Influence of Sub-Chronic Cigarette Smoke Exposure on the Progression of Myocardial Hypertrophy in Spontaneously Hypertensive Rats (SHR)

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Introduction and Objective

In search of an experimental model for cardiac risk by smoking, we investigated the development of myocardial hypertrophy in Spontaneously Hypertensive Rats (SHR) exposed to cigarette mainstream smoke.

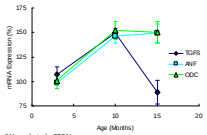
Why Myocardial Hypertrophy?

- Myocardial hypertrophy in response to hemodynamic overload is an established risk factor for cardiovascular morbidity and mortality. Pressure overload hypertrophy is characterized by a period of compensation, followed by transition to cardiac failure.
- On the molecular level, ventricular hypertrophy shows up as specific changes in cardiac gene expression, including the induction of "immediate-early genes" and hypertrophy-associated proteins such as **atrial natriuretic factor (ANF)**, leading to a "fetal" phenotype.
- Up-regulation of ventricular transforming growth factor β_1 **(TGF-b_1)** expression and **ornithine decarboxylase (ODC)** is a characteristic molecular feature of the transition from myocardial hypertrophy to heart failure.
- Several cardiac diseases are associated with an increased expression of TGF-β_1 mRNA, particularly during the transition from stable cardiac hypertrophy to heart failure.
- TGF-β_1 mRNA expression is increased in the left ventricular myocardium of patients with idiopathic hypertrophic cardiomyopathy and dilated cardiomyopathy.
- Left ventricular hypertrophy appears to be induced by either pressure overload or excess neurohumoral activation including the release of angiotensin II, norepinephrine, and aldosteron.
- The progression of myocardial hypertrophy is sensitive to life style factors, e.g., **smoking**.

Why SHR?

- SHR are a suitable model for studying transcriptional alterations in failing hearts compared to normal hearts because SHR exhibit symptoms similar to the clinical symptoms seen in human patients suffering from high blood pressure.

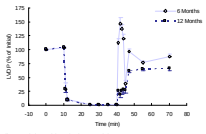
Growth Factor Expression in SHR-Stroke Prone



(Wenzel et al., 2001)

Error bars represent standard error of the mean.

Post-Ischemic Recovery of Male SHR During Ageing



(internal data; historical controls)
LVDP: left ventricular developed pressure
Error bars represent standard error of the mean.

Objective: Investigate smoke-induced progression of myocardial hypertrophy as well as induction parameters normally associated with heart failure in SHR.

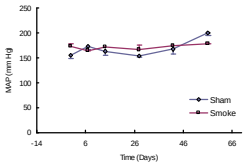
Study Design and End Points

- 60 male SHR
  - 30 rats exposed to cigarette mainstream smoke (450 µg total particulate matter/l) for 30, 60, or 90 days (Smoke), 5 days per week, 2 x 1 h with a 30-min fresh air exposure break
  - 30 rats exposed to fresh air (Sham)
- The study was conducted in accordance with the American Association for Laboratory Animal Science Policy on the Human Care and Use of Laboratory Animals (1991).

End Point	What	How
hypertension	mean arterial pressure (MAP)	telemetric analysis: during exposure period
hypertrophy marker	ratio: heart weight (HW) to body weight (BW)	gravimetry: wet weight ratios 30, 60, and 90 days
	ANF expression	mRNA (real time-PCR): 30 and 90 days, protein (immunoblot): 60 days
changes in growth factor response	TGF-ß, ODC	mRNA (real time-PCR): 30 and 90 days, protein (immunoblot): 60 days
ischemic tolerance	post-ischemic recovery after 30 min no-flow	LVDP: ex vivo (Langendorff technique): 30, 60, and 90 days

