Impact of cigarette smoke on NO bioavailability in endothelial cells: role of vascular NADPH oxidase

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Introduction

Dysfunctional endothelium is recognized as one of the earliest steps in the development of atherosclerosis. Cigarette smoking has been shown to cause endothelial dysfunction characterized by a reduced synthesis and/or enhanced inactivation of vasoprotective nitric oxide (NO) produced by endothelial NO synthase (eNOS). NO prevents oxidative modification, e.g., induced by low-density lipoprotein (LDL) which is considered a major cause of endothelial cell dysfunction; however, the mechanisms involved are not completely understood.

Objective

Determine the effect of cigarette smoke (CS) by measuring:
1) NO levels in human umbilical vein endothelial cells (HUVECs)
2) Bioavailability of NO by measuring the cGMP level
3) Oxidation product of NO (nitrite [NO_2^-] in HUVECs
4) Reaction product of superoxide and NO peroxynitrite
5) Endothelial function in rat aortic rings

Materials and methods

Aqueous CS extract: Mainstream smoke was generated from the Reference Cigarette 3R4F (University of Kentucky, 2003) on a Borgwaldt RM 20-port smoking machine, bubbled through PBS, and diluted in medium (0.03 puffs/ml).

Cell treatment: HUVECs (Promocell) were cultured in growth medium to confluency on 6 cm dishes. Treatment was performed in experimental medium (2% FCS) with PBS, LDL (100µg/ml), oxidized LDL (100µg/ml), and aqueous CS extract (0.03 puffs/ml) for another 24h.

NO measurement: Supernatant of HUVECs was evaluated by measuring the accumulation of the stable NO-metabolism product nitrite with a chemiluminescence analyzer. Cells were stained with the NO-specific fluorescent dye DAF (Diaminofluorescein). Fluorescence was quantified with a plate reader (Arrayscan, Cellomics; N = 4 in triplicates).

cGMP measurement: Cell lysates were analyzed with a cGMP ELISA (Cayman). Values (N=4 in triplicates) obtained after 24h treatment. Positive control: papanoate (NO donor); negative control: morpho-sydnonimine (SIN).

Nitrite measurement: HUVEC supernatants were analyzed with an NO analyzer. L-NAME: monomethyl-L-arginine; NOx inhibitor: apocynin. Values (N = 4 in triplicates).

Reaction products of NO: Formation of peroxynitrite was evaluated with a 3-Nitrotyrosine antibody in a dot blot.

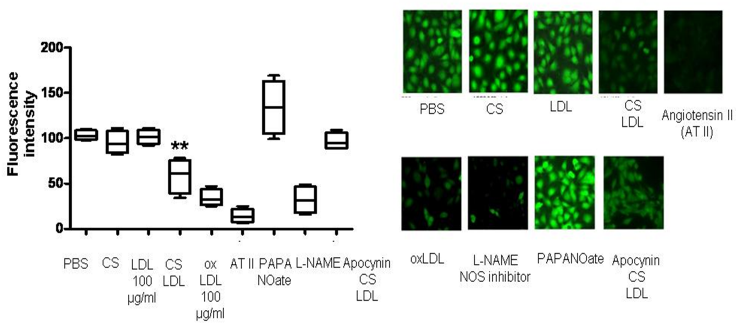
Aortic ring assay: All animal procedures were ethically approved by our in-house animal welfare committee and the German authorities. Thoracic aortic rings from male Wistar Unilever rats (Harlan Winkelmann, Germany) were analyzed with an ADInstrument organ bath system, model LEO1.046. Pretreatment with apocynin / CS ≥ 30min; apocynin (NADPH oxidase inhibitor):100 µM; n=7.

Statistics: Comparisons between groups (PBS treatment / CS and LDL treatment) were performed by means of standard t-tests. No confirmatory tests were performed. Data are presented as mean ± SE.
* p <0.05 ; ** p <0.01.

Results

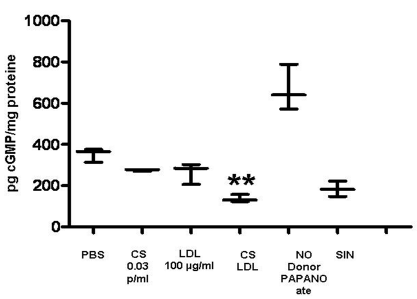
1) NO levels

• **decrease in nitric oxide (steady state) by CS/LDL**



2) Bioavailability of NO levels

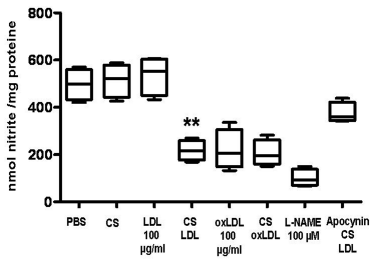
• **impaired bioavailability of NO (cGMP) by CS/LDL**



Results

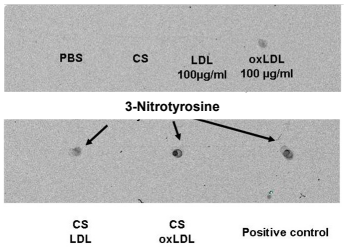
3) Oxidation product of NO

• **decrease in nitrite by CS/LDL**



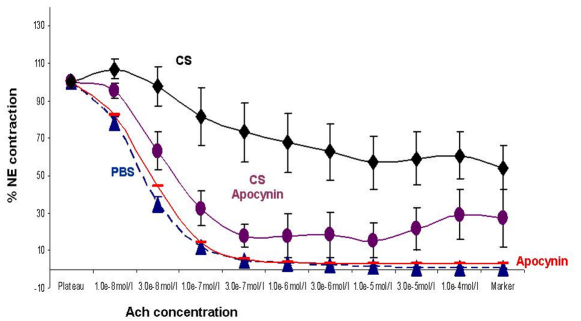
4) Reaction product of superoxide and NO peroxynitrite

• **increase in peroxynitrite by CS/LDL**



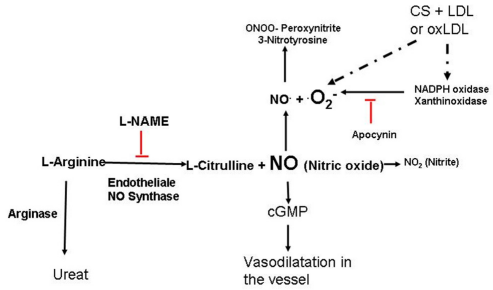
5) Endothelial function

• **impairment of endothelial function in rat aortic rings by CS**



Summary and Conclusion

Treatment with cigarette smoke resulted in reduced levels of nitrite, increased levels of superoxide anion and 3-nitrotyrosine, and enhanced arginase-1 activity. From this, we conclude that cigarette smoke combined with LDL increases endothelial superoxide production, resulting in decreased nitric oxide (NO) bioavailability and increased generation of peroxynitrite similar to the effects of oxLDL. Both mechanisms may contribute to a decreased bioavailability of vasoprotective NO and thereby may be involved in the cigarette-smoke-induced development of atherosclerosis.



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