Effects of nicotine on vasomotor function of rat aortic rings in vitro

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Introduction and Objective

Cigarette mainstream smoke (MS) is a known risk factor for atherosclerosis. One of the early steps in the development of atherosclerosis is a dysfunctional endothelium. Clinical studies have shown that smokers without atherosclerotic disease have a significant reduction in endothelium-dependent vasodilatation compared to non-smokers. This association is dose-dependent, i.e., the vasodilatation decreased with number of pack-years[1]. An *in vitro* test system to measure cigarette-smoke-dependent endothelial dysfunction is the treatment of rat aortic rings with aqueous solutions of MS. Experiments with this test system show that MS enhances the vasoconstriction and diminishes the vasorelaxation properties of rat aortic rings. The objective of this study was to evaluate whether nicotine, alone, has the same effect as MS on vessel function *in vitro*.

Materials and Methods

Cigarettes and Smoke Generation

- ❑ MS from Reference Cigarettes[2]: 2R4F, 3R4F, and 1R5F
- ❑ MS generated on 20-port Borgwaldt-smoking machine: ISO Standard 3308[3] or Massachussettes[4] (MCTPS) conditions

Parameter	ISO[3]	MCTSP[4]
Puff volume (ml)	35	45
Puff duration (s)	2	2
Puff number per min	1	2
Filter ventilation blocked (%)	0	50

Test Substances

- ❑ (-)-nicotine (Sigma Aldrich, Germany)
- ❑ MS-bubbled phosphate-buffered saline (sbPBS): MS bubbled through buffer:
- ❑ gas/vapor phase (GVP): MS passed a Cambridge filter and bubbled through buffer
- ❑ total particulate matter (TPM): MS trapped a Cambridge filter and eluted with DMSO (note: most nicotine in MS is found in TPM)

Characterization of sbPBS

- ❑ nicotine measured by gas chromatography
- ❑ aldehydes measured by HPLC after derivatization with 2,4-dinitrophenylhydrazine

Aortic ring assay

- ❑ Male Wistar Unilever rats (Harlan Winkelmann, Netherlands)
- ❑ Thoracic aortic rings mounted in an organ bath system (ADInstrument, Germany)
- ❑ Incubation of aortic rings with test substances (different concentrations) for 45 min followed by relaxation or constriction:
  - -relaxation with acetylcholine (ACh, 10^{-8} M to $3*10^{-4}$ M) after pre-constriction with 10^{-7} M NE
  - -constriction with norepinephrine (NE, 10^{-8} M to 10^{-4} M)

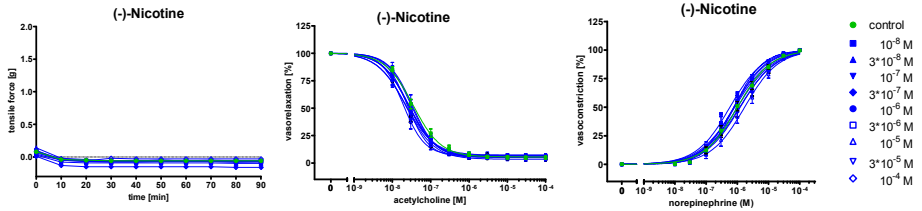
Characterization of sbPBS

	Nicotine [µg/ml]	Acetaldehyde [µg/ml]	Acrolein [µg/ml]	Propionaldehyde [µg/ml]
2R4F (ISO)	12.2 +/- 0.8	125.8 +/- 4.8	16.6 +/- 0.9	7.8 +/- 0.3
1R5F (MTCSP)	8.4 +/- 0.8	150.4 +/- 4.8	18.8 +/- 0.6	8.6 +/- 0.5

1R5F vs. 2R4F (%)			
Acetaldehyde	Acrolein	Propionaldehyde	
119.6	113.6	109.5	

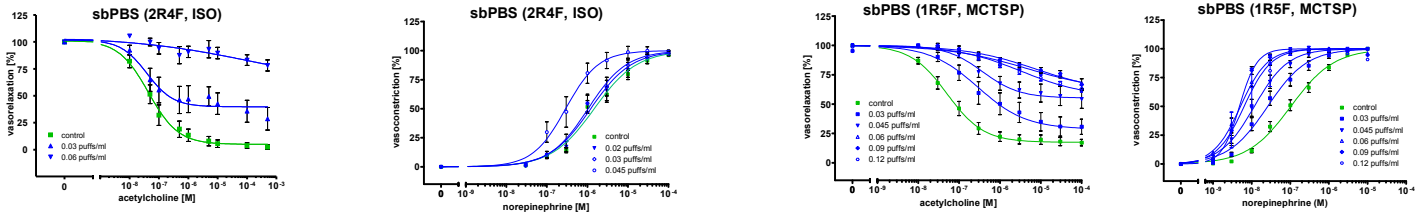
❑ 1R5F-sbPBS (MCTSP) had lower nicotine but similar aldehyde levels compared to 2R4F-sbPBS (ISO)

Results: Effect of nicotine on vasomotor function



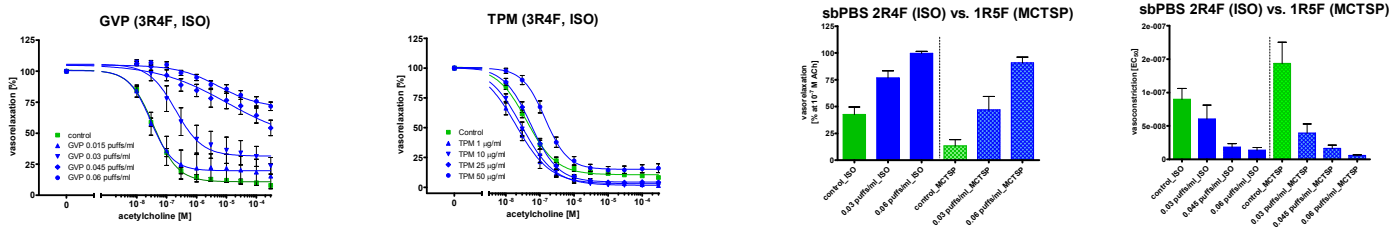
❑ (-)-nicotine has no effect on the tensile force of rat aortic rings
❑ (-)-nicotine has no effect on ACh-induced vasorelaxation or NE-induced vasoconstriction

Results: Effect of MS on vasomotor function



❑ sbPBS (2R4F, ISO) diminishes ACh-induced vasorelaxation and enhances NE-induced vasoconstriction

❑ sbPBS (1R5F, MCTSP) diminishes ACh-induced vasorelaxation and enhances NE-induced vasoconstriction



❑ GVP (3R4F, ISO) diminishes ACh-induced vasorelaxation
❑ TPM (3R4F, ISO) has no effect on ACh-induced vasorelaxation

❑ No difference between the 2R4F-sbPBS (ISO) and 1R5F-sbPBS (MCTSP) on ACh-induced vasorelaxation or NE-induced vasoconstriction

Acknowledgement

The authors wish to thank Kerstin Landwehr, Gabriele Kirsch, Dorothea Klinghardt, Nadine Gorges, and Josef Severic for technical assistance.

References

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Conclusion

Results suggest that the endothelial dysfunction of rat aortic rings after treatment with aqueous solutions of cigarette mainstream smoke *in vitro* is not mediated by nicotine.

Further research is necessary to identify the compounds that evoke this effect.

Keystone Symposia: Dissecting the Vasculature: Function, Molecular Mechanisms and Malfunction (J8), Feb 24 - Mar 1, 2009, Vancouver, British Columbia