

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

Christian H. Coyle, Neal L. Weintraub, Khalid N. Kader, University of Iowa, Iowa City, IA, University of Iowa Hospitals and Clinics, Iowa City, IA

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 μ M 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

Christiane P. Tiefenbacher, Chen Xiaobo, University of Heidelberg, Heidelberg, Germany

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, activity 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

Michael Merten, Frank C. Arnett, Perumal Thiagarajan, Baylor College of Medicine, Houston, TX, University Clinic of Hamburg-Eppendorf, Hamburg, Germany

Background Sulfatides are sulfated glycosphingolipids expressed on the surface of erythrocytes, leukocytes and platelets. Sulfatides interact with several cell adhesion molecules involved in hemostasis. β 2-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 β 2-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, β 2-glycoprotein I was isolated by gel filtration.

Results β 2-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 μ g/well, β 2-glycoprotein I reached half-maximal binding at 2.5 μ g/ml and the binding saturated at 10 μ g/ml. The binding of β 2-glycoprotein I also depended on the coating density of sulfatides in the well. At a constant β 2-glycoprotein I concentration of 5 μ g/ml, maximal binding of β 2-glycoprotein I was observed at a sulfatide coating density of 1 μ g/well. β 2-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 β 2-glycoprotein I. All of these sera interacted with sulfatide-bound β 2-glycoprotein I as efficiently as with cardiolipin-bound β 2-glycoprotein I. In addition, some of these patients have antibody species that are specific for sulfatide- β 2-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?

Andreas Zirk, Anne Leugers, Sandra Ernst, Christoph Bode, Burton E. Sobel, Thomas K. Nordt, University of Freiburg, Freiburg, Germany, University of Vermont College of Medicine, Burlington, VT

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- α (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 gemfibrozil and fenofibrate (100, 350, 700 μ M) in the presence or absence of 40pM transforming growth factor- β (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, gemfibrozil and fenofibrate reduced PAI-1 protein expression by up to 39 \pm 10% (n=6, P=0.01) and 27 \pm 8% (n=6, P=0.01) under basal conditions and up to 35 \pm 6% (n=6, P=0.001) and 18 \pm 5% (n=6, P=0.04) when stimulated with 40pM TGF- β . Coincubation of 350 μ M gemfibrozil and 5 μ M 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 persistent viability of cells. Quantification of mRNA proved 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

Ryushi Komatsu, Takahiko Naruko, Akira Itoh, Kazuo Haze, Nobuyuki Shirai, Eishu Hai, Yoshihiro Ikura, Masahiko Ohsawa, Makiko Ueda, Osaka City General Hospital, Osaka, Japan, Osaka City University Graduate School of Medicine, Osaka, Japan

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

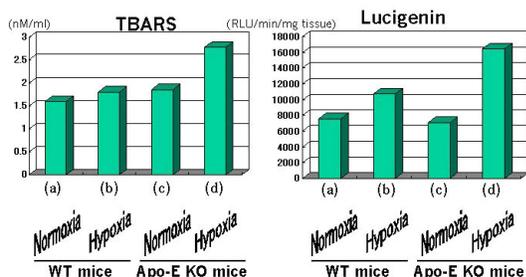
Nobuaki Okuda, Tetsuya Hayashi, Tatsuhiko Mori, Koichi Sohmiya, Sakiko Inamoto, Yasushi Kitaura, Naoko Tazawa, Daisuke Nakano, Yasuo Matsumura, Osaka Medical College, Takatsuki, Japan

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

(MMP) activity and superoxide production in apolipoprotein E-knockout mice (ApoE-KO mice).

Methods and Results: Female apoE-KO mice and wild-type (WT) mice at 6 weeks of age were exposed to hypoxia (10.0±0.5% oxygen) for 3 weeks. Following 4 groups (5 mice each) were studied: (a) WT mice in normoxia, (b) WT mice under hypoxia, (c) apoE-KO mice in normoxia, and (d) apoE-KO mice under hypoxia. Low-density lipoprotein (LDL), total cholesterol (TC) and thiobarbituric acid-reactive substances (TBARS) increased in apoE-KO mice. The MMP activity by zymography and vascular superoxide production assessed by lucigenin chemiluminescence in aorta were increased both in apoE-KO mice and WT mice under hypoxia.

