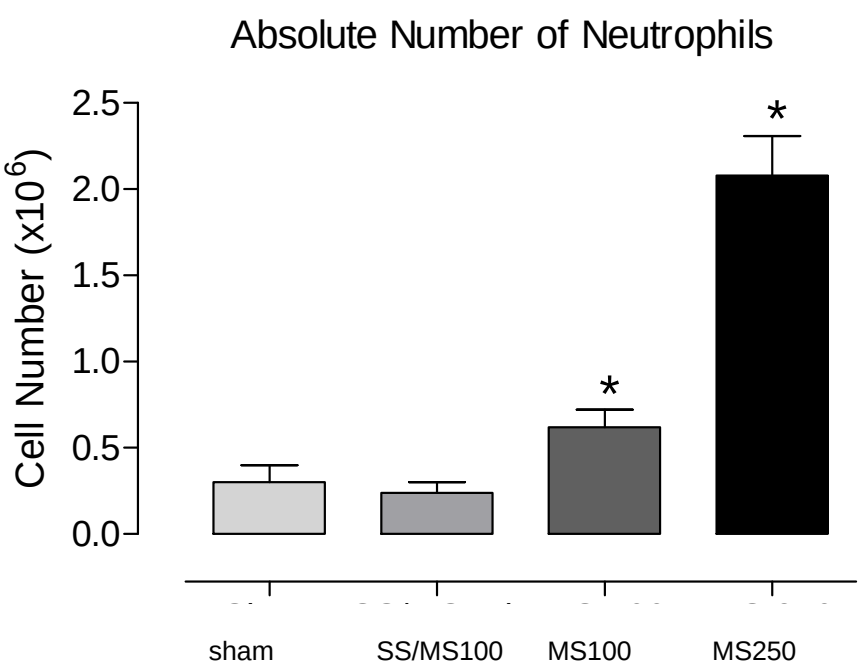
mean ± SE, N=8
p< 0.01 vs. sham

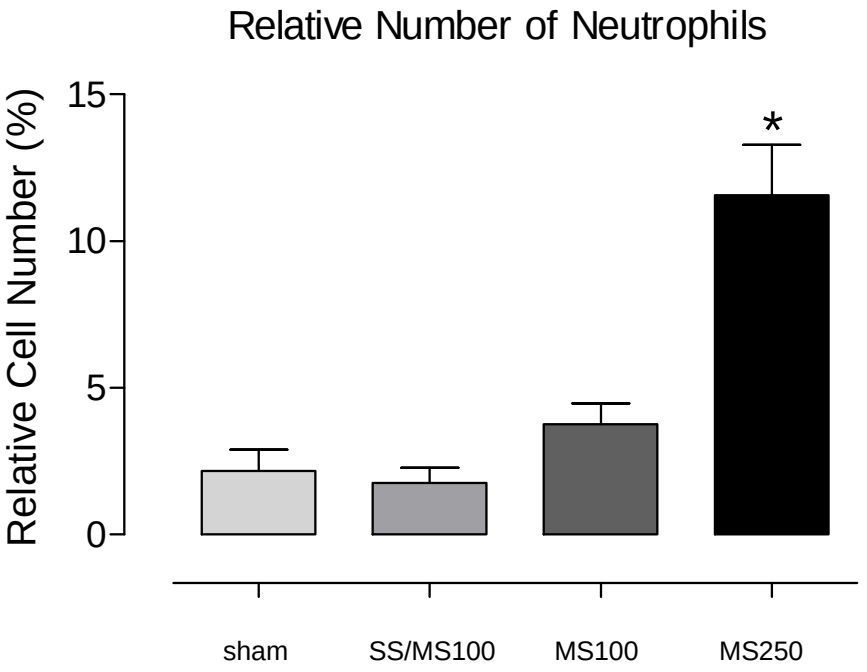
#: 4-ABP content in smoke x exposure time x respiratory minute volume.

