totic agent against myocardial cell apoptosis and that aspirin differentially regulates MAPKs; activation of protective ERK1/2 pathway and inhibition of pro-apoptotic p38 MAPK pathway.

ORAL CONTRIBUTIONS

875 Circulating Cells in Atherosclerosis

Wednesday, April 02, 2003, 8:30 a.m.-10:00 a.m.
McCormick Place, Room S405

8:30 a.m.

875-1 Acute Coronary Syndromes Produce Increased Levels of Platelet-Monocyte Binding: Possible Novel Mechanisms in Vascular Injury

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Background: Inflammatory cellular mechanisms underlie acute coronary syndromes. Current therapies limit platelet-platelet adhesion and aggregation. However, leukocyte-platelet adhesion may alter plaque dynamics. Previous studies showed that platelet-monocyte binding (PMB) is mediated by P-selectin and glycoprotein ligand-1 (PSGL-1). We assessed the anti-coagulant and anti-platelet effects on binding levels, and examined the molecular mechanisms of PMB. We then measured PMB levels in patients with acute coronary syndromes to gauge if they correlated to clinical outcomes.
Methods and Results: Two-colour flow cytometric analysis showed that P-selectin-PSGL-1 interactions subserve most of the binding of platelets to monocytes in fresh peripheral blood from normal donors. However, a component of the observed adhesion was calcium-independent, not involving PSGL-1 or P-selectin. PMB was not reduced by anti-coagulant and anti-platelet drugs. PMB levels were examined in 52 patients admitted within 12 hours of symptom onset with acute coronary syndromes defined as unstable angina (UA) (n=12) and acute myocardial infarction (AMI) (n=13), or non-cardiac chest pain (NCCP) (n=27). When compared to subjects with NCCP, significantly elevated levels of PMB were found in patients with AMI (70.1% ± 15.4% SD vs. 45.4% ± 23.3% SD p<0.01), and UA(67.4% ± 12.9% SD vs. 45.4% ± 23.3% SD p>0.01). Calcium independent PMB was significantly elevated in AMI patients alone (14.7% ± 7.7%SD vs. 6.1% ± 5.96% SD p<0.001). **Conclusions:** A significant P-selectin-independent adhesion component underlies the molecular basis of PMB in peripheral blood. Patients with myocardial infarction and unstable angina show increased total PMB levels. Calcium-independent adhesion was significantly elevated in patients with evidence of myocardial infarction. These findings suggest that PMB inhibitors might be therapeutically useful adjunctive to glycoprotein IIb-IIIa antagonists in vascular injury scenarios associated with myocardial infarction. We also demonstrate that other novel and hitherto undescribed cation-independent adhesion mechanisms may modulate PMB, opening up new therapeutic possibilities.

8:45 a.m.

875-2 In Monocyte Culture Apoptosis and Tissue-Factor Expression Are Induced by Oxidized Low-Density Lipoprotein and Can Be Attenuated by Caspase-Inhibition

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Background: Lipid lowering has been demonstrated to reduce blood thrombogenicity. Resident vascular cells undergoing apoptosis in lipid-rich plaque could contribute to blood thrombogenicity through secretion of tissue-factor (TF). However, circulating monocytes exposed to hyperlipidemic conditions and oxidized LDL (oxLDL) in the blood might also undergo apoptosis and secrete tissue-factor systemically. Our objective has been to evaluate whether peripheral blood-derived monocytes undergo apoptosis and up-regulate tissue-factor when exposed to oxLDL *in vitro*.
Methods: Mononuclear cells from peripheral blood of healthy human subjects were isolated by Ficoll Paque density gradient centrifugation and MACS cell sorting procedure (purity of CD14 positive cells > 97% of total cells). Cells were cultured in RPMI 1640 medium (Gibco) containing 100 units/ml penicillin, 100 µg/ml streptomycin and 1 % FCS. Human LDL was commercially obtained (Sigma) and oxidized *in vitro*. Cells were treated either with oxLDL (75 µg/ml) alone or in combination with the caspase-inhibitor Z-VAD-FMK (Trevigen) (20 µM). Active-Caspase-3 (aCS) as marker of apoptosis and TF antigen were detected by immunofluorescence labeling.
Results: In oxLDL treated monocytes aCS expression increased gradually from 0% of cells at baseline to 2% ± 2 at 2h, 58% ± 3 at 4h, and up to 90% ± 3 of cells at 6h (P<0.05). Cellular apoptosis at 6h of treatment was confirmed by detecting DNA fragmentation. The expression of TF increased in parallel from a low baseline of 8% ± 1 of cells to 16% ± 3 at 2h, 45% ± 13 at 4h, and 77% ± 13 of cells at 6h of treatment with oxidized LDL (P<0.05). In addition, double-labeling demonstrated the co-expression of aCS and TF in single cells. Co-incubation of monocytes with oxLDL and the general caspase-inhibitor Z-VAD-FMK decreased the expression of aCS from 90% ± 3 to 33% ± 3 of cells (P<0.05) and the expression of TF from 77% ± 13 to 6% ± 6 of cells (P<0.05) at 6h. Untreated cells did not express aCS or TF at 6h (P<0.05).
Conclusion: Peripheral blood-derived monocytes undergo apoptosis and up-regulate TF when exposed to oxLDL *in vitro* pointing to apoptotic monocytes as a potential systemic source of circulating TF in hyperlipidemic blood.

