Patients with increased ExBP showed significantly higher ($p<0.05 - 0.001$) ldl-cholesterol and triglyceride levels and significantly lower hdl-cholesterol levels.
Conclusions: Despite normotensive BP during ABPM, obese patients frequently show a pathologically increased BP response during standardized ergometry. This increased BP response to exercise has an influence not only on LVMI, but also on cardiovascular mortality, and should therefore be assessed in obese patients.

POSTER SESSION

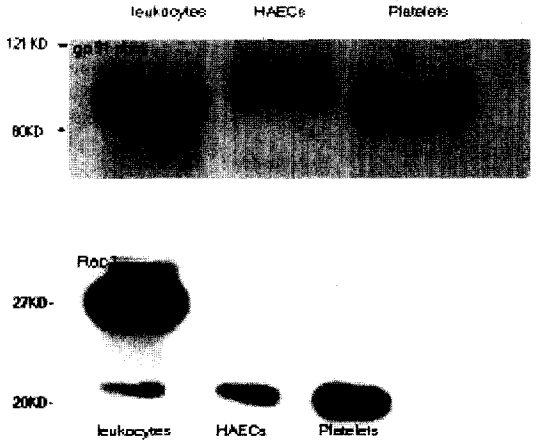
1032 Novel Insights Into Thrombosis

Sunday, March 30, 2003, Noon-2:00 p.m.
McCormick Place, Hall A
Presentation Hour: 1:00 p.m.-2:00 p.m.

1032-120 Expression of NAD(P)H Oxidase Components in Human Platelets

Naglaa A. El-Sherbeeny, Lawrence T. McGrath, Lana J. Dixon, Siobhan Brennan, Gary E. McVeigh, Queen's University Belfast, Belfast, United Kingdom

Background. Nitric oxide (NO) is involved in vasodilation. This is related to the bioavailability of NO, rather than absolute production. A major scavenger of NO is the superoxide anion which is released by endothelial and vascular smooth muscle cells. Platelets release both NO and superoxide and share many pathways found in endothelial cells. We examined human platelets for components of of the superoxide generating system, NAD(P)H oxidase. **Methods.** Platelets and leukocytes were prepared by sequential centrifugation and lysed in buffer containing n-octyl-D-glucopyranoside (ODG). Early passage human aortic endothelial cells (HAECs) were cultured until confluence, washed and lysed in ODG containing buffer. Standardised amounts of each lysed cell type were analysed by SDS-PAGE and Western blotting using anti gp 91 phox and anti rac1 primary antibodies and visualised by enhanced chemiluminescence. **Results.** We demonstrated gp 91 phox and Rac 1 in human platelets in amounts equal to or greater than that found in cultured human HAECs. These componets of NAD(P)H oxidase were found in all samples of platelets examined (n=10). **Conclusion.** Other workers have demonstrated the

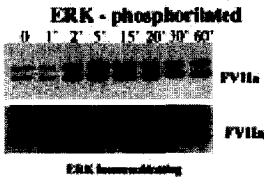


presence of p22 phox and p67 phox which along with our observations comprise the major components of NAD(P)H oxidase found in human endothelial cells. We suggest this makes isolated platelets a suitable model to study changes in endothelial cell superoxide pathway components in response to various interventions.

1032-121 Binding of FVIIa to TF Promotes Smooth Muscle Cell Proliferation In Vitro via Activation of the Extracellular Regulated Kinases

Plinio Cirillo, Paolo Golino, Paolo Calabro, Gaetano Cali, Lavinia Forte, Salvatore De Rosa, Mario Pacileo, Francesco Scopacasa, Massimo Chiariello, University of Naples, Naples, Italy, 2nd University of Naples, Naples, Italy

Restenosis following PTCA recognizes smooth muscle cell (SMC) proliferation as a key event. However, the mechanisms involved are not completely known. Tissue Factor (TF), a transmembrane glycoprotein, forms a complex with factor VII (FVII), thus activating the coagulation pathway. However, TF may function also as a membrane receptor and we have previously shown that binding of activated FVII (FVIIa) to TF induces SMC proliferation. In the present study we assessed whether this phenomenon is the result of activation of the extracellular signal regulated kinase (ERK) pathway. SMCs from rabbit aortas were made quiescent by serum deprivation. After 24 hrs, SMCs were stimulated with FVIIa (100nM) and processed for total protein isolation at baseline (no stimulation), and after 1, 2, 5, 15, 20, 30 and 60 min following stimulation. Phosphorylation (activation) of ERKs (pERKs) was assessed by immunoblotting using anti-pERK antibody. To better define the role of pERK in SMC proliferation, in additional experiments, SMCs were pre-incubated with U0126 (10μM), an inhibitor of ERK phosphorylation; SMCs were stimulated with FVIIa as above and proliferation (thymidine incorporation) and ERK phosphorilation (immunoblotting) evaluated. SMC proliferation appeared to be related to ERK activation, as suggested by the observation that U0126 inhibited it. This effect was associated with the inhibition (delay) of ERK phosphorilation. Thus, TF/FVIIa complex leads to SMC proliferation via activation of ERKs.