Endpoints responsive mainly to SS/MS-mix: Body weight



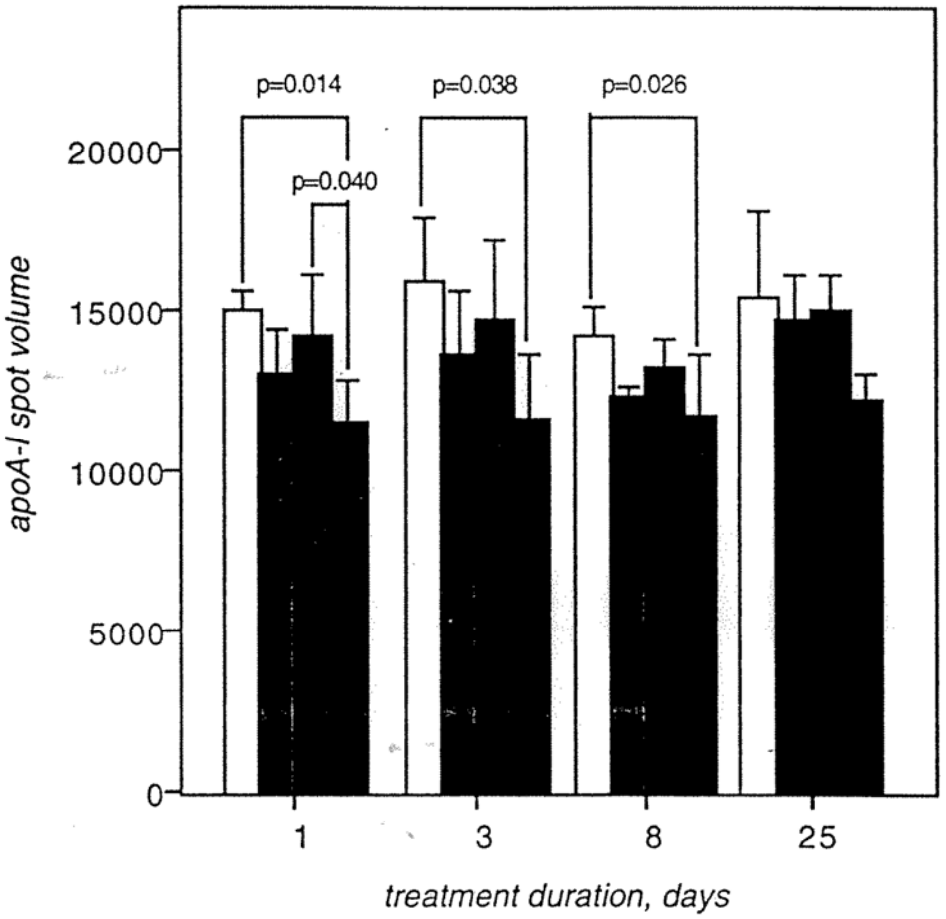
Endpoints responsive mainly to MS: Neutrophils in BALF





mean ± SE, N=8
p< 0.05 vs. sham

Endpoints responsive to MS 250 only: Proteomics approach: apolipoprotein-A1



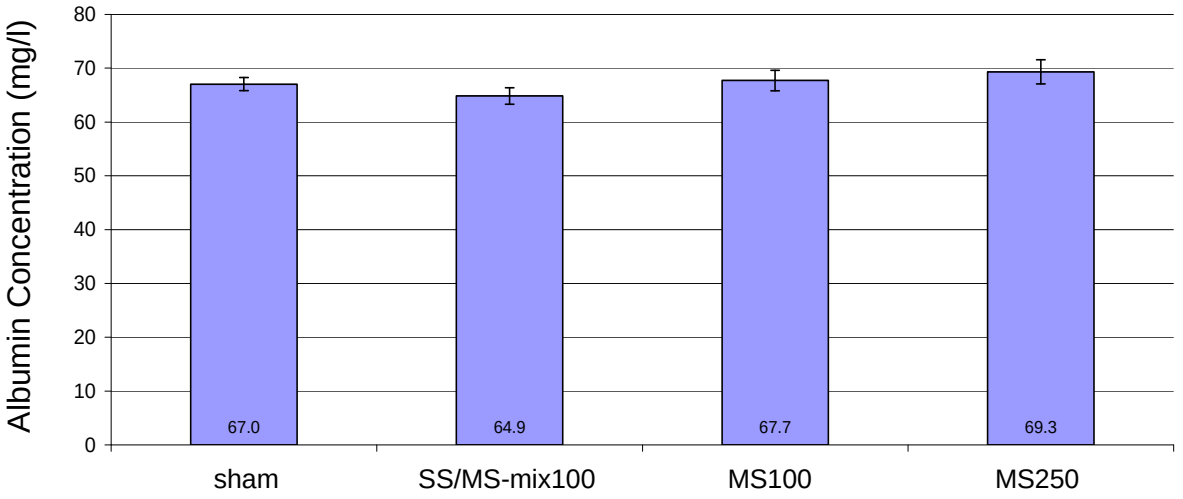
Sham
SS/MS100
MS100
MS250

~20% decrease of Apolipoprotein-A1 in serum compared to sham.

Endpoints not responsive to cigarette smoke

- Lung epithelial permeability (albumin in BALF)
- Micronuclei (bone marrow, blood cells)
- CYP2E1 activity
- DNA adducts

