Mainstream cigarette smoke (MS) affects the cardiovascular system of spontaneously hypertensive rats (SHR)

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Abstract # 912

Introduction

According to the American Heart Association (2009), smoking accounts for more than 440,000 of the more than 2.4 million annual deaths in the United States. In 2004, the Surgeon General reported that heart disease and stroke, which are the main types of cardiovascular disease caused by smoking, were the first and third leading causes of death in the United States.

Spontaneously hypertensive rats (SHR) have been widely used as an animal model to study the transition from hypertension-derived hypertrophy to heart failure. Due to their high blood pressure, these animals develop general hypertrophy of the heart and show several aspects that are similar to the human heart under conditions of increased blood pressure, such as activation of the renin-angiotensin-system and the ventricular expression of transforming growth factor (TGF)-β1.

Although SHR have been used to investigate the basic mechanisms by which hypertension contributes to the development of heart failure, only limited data are available on the combined effect of additional risk factors such as age, gender, smoking, diabetes, and obesity.

Results from previously performed inhalation studies showed impaired heart function in SHR exposed to cigarette mainstream smoke (MS): a significant difference in left ventricular function, an increase in heart weight to body weight ratio, an increase in expression of hypertrophy related genes (whole heart), and a loss of ischemic tolerance (Meurrens et al., 2007).

Objective

Investigate effects of MS on cardiac functional and phenotypic changes, as well as on cardiovascular gene expression, in SHR.

Materials and Methods

The *in vivo* study was performed at PHILIP MORRIS Research Laboratories bvba, Leuven, Belgium. Care and use of the animals was in conformity with 'The Guide for the Use and Care of Laboratory Animals' published by the US National Institute of Health (NIH publication, NIH 85-23, revised 1996). All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC).

Male SHR, 5-9 weeks old, were nose-only exposed to filtered, conditioned air (sham) or to MS from the Reference Cigarette 2R4F at a total particulate matter (TPM) concentration of 450 µg for 1, 2, or 3 h/day (MS-450, MS-900, and MS-1350, respectively), 5 days/week for 13 weeks (including an adaptation phase of 3 days).

The biological endpoints analyzed were:

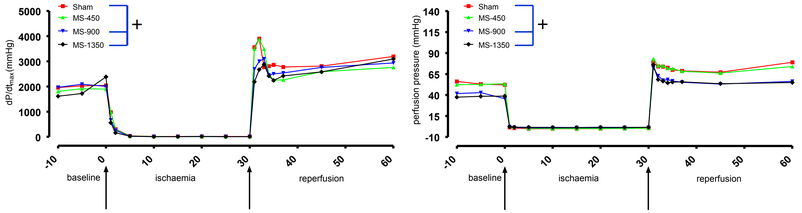
- Heart function (Langendorff-Heart, ischemic-reperfusion protocol with constant coronary flow, modified from Meurrens et al., 2007)
- Heart hypertrophy (ratio heart/left ventricle weight normalized to body weight or tibia length)
- Gene expression (left ventricle tissue: whole genome array analysis [Affymetrix] and quantitative real-time polymerase chain reaction [qRT-PCR])
- Protein analysis (serum)
- Thromboxane metabolites ([2,3-dinor-thromboxane B_1 (2,3-dinor TXB1)] urine)

Results: Heart Function

Parameters

Exposure	Heart Rate (beats/min)	Developed Pressure (mmHg)	dP_{max}/dt (mmHg/sec)	Perfusion Pressure (mmHg)	Group Code
Sham	371.4 ± 19.3	82.4 ± 4.0	2021 ± 86	53.0 ± 2.1	Sham
450 µg TPM/d	368.3 ± 23.1	77.6 ± 6.1	1909 ± 146	52.6 ± 5.5	MS-450
900 µg TPM/d	381.0 ± 17.7	87.0 ± 7.4	2088 ± 129	42.8 ± 1.4 +	MS-900
1350 µg TPM/d	387.7 ± 3.2	73.3 ± 2.8	1719 ± 61	38.4 ± 2.0 +	MS-1350

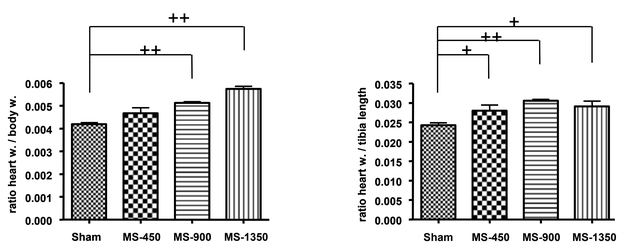
Ischemic Reperfusion



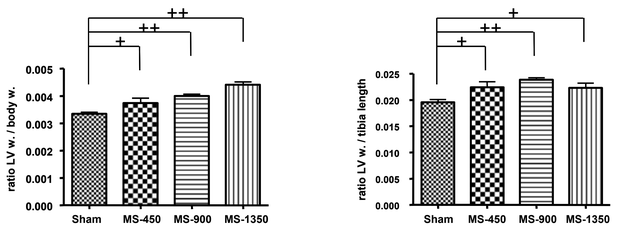
Remarks: Table shows heart function parameters at experimental time point 0 min. Figure shows heart function (dP/dtmax [mmHg] and perfusion pressure [mmHg]) over 60 minutes: 30-min ischemic period and 30 min reperfusion period; +: p ≤0.05.

