Relations of Left Ventricular Hypertrophy to Markers of Systemic Inflammation and Microangiopathy in Adults With Type II Diabetes: The Strong Heart Study

Background: Left ventricular (LV) hypertrophy (H) is a marker of proracrical cardiovascular disease (CVD) associated with elevated fibrinogen and microangiopathy. In adults with diabetes but without clinical CVD, we evaluated whether relations of LV mass (M) to fibrinogen and CRP were explained by microangiopathy.

Methods: We selected 1,414 American Indians aged 20 years or older with diabetes (WHO criteria; 6.57% of hypertensives (HTN)) without clinically overt CVD. LV mass index ≥49.2 g/m² in men or ≥45.7 g/m² in women defined LVM. Usual antibody/macroangiopathy (UACR) ≥50 mg/dL defined microangiopathy.

Results: LVM (n=4,924) was associated with higher levels of fibrinogen (104 vs 721 mg/dL), CRP (9.4 vs 6.98 mg/L) and UACR (1.412 vs 459 mg/L, p<0.01). In a multiple regression analysis adjusted for gender, body mass index (BMI), systolic blood pressure (SBP), HTN, Diabetic stroke volume (SV) and microangiopathy, LVM index was associated with fibrinogen (β=0.06, p<0.01) but not with CRP (β=0.02, p<0.02). LVM was related to LVM index (β=0.07, p<0.01) only when both fibrinogen and albuminuria status were excluded from the set of covariates (multiple R²=0.50, p<0.01). After controlling for gender, age, SBP and HTN, log-UACR was more closely related to fibrinogen (partial β=0.07, p<0.01) than to CRP (partial β=0.05, p<0.01). BMI was related to both fibrinogen (partial β=0.19) and CRP (partial β=0.20, p<0.01), independent of age, gender, SBP, HTN and Log-UACR.

Conclusion: In a population-based cohort of adults with diabetes, but without clinical CVD, LVM index was higher with LVH independently of microangiopathy. The association of LVH to higher fibrinogen and microangiopathy obscured the urine association between LVH and CRP. Higher fibrinogen in the presence of LVH suggests higher blood viscosity and prothrombotic tendency associated with increased LVH. Fibrinogen was more closely related to albuminuria than CRP. Obesity, a strong stimulus to LVH and source of profibrinolytic cytokines, was independently associated with