Hydrogen Peroxide-Induced Superoxide Production in Endothelial Cells: Role of Nitric Oxide Synthase

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Background: Hydrogen peroxide causes oxidant injury to vascular cells and may contribute to the pathogenesis of vascular disease and endothelial dysfunction. In smooth muscle cells (SMC), hydrogen peroxide causes oxidant injury via NAD(P)H oxidase-dependent production of superoxide and superoxide scavengers protect against hydrogen peroxide-induced SMC cytotoxicity. We investigated the potential enzymatic sources and consequences of hydrogen peroxide-induced superoxide production in quiescent endothelial cells exposed to physiological shear.

Methods: Superoxide production was examined by dihydroethidium fluorescence. Nitric oxide production was quantified via Greiss assay and intracellular staining with DAF-FM diacetate. Cell viability was determined by propidium iodide staining, and cell detachment with lower activity of calcein and PI staining.

Results: Under static conditions, 60 µM hydrogen peroxide induced superoxide production in porcine aortic endothelial cells (PAEC). In the presence of L-NAME (1 mM), a specific inhibitor of nitric oxide synthase (NOS), there was a sixty percent reduction in cellular superoxide production (n=9, p<0.001). Apocynin (0.5 mM), an inhibitor of NAD(P)H oxidase, combined with L-NAME (1 mM) was also found to reduce superoxide production. Manganese superoxide dismutase (MnSOD) overexpression by adenoviral-mediated gene transfer reduced hydrogen peroxide-induced superoxide levels five-fold (n=7, p<0.05), whereas overexpression of MnSOD failed to produce a significant effect when physiological shear (n=4, p=0.03) conditions as assayed by hydroethidine fluorescence. Hydrogen peroxide induced endothelial cytotoxicity and detachment under static and shear conditions, which was reduced by MnSOD overexpression or L-NAME or the combination of L-NAME and MnSOD.

Conclusions: In conclusion, in culture and shear-exposed PAEC, hydrogen peroxide induces production of superoxide, which contributes to cytotoxicity in these cells. eNOS appears to be an important enzymatic source of hydrogen peroxide-induced superoxide production in PAEC.

Elevated Gene Expression of Asymmetric Dimethylarginine-Generating Enzymes in Atherosclerosis

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Background: Endothelial function is altered early in the course of atherosclerosis resulting in reduced biological activity of nitric oxide. NO-synthase I-III as well as GTPC1-1 (formylating tetrahydrobiopterin, an essential cofactor for all NO-synthase isoforms) contribute to the generation of NO. Arginase II and I lead to arginine, the substrate of NO. PRMT I-III contribute to the synthesis of ADMA (inhibitor of NO-synthesis) whereas DDAH2 reduces ADMA. SOD I-III and p22phox, a subunit of NADPH oxidase, are involved in the production of ADMA. We hypothesized that the expression of these enzymes is involved in the reduction of NO-availability in atherosclerosis.

Methods. Gene and protein expression were determined by western blot and real-time PCR in human myocardium from patients with and without atherosclerosis.

Results. All enzymes could be detected in myocardial tissue. Expression of p22phox, SOD I-III as well as arginase I and II and PRMTII was unaltered in atherosclerotic tissue. expression of all NO-synthase isoforms as well as DDAH2 was significantly decreased. In contrast, the level of PRMT I and III was increased in tissue from patients with atherosclerosis.

Conclusion. In myocardial tissue from patients with atherosclerosis, activity of NO-synthase is severely decreased. Additionally, an enhanced activity of PRMT I in combination with reduced activity of DDAH2 could induce an increased formation of ADMA, a potent inhibitor of NO-synthesis. The altered gene expression pattern as observed in our investigation could contribute to the known reduction of NO-availability in atherosclerosis.

Sulfatides Are Targets for Antiphospholipid Antibodies

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Background: Sulfatides are sulfated glycolipids such as sphingomyelin, cerebrosides, galactosylceramide and cerebroside. At a sulfatide coating density of 5 µg/ml, 12-glycoprotein I reached a half-maximally binding to 2.5 µg/ml and binding density was saturated at 10 µg/ml. The binding of 12-glycoprotein I also depended on the coating density of sulfatides in the well. At a constant 12-glycoprotein I concentration of 5 µg/ml, maximal binding of 12-glycoprotein I was observed at a sulfatide coating density of 1 µg/ml. 12-glycoprotein bound also to vesicles containing sulfatides and aggregated these vesicles in a concentration-dependent manner. Furthermore, we examined the sera of 14 patients with antiphospholipid antibodies, a subset of antiphospholipid antibodies, for their binding to sulfatide-bound 12-glycoprotein I. All of these sera interacted with sulfatide-bound 12-glycoprotein I as efficiently as with cardiac troponin I-2-glycoprotein I. In addition, some of these patients have antibody species that are specific for sulfatide-12-glycoprotein I complex. Conclusion: These results show that not only anionic phospholipids, as commonly known, but also sulfatides are targets for antiphospholipid antibodies, thereby possibly contributing to some of the clinical symptoms of the antiphospholipid syndrome.