Results: Heart Hypertrophy

Global Heart Hypertrophy



Left Ventricle Heart Hypertrophy



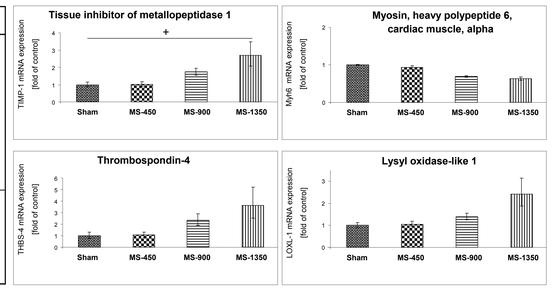
Remarks: Weight of whole heart and weight of left ventricle were normalized to body weight (left side) and tibia length (right side); mean ± SE; n: 6 to 8; +: p≤ 0.05, ++: p≤ 0.01. Results comparable for ratio left ventricle weight to brain weight. Body weight was significantly decreased in the MS-1350 group compared to all other groups.

Results: Gene Expression

Affymetrix Chip

Gene	FC (fold change)	p-value
ribonuclease, RNase A family, 1	6.6	0.0269
thrombospondin 4	6.2	0.0304
orosomucoid 1	6.1	0.0002
aurora kinase B	6.1	0.0121
galectin 3	5.2	0.0172
tissue inhibitor of metalloproteinase 1	4.7	0.0149
defensin beta 1	4.6	0.0160
musculoskeletal, embryonic nuclear protein 1	4.5	0.0350
similar to myotilin	3.7	0.0373
lipocalin 2	3.5	0.0310
calpastatin isoform a	3.5	0.0086
myosin heavy chain, polypeptide 6	-1.6	0.0060
angiopoietin-like protein 4	-4.1	0.0269
endothelial cell-specific molecule 1	-5.3	0.0057
interferon regulatory factor 7	-5.7	0.0325
myxovirus resistance 1	-6.9	0.0040
similar to Interferon, alpha-inducible protein	-9.8	0.0059

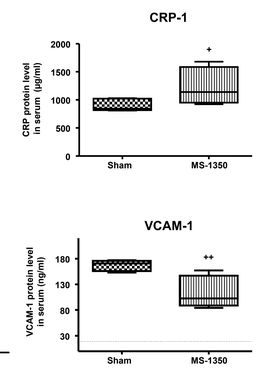
qRT-PCR



Remarks: (Left) Partial list of up- and down-regulated genes of the left ventricle (Affymetrix Rat Genome 230 2.0 Array with around 31 000 analyzed transcripts; complete analysis revealed Fold Change (FC) ≥ 1.6, p≤ 0.05: 113 gene probes; FC ≤ 1.6, p≤ 0.05: 151 gene probes). (Right) qRT-PCR analysis of four selected genes; Standard Error bars represent the maximum (RQmax) and minimum (RQmin) of the relative quantification (RQ). Statistical analysis was performed with ΔCT (threshold cycle) values; n ≥6 animals per group; + p ≤ 0.05.

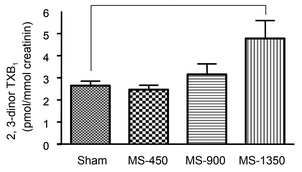
Results: Serum Proteins

Serum Proteins	Sham	MS-1350	Unit	MS-1350 versus Sham
CRP (C Reactive Protein)	844.5	1140	µg/ml	p ≤ 0.05
GCP-2 (Granulocyte Chemotactic Protein-2)	0.23	0.18	ng/ml	p ≤ 0.05
Growth Hormone	96	8.65	ng/ml	p ≤ 0.05
Haptoglobin	627.5	859	µg/ml	p ≤ 0.01
IL-10 (Interleukin-10)	197.5	265.5	pg/ml	p ≤ 0.05
MCP-1 (Monocyte Chemoattractant Protein-1)	911.5	1390	pg/ml	p ≤ 0.05
MCP-3 (Monocyte Chemoattractant Protein-3)	437.5	692	pg/ml	p ≤ 0.05
Myoglobin	331.5	119	ng/ml	p ≤ 0.05
NGAL	77.5	1028	ng/ml	p ≤ 0.01
SCF (Stem Cell Factor)	82	194	pg/ml	p ≤ 0.01
SGOT (Serum Glutamic-Oxaloacetic Transaminase)	18.5	64	µg/ml	p ≤ 0.01
TIMP-1 (Tissue Inhibitor of Metalloproteinase Type-1)	2.05	12	ng/ml	p ≤ 0.01
VCAM-1 (Vascular Cell Adhesion Molecule-1)	170.5	102.5	ng/ml	p ≤ 0.01
vWF (von Willebrand Factor)	285	202.5	ng/ml	p ≤ 0.01



Remarks: All 67 serum proteins were analyzed by Rules Based Medicine, USA; n = 6 samples per group; +: p≤ 0.05, ++: p≤ 0.01. Table shows median values; red = up-regulated; green = down-regulated.

Results: 2,3-dinor TXB_1



Remarks: Urine was collected over 16h under cooled conditions and 2,3-dinor TXB_1 was analyzed by liquid chromatography/mass spectrometry/mass spectrometry; mean ± SE; n = 7 to 8 samples (each sample includes urine from 2 rats) per group; + = p≤ 0.05.

Conclusion

Exposure to cigarette smoke revealed a dose-dependent impact on the cardiovascular system of SHR: decrease in heart function parameters, increase in hypertrophy status, changes in gene expression, and increase in TXB_1 in urine.

The data show that this model system is responsive to cigarette smoke and therefore may be useful in dissecting the mechanisms involved in smoke-induced cardiovascular disease and potentially for screening reduced-risk tobacco products. It is important to remember, however, that increased hypertrophy of the left ventricle is not described as a disease caused by cigarette smoke in humans.

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Acknowledgement

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