plaque: large lipid core, extensive macrophage density and neoangiogenesis. Large areas of calcification, on the other hand, have lower (or absent) lipid cores, macrophage and angiogenesis density.

1140-170 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 and in cells exposed to physiologic 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 was assessed under static and shear conditions.
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 seventy-five percent under static (n=12, p-value less than 0.001) and forty-seven percent under physiological shear (n=4, p=0.03) conditions as assayed by hydroethidium 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 Apocynin.
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.

1140-171 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 GTPCH–1 (formation of tetrahydrobiopterin, an essential cofactor of all NO-synthase isoforms) contribute to the generation of NO. Arginase I and II reduce L-arginine, the substrate of NO. PRMT I - III contribute to the synthesis of ADMA (inhibitor of NO-synthase) whereas DDAH2 reduces ADMA. SOD I - III and p22phox, a subunit of NADPH oxidase, are involved in the metabolism of oxygen free radicals. We hypothesized that alterations in the gene 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. Whereas 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 PRMTI and III was increased in tissue from patients with atherosclerosis.
Conclusion. In myocardial tissue from patients with atherosclerosis, acitivity of NO-synthase is severely decreased. Additionally, an enhanced activity of PRMT in combination with lower activity of DDAH2 could induce an increased formation of ADMA, a potent inhibitor of NO-synthase. The altered gene expression pattern as observed in our investigation could contribute to the known reduction of NO-availability in atherosclerosis.

1140-172 Sulfatides Are Targets for Antiphospholipid Antibodies

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Background Sulfatides are sulfated glycosphingolipids expressed on the surface of erythrocytes, leukocytes and platelets. Sulfatides interact with several cell adhesion molecules involved in hemostasis. β2-glycoprotein I is an anionic phospholipid-binding plasma protein and the phospholipid-bound form is the target for most antiphospholipid antibodies that are associated with recurrent thrombosis, miscarriages and neurological symptoms. In this study, we examined whether β2-glycoprotein I forms a complex with sulfatides, and thereby becomes a target for antiphospholipid antibodies.
Methods We used ELISA-assays for the binding studies, and spectrophotometry to measure vesicle aggregation. Anticardiolipin antibodies were isolated by immunoaffinity purification, β2-glycoprotein I was isolated by gel filtration.
Results β2-glycoprotein I bound saturably to surface-bound sulfatides, but not to other glycolipids such as ceramide, cerebrosides, sphingomyelin or ganglioside. At a sulfatide coating density of 1 μg/well, β2-glycoprotein I reached half-maximal binding at 2.5 μg/ml and the binding saturated at 10 μg/ml. The binding of β2-glycoprotein I also depended on the coating density of sulfatides in the well. At a constant β2-glycoprotein I concentration of 5 μg/ml, maximal binding of β2-glycoprotein I was observed at a sulfatide coating density of 1 μg/well. β2-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 anticardiolipin antibodies, a subset of antiphospholipid antibodies, for their binding to sulfatide-bound β2-glycoprotein I. All of these sera interacted with sulfatide-bound β2-glycoprotein I as efficiently as with cardiolipin-bound β2-glycoprotein I. In addition, some of these patients have antibody species that are specific for sulfatide-β2-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.

1140-173 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 activated 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 40pM 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 cycler RT-PCR. Parallel experiments were performed in cultures of 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 39±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 40pM TGF-β. Coincubation of 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). Total protein quantification indicated persistant viability of cells. Quantification of mRNA proofed that regulation was at least in part at the transcriptional level (n=3, P<0.01 each). Incubation with nonfibrate PPAR-α agonists showed similar reductions in PAI-1 expression suggesting that effects were mediated by PPAR-α (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 down-regulation of PAI-expression in adipose tissue.

1140-174 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 oxidative stress in coronary atherosclerotic lesions relate to rapidly progressive plaque destabilization. We have recently demonstrated that neutrophils play a role in mediating destabilization of atherosclerotic plaques (Naruko T et al, *Circulation* 106, 2002). Recent studies have demonstrated the presence of active pro-oxidant enzyme myeloperoxidase (MPO) and 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=74) with fibrolipid plaque (FLP; n=31), fibrous plaque (FP; n=30) , ruptured plaques (RP; n=8), and eroded plaques (EP; n=5) were stained with antibodies against smooth muscle cells, macrophages, endothelial cells, MPO and neutrophils(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 was 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.

1140-175 Hypoxia Increased Matrix Metalloproteinase Activity and Superoxide Formation Before Atherosclerosis in 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