Conclusion: The increase of oxidative stress in aorta and serum caused by hypoxia was more prominent in atherogenic apoE-KO mice than WT mice in female.



1140-176

Expression of HMG-1, a Novel Mediator of Inflammation, by Human Smooth Muscle Cells of Atherosclerosis and Percutaneous Transluminal Coronary Angioplasty-Restenosis Lesions

Katsumi Inoue, Masakiyo Nobuyoshi, Kazuaki Mitsudo, Kouichi Kawahara, Ikuro Maruyama, Kokura Memorial Hospital, Kitakyushu, Japan, Kagoshima University School of Medicine, Kagoshima, Japan

Background: High mobility group-1 (HMG-1) protein is a ubiquitous and abundant chromatin component with chemoattractant effects leading to neuronal and tumor cell migration. HMG-1 is also secreted by activated macrophages (MACs), acting as a mediator of inflammation and endotoxic lethality (Wang H et al. Science, 285: 248, 1999). However, HMG-1 expression under various pathophysiological conditions of vascular lesions, is still unknown. We investigated the expression of HMG-1, particularly by smooth muscle cells (SMCs) of human atherosclerotic and restenotic coronary arteries after balloon angioplasty (PTCA).

Methods: Specimens were obtained at the time of directional coronary atherectomy of 26 restenotic (2.5 to 4 months post-PTCA) and 15 primary lesions tissue samples. All specimens were immunohistochemically stained with antibodies against HMG-1, MACs, C-reactive protein (CRP), nuclear factor kappa B (NF-KB), plasminogen, matrix metalloproteinases (MMP-3 and MMP-9) and 3 types of SMC myosin heavy chains (SM1, SM2 and SMemb).

Results: Intense HMG-1 immunoreactivity was observed in nuclei and/or cytoplasm of lipid-laden foamy SMCs and MACs from the intima of atherosclerotic plaques. HMG-1 positive SMCs expressed both NF-KB and CRP immunoreactivities. Co-expression of HMG-1 and plasminogen and/or MMPs was also observed in these SMCs by immunodouble staining. These immunohistochemical findings were clearly observed in polymorphic SMemb-positive SMCs from coronary restenotic neointima, but could not be observed in atrophic and spindle-shaped SMCs from fibrous plaques with scanty cellularity and normal medial SMCs.

Conclusions: HMG-1 is expressed by MACs and activated SMCs. Thus, it may play important roles in progression and vulnerability of primary coronary atherosclerotic lesions by enhancing inflammatory responses, and in induction of restenosis post-PTCA by increasing SMC migration.

1141

Pharmacologic Interventions in Cardiovascular Diseases

Tuesday, March 09, 2004, Noon-2:00 p.m.

Morial Convention Center, Hall G

Presentation Hour: 1:00 p.m.-2:00 p.m.

1141-189

Limited Antiplatelet Effect of Aspirin and Clopidogrel in Patients With Stable Coronary Artery Disease: The Platelet Glycoprotein Ia/Ia and Glycoprotein IIb/IIIa Genetics and Female Sex

Aino Lepantalo, **Jussi Mikkelsson**, Julio Resendiz, Pekka J. Karhunen, Riitta Lassila, Wihuri Research Institute, Helsinki, Finland, University of Tampere, Tampere, Finland

Impaired antithrombotic effect of aspirin has been suggested to be a risk factor for coronary events in stable coronary artery disease (CAD). In the current series, we have analysed functional platelet phenotypes in stable CAD patients using aspirin as well as combined aspirin and clopidogrel. These phenotypes were related to patient characteristics as well as platelet GPIa/Ia, GPIb-IX-V and GPIIb/IIIa genotype status.

We took samples from 101 patients with stable CAD on aspirin at baseline and again after a 300mg loading dose of clopidogrel. The antiplatelet effect of the treatment was studied in whole blood with PFA-100 and in platelet-rich plasma with turbidometric aggregations. We used 170s. cut-off limit for epinephrine closure time (CT-CEPI) and aggregation slope >10%/min for arachidonic acid (AA) (1.5mmol/L) as predetermined cut-off points to identify aspirin non-responders. Clopidogrel response was determined by percentage inhibition of ADP (5 µmol/L)-induced aggregation from the pre-clopidogrel value by aggregometry.

7% (AA) to 22% (CT-CEPI) of patients were defined as poor aspirin responders. 41% of these aspirin non-responders were GPIIIa A2/A1 heterozygotes as opposed to 17% among aspirin responders (p=0.02) using the PFA cut-off values. In addition, 45% of poor aspirin responders were women as opposed to 20% of aspirin responders (p=0.02) with PFA. Genotype and sex were not associated with aspirin response defined by AA-induced aggregation.

24% of patients were poor responders to clopidogrel (with the 10% cut-off). The mean inhibition of ADP-induced aggregation in GPIIIa A2/A1 heterozygotes was 16% and in GPIIIa A1/A1 22% (p=0.04). The mean inhibition was 33% in GPIa/Ia A1A1 homozygotes as opposed to 21% in other GPIa/Ia genotypes (p=0.02).

We conclude that poor response to aspirin and clopidogrel is common among individuals with angiographically proven severe CAD. Female sex and the common platelet GPIIb/IIIa receptor A2 allele are associated with a poor response to aspirin and the GPIIIa A2 allele and the rare GPIa A1/A1 genotype associate with clopidogrel response.

1141-190

Current Enoxaparin Dosing Strategies Yield Supratherapeutic Anti-Xa Activity in Many Elderly Patients

Frederick Leri, Stephen J. Voyce, Salvatore Scialla, William Glavich, Moses Taylor Hospital, Scranton, PA

Background: Enoxaparin (E) is used to treat a wide range of thromboembolic disorders. Therapeutic anti-Xa activity has been defined as 0.5 -1.0 IU/ml with increased hemorrhagic risk reported at anti-Xa activity ≥1.5 IU/ml. Current dosing recommendations (1 mg/kg subcutaneous every 12 hours) do not adjust for age, gender, or creatinine clearance (CrCl) >30 ml/min. Accordingly, we sought to determine anti-Xa activity in a cohort of elderly patients receiving E.

Methods: We identified 36 consecutive patients greater than 65 years old (12 male, 24 female) admitted to our institution and treated with E for various clinical indications. All patients had CrCl ≥ 30ml/min. Blood samples for peak Anti-Xa activity were obtained at steady state.

Results: Subjects had a mean age of 79 ± 6 years, mean weight of 81.3 ± 17.4 kg, mean serum creatinine of 1.0 ± 0.3 mg/dl and mean CrCl of 46 ± 11 ml/min. Fifty-nine percent of patients were treated for acute coronary syndrome, 22% for atrial fibrillation and 11% for deep venous thrombosis/pulmonary embolism. The mean anti-Xa activity for these study patients was 1.3 ± 0.4 IU/ml. Anti-Xa activity was >1.0 IU/ml in 25/36 patients (69%) and >1.5 IU/ml in 10/36 patients (28%). Analysis by gender revealed 18/24 (75%) of female patients with anti-Xa activity >1.0 IU/ml and 8/24 (33%) with anti-Xa activity ≥1.5 IU/ml. Values for male patients were 7/12 (58%) and 2/12 (17%) respectively.

Conclusion: Current E dosing recommendations lead to supratherapeutic anti-Xa activity in a high percentage of elderly patients. Twenty-eight percent of elderly patients demonstrate Anti-Xa activity >1.5 IU/ml. Elderly female patients are more likely to exhibit elevated anti-Xa activity.

1141-191

Beneficial Effect of Oral Acetylcysteine for Prevention of Contrast-Induced Nephropathy in Patients With Renal Insufficiency: A Meta-Analysis

Ramin Ebrahimi, **Christopher Wolf**, Jahandar Saleh, West Los Angeles VA, Los Angeles, CA

Background: Contrast Induced Nephropathy (CIN) is a well known complication of coronary angiography and angioplasty in patients with renal insufficiency. Prophylactic use of Acetylcysteine in such patients has been reported to ameliorate CIN in multiple small studies. The purpose of this report is to evaluate the cumulative evidence from reported