9:00 a.m.

875-3 Pulsatile Flow Regulates the Extent of Native Low-Density Lipoprotein Oxidation: Implication for Monocyte/Endothelial Cell Binding Kinetics

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Introduction The extent of oxidant/antioxidant production influences the oxidative stress imposed by vascular cells on LDL, and in turn the formation of electronegative LDL particles, LDL^- and LDL^{-2}. Both are the *in vivo* oxidatively modified LDL, known to induce proinflammatory factors, with LDL^{-2} being more electronegative and oxidatively modified. It is not known whether pulsatile vs. oscillatory flow can modulate LDL oxidation, which in turn regulates MCP-1 mRNA expression and monocyte/endothelial cell binding.
Methods and Results Bovine aortic endothelial cells (BAEC) were exposed to flow conditions in 50 µg/ml of native LDL (nLDL) at 60 cycles/min: (1) pulsatile flow at time-averaged shear stress (t_{ave}) = 25 dynes/cm^2, and (2) reversing oscillating flow (0 ± 3 dynes/cm^{2}sec). After 4 hours, aliquots of culture medium were collected for HPLC analyses of LDL^- and LDL^{-2} formation using an anion exchange column. RT-PCR and quantification were performed for MCP-1 mRNA expression. Monocyte adhesion assay was performed. Pulsatile flow significantly reduced the ratios of oxidatively modified forms of LDL relative to static conditions by 51 ± 12 % for LDL^- and 30 ± 7 % for LDL^{-2}, whereas oscillating flow increased LDL oxidation by 30 ± 7 %, and 67 ± 17 %, respectively (P< 0.05, N=3). The reduction in LDL oxidation coincided with the down-regulation of MCP-1 mRNA by three-fold compared to static conditions (MCP-1 to GAPDH density ratio in response to pulsatile flow was 0.18 ± 0.06, while under static conditions it was 3.4 ± 0.25; P < 0.05, N = 3) and decreasing monocyte binding to EC (control = 19 ± 3 monocytes/HPF, pulsatile flow = 27 ± 1; P > 0.05). In contrast, the increase in LDL oxidation coincided with the up-regulation of MCP-1 expression by 6.6-fold compared to controls (MCP-1 to GAPDH density ratio: 4.2 ± 0.25, P < 0.38, N = 3), and increasing monocyte/EC binding (control = 24 ± 2 monocytes/HPF, oscillatory flow alone = 29 ± 1, P < 0.05).
Conclusion The present study suggests that shear stress modulates inflammatory responses via LDL^- and LDL^{-2} formation, mediated by endothelial cells. The formation of bioactive oxidized lipids in LDL may account for this reciprocal effect on endothelial cell function.

9:15 a.m.

875-4 Heme Oxygenase-1 Is Repressed by Bach1: A Novel Heme-Binding Transcriptional Repressor in Smooth Muscle Cells and Macrophages

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Background: Heme oxygenase-1 (HO-1) protects cells from various insults including oxidative stress and its transcriptional induction by heme and various stresses provides an important cellular adaptive defense mechanism. Recently we found that Bach1 is a physiological repressor of HO-1 and its repression is inhibited by increased level of heme (EMBO J., in press). The aim of this study is to elucidate the association between Bach1 and HO-1 expression in various cultured cells.
Methods: We have developed bach1 knockout (KO) mice and isolated aortic smooth muscle cells (SMC), embryonic fibroblasts (EF), and macrophages. Using RT-PCR method, western blotting and indirect immunofluorescence staining, we analyzed the expression of HO-1 with or without oxidative stress comparing them with that in wild type (WT) cells. We also studied proliferative effect of bach1 ablation with BrdU labeling and MTT assay.
Results: With KO SMC, EF, and macrophages, the expression of HO-1 was increased compared with WT cells (3.6-fold and p<0.01; 5.6-fold and p<0.05, respectively). While HO-1 expression was induced in WT cells when stimulated with Cd or Hemin, no further induction was observed in the KO cells. Thus, HO-1 is fully activated in the absence of bach1 function. Proliferation of KO EF decreased 0.64-fold (p<0.05) in BrdU labeling assay and 0.73-fold (p<0.05) with MTT assay.
Conclusion: Bach1 regulates the expression of HO-1 and have a prolifarative effect. Inhibition of Bach1 may lead to anti-atherosclerotic effect.