Fibrates Inhibit Plasminogen Activator Inhibitor Type-1 Expression in Human Adipose Tissue: An Antiatherogenic Phenomenon?

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Background: Plasminogen Activator Inhibitor Type-1 (PAI-1) is an independent risk factor for cardiovascular disease and is extensively produced by adipose tissue. Fibrates are lipid lowering agents activating peroxisome proliferator activator receptor-alpha (PPAR-α). Recent experimental as well as clinical data attributed additional antiatherosclerotic properties to these substances. The mechanisms, however, remain to be fully understood. The current study investigates a possible modulation of PAI-1 expression in adipose tissue by fibrates.

Methods. Human preadipocytes in primary culture were exposed to selected concentrations of gemfibrocil and fenofibrate (100, 350, 700µM) in the presence or absence of 40µM transforming growth factor-beta (TGF-β), an established stimulus for PAI-1 expression. PAI-1 protein was measured by ELISA, PAI-1 mRNA by light cycle RT-PCR. Parallel experiments were performed in cultured differentiated human adipocytes.

Results: In both cell fractions of human adipose tissue, in preadipocytes and adipocytes, gemfibrocil and fenofibrate reduced PAI-1 protein expression by up to 93±10% (n=6, P=0.01) and 27±8% (n=6, P=0.01) under basal conditions and up to 35±6% (n=6, P<0.001) and 18±5% (n=6, P<0.04) when stimulated with 40µM TGF-β. Coincubation with 350µM gemfibrocil and 5µM troglitazone, a PPAR-γ agonist known to downregulate PAI-1 expression in adipose tissue, did not produce an additional effect compared to either of the substances alone suggesting a common final pathway (n=4, P=n.s.). Time course experiments showed a maximal effect after 24 hours (n=3, P<0.05 each), and not merely pleiotropic.

Conclusions: Our data support the view that PPAR-α activation is an antiatherosclerotic phenomenon that may diminish cardiovascular risk, possibly by downregulation of PAI-1 expression in adipose tissue.

Enhanced Expression of Pro-Oxidant Enzyme Myeloperoxidase Is Associated With Plaque Destabilization in Human Coronary Atherosclerotic Lesions

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Background: Presently, a growing body of literature have shown that inflammation and oxidant stress in coronary atherosclerotic lesions relate to plaque rupture and subsequent acute plaque destabilization. We have recently demonstrated that neutrophils play a role in mediating plaque destabilization of atherosclerotic plaques (Naruko T et al, Circulation 106, 2002). Recent studies have demonstrated the presence of active pro-oxidant enzyme myeloperoxidase (MPO). The products of MPO-mediated reaction in human atherosclerosis. To investigate the potential significance of MPO in human coronary atherosclerosis, we immunohistochemically studied the expression of MPO in human coronary atherosclerotic lesions.

Methods: Frozen sections of normal coronary artery segments (Normal, n=26), and coronary atherosclerotic segments (n=76) with fibroplid plaque (FLP; n=31), fibrous plaque (FP; n=30), ruptured plaques (RP; n=8), and eroded plaques (E; n=5) were stained with antibodies against smooth muscle cells, macrophages, endothelial cells, MPO and neoprithin(CD66b, CD11b, and elastase). We used computer-aided planimetry which quantified the immunoreactivity of macrophages, neutrophils and MPO positive area. Moreover for the identification of cell types which stain positive for MPO, immunodouble staining with MPO and macrophages or MPO and neutrophils was also performed. Results: Quantitative analysis demonstrated that neutrophil, macrophages, or MPO positive area as significantly (P<0.0001) higher in EP and RP than in FLP and FP. Immunodoublestaining for MPO and neutrophils or macrophages revealed that the majority of MPO-positive cells were neutrophils, and occasional macrophages were also positive for MPO. Conclusions: These findings strongly suggest that strong expression of MPO positive neutrophils plays an important role in the pathogenesis of plaque destabilization in human coronary arteries.

Hypoxia Increased Matrix Metalloproteinase Activity and Superoxide Generation in Vascular Smooth Muscle Cells of Female Artery of Apolipoprotein E-Knockout Mice

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Background: Arterial wall hypoxia and the associated vascular smooth muscle cell proliferation might be implicated in the development of atherosclerosis. To evaluate the effect of hypoxia on the artery before atherosclerosis, we examined matrix metalloproteinase activities including gelatinase, collagenase, and stromelysin activities in the aorta and the coronary artery of female ApoE-KO mice.