e.g., Albumin



mean ± SE, N=8

Gene expression: MS100

Stress genes

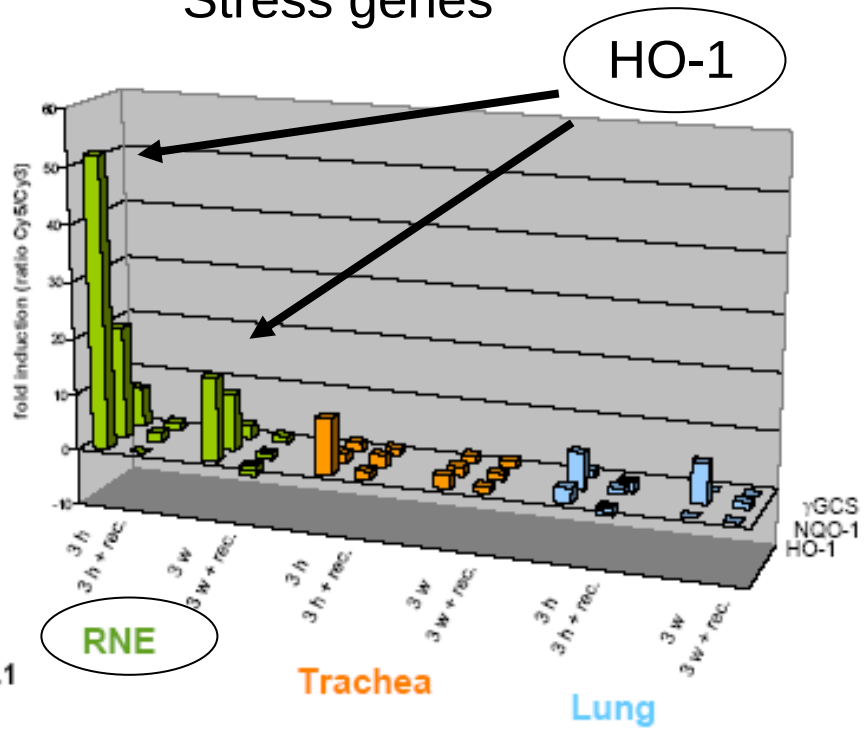
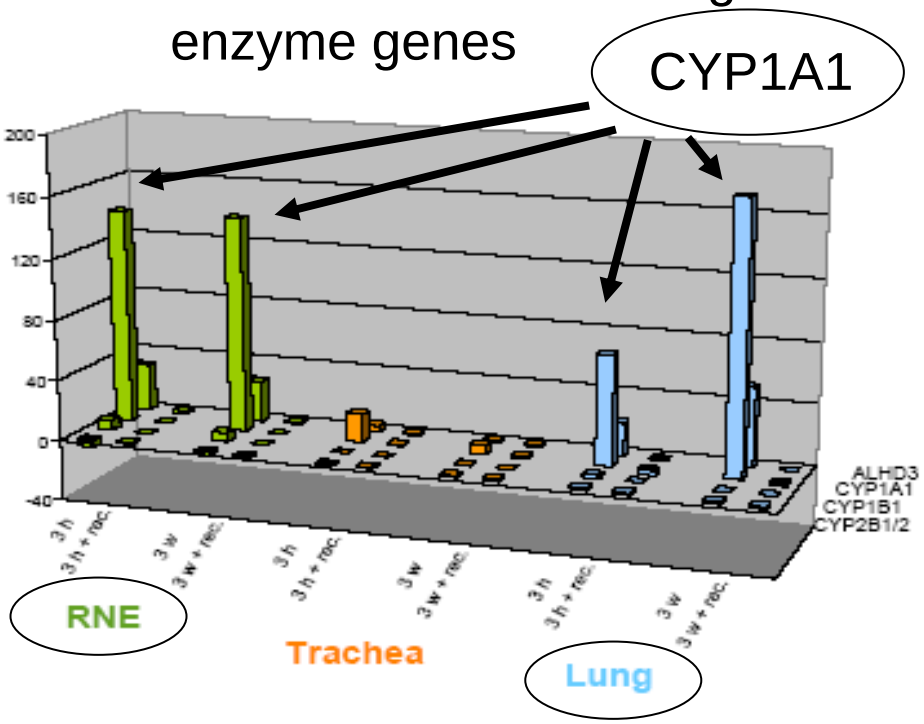


Fig.1

Xenobiotic-metabolizing enzyme genes



From: Gebel et al. (2004)
Carcinogenesis 25:169-178

Novel or confirmed endpoints

- Respiratory Tract

Neutrophils in BALF	confirmed
LDH	confirmed
Oxidative Stress (8-OHdG)	novel methodology
Ratio CYP1A1/CYP 2B1	novel finding
CYP 2E1	no effect
DNA adducts	no effect
Gene expression profiling	novel findings

- Systemic

Body weight	confirmed
Hemoglobin adducts (4-ABP)	confirmed
Apolipoprotein A1	novel finding
Micronucleus bone marrow	no effect

Summary

- We have identified novel biomarkers in the rat which are responsive to cigarette smoke.
- Inflammatory response to cigarette smoke is generally local (e.g., lung neutrophils) rather than systemic.
- Stress-related response to cigarette smoke (i.e, HO-1) is found mainly in rat nasal epithelium.
- SS/MS and MS do not always induce the same response.
- For some endpoints (e.g., DNA adducts), it may be better to extend the exposure period or use assays which are more sensitive.

Conclusion

This multi-endpoint approach, which incorporates novel endpoints and can discriminate SS/MS-mix and MS at similar TPM concentrations, may be useful for evaluating potentially reduced exposure/risk prototypes.

Contributors to this work

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