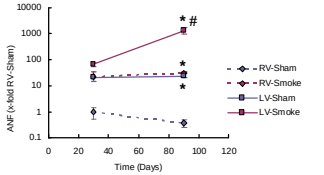
Results

Spontaneous Hypertension



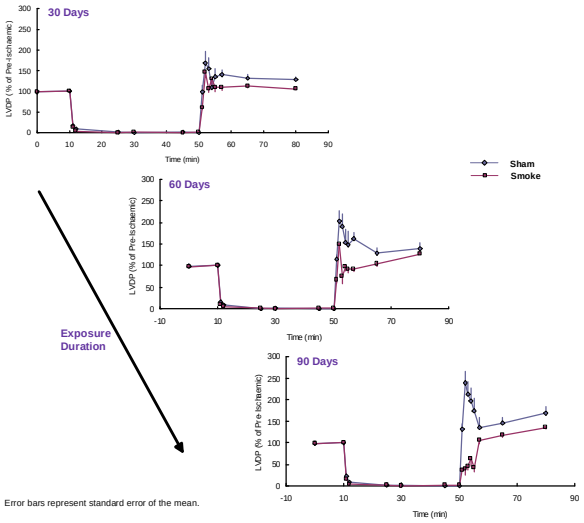
MAP: mean arterial pressure
Error bars represent range.

Hypertrophy Marker (ANF expression (mRNA))



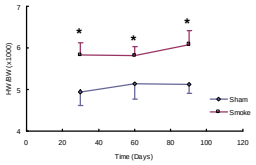
Statistical significance (p <0.01): * = LV-Smoke vs RV-Sham
RV-Smoke vs RV-Sham
LV-Sham vs RV-Sham
= LV-Smoke 90 days vs LV-Smoke 30 days
Error bars represent standard deviation.

Post-Ischemic Recovery



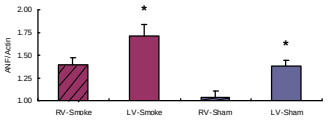
Error bars represent standard error of the mean.

Hypertrophy Marker (heart weight to body weight ratio)



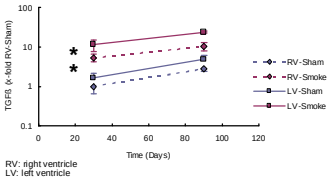
Statistical significance (p <0.01): * = Smoke vs Sham
Error bars represent standard deviation.

Hypertrophy Marker (ANF protein levels)



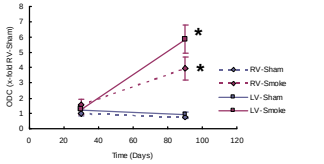
Statistical significance (p <0.01): * = LV-Smoke vs RV-Sham
LV-Sham vs RV-Sham
Error bars represent standard deviation.

Growth Factors (TGF-β_1 expression (mRNA))



RV: right ventricle
LV: left ventricle
Statistical significance (p <0.01) 30 days: * = RV-Smoke vs RV-Sham
LV-Smoke vs RV-Sham
Error bars represent standard deviation.

Growth Factors (ODC expression (mRNA))



Statistical significance (p <0.01) 90 days: * = RV-Smoke vs RV-Sham
LV-Smoke vs RV-Sham
Error bars represent standard deviation.

Summary and Conclusion

- Sub-chronic cigarette smoke exposure in male SHR
  - has no impact on mean arterial pressure,
  - increases the extent of hypertrophy in both ventricles (as measured by ANF),
  - accelerates the disease-specific characteristic impairments of growth factor pathways, and
  - accelerates the loss of ischemic tolerance.

The progression of myocardial hypertrophy as well as parameters normally associated with heart failure in Spontaneously Hypertensive Rats are accelerated by cigarette smoke.

References
American Association for Laboratory Animal Science Policy on the Humane Care and Use of Laboratory Animals. *Lab. Animal Sci.* 41: 91 (1991)
Wenzel et al., *J. Mol. Cell Cardiol.* 33(9): 1181-90 (2001)

SOT 2005, New Orleans, LA, U.S.A.
Data also presented at 26th Annual Congress of the European Society of Cardiology, August 2004 in Munich, Germany.