1032-122 The Molecular Mechanisms of Calcium Dependent Tissue-Type Plasminogen Activator Release

James A. Muldowney, III, Corrie A. Painter, Elaine Sanders-Bush, Nancy J. Brown, Douglas E. Vaughan, Vanderbilt University Medical Center, Nashville, TN

Physiologic release of tissue-type plasminogen activator (t-PA) from the endothelium is critical for vascular homeostasis. Atherosclerosis and cigarette smoking impair coronary release of t-PA. Thrombin-induced t-PA release is a g-protein coupled, calcium-dependent phenomenon. G_q is the g-protein primarily responsible for GPCR-dependent calcium signaling. In this study, we tested the hypothesis that thrombin-induced t-PA release is a G_q, phospholipase-Cβ (PLCβ), and inositol-triphosphate (IP_3)-dependent phenomenon. Human microvascular endothelial cells were incubated with 2mM sodium butyrate for 24 hours. Cells were washed and incubated for 30 minutes in M199+0.03% albumin and inhibitors in triplicate. Cells were stimulated with 10 U/ml thrombin, agonist, or vehicle. Medium was harvested at 0 and 5 minutes, and t-PA antigen was measured by ELISA. Cells were pretreated with a decoy peptide identical to the G_q c-terminus linked to a membrane permeable sequence (MPS-G_q) prior to thrombin treatment. A peptide containing the G_s sequence was a negative control (MPS-G_s). Separately, cells were stimulated with Pasteurella multocidia toxin (rPMT), a G_q agonist. MPS-G_q obliterated thrombin induced t-PA release while MPS-G_s did not. Treatment with rPMT, trended towards induction of t-PA release at 140 pM. Thrombin-induced t-PA release was inhibited in a dose-dependent manner by U73122, a PLC-β antagonist (IC_{50}=6μM). U73343, an inert analog, had no effect. The IP_3 receptor antagonist 2-APB also inhibited thrombin-induced t-PA release in a dose-dependent manner (IC_{50}=50μM). Thrombin-induced t-PA release in human endothelial cells is G_q, PLCβ, and IP_3-dependent. Acquired impairment of coronary t-PA release in smokers may involve alterations in one or more messengers in this pathway, which may partially explain the increased risk of coronary thrombosis in these groups.

1032-123 Altered Balance of Matrix Metalloproteinase and Tissue Inhibitor of Metalloproteinase in Human Atherosclerotic Plaque: Evidence From Quantitative Analysis of Gene Expression Using Real-Time RT-PCR Method

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Background: Although gene expression survey for atherosclerotic lesion has been recently conducted by cDNA array, few data exist regarding quantitative measurement of key genes in human atherosclerotic tissue. We developed an efficient procedure that makes it possible to quantitate expression levels of various genes in the scarce amount of human tissue available. And we tested a hypothesis that altered expression balance of matrix metalloproteinases (MMPs) to tissue inhibitors of metalloproteinase (TIMPs) plays a pivotal pathogenetical role in human atherosclerosis.
Methods: Samples of the plaque shoulder region and minimally affected intima were obtained from atherosclerotic carotid arteries in 24 patients with carotid endoarterectomy. Total RNA was extracted from the samples and subjected to cDNA synthesis with random primer. Amount of the cDNAs for MMP-1, 2, 3 and 9, and TIMP-1, 2, 3 and 4 in each cDNA mixture were determined by real-time RT-PCR method using ABI 7700 sequence detection system (Appliedbiosystems), and normalized with that of GAPDH (glutaraldehyde 3-dehydrogenase) as a reference gene.
Results: Total RNA (0.49±0.22μg) was successfully extracted from small amount of the human samples weighing 11.7±2.64mg, which was adequate for cDNA synthesis. In shoulder region, the genes for MMP-1 (1.27±0.27) and MMP-9 (2.20±0.55) were significantly augmented than those in minimally affected intima (0.30±0.12 and 0.62±0.24, respectively, $p<0.01$). Also, the expression levels of TIMP-1 and TIMP-3 genes were higher in shoulder region (1.22±0.22 vs. 0.81±0.47, and 0.47±0.12 vs. 0.26±0.09, respectively). The indices of MMP-1/TIMP-1 and MMP-9/TIMP-1 were 2.8 and 4.5 times higher in plaque shoulder region than in minimally affected intima.
Conclusion: Upregulation of MMPs in plaque shoulder region was disproportional to that of TIMPs, suggesting that active degradation of extracellular matrix persists in advanced atherosclerotic lesion. We consider that quantitative measurement of gene expression with the present method in human atherosclerotic tissue gives us deep understanding for its pathogeneses.

1032-124 Fibrinolytic Activity and Protein Gene Polymorphisms: Association With Coronary Artery Disease

Raymond L. Benza, University of Alabama at Birmingham, Birmingham, AL

Background: Impaired fibrinolytic activity (FA) is associated with atherosclerosis and may be determined, in part, by specific fibrinolytic protein (FP) gene polymorphisms (PMs), yet interrelationships between these PMs, Fas , and clinical atherosclerosis remain unclear. We determined whether associations existed between FP gene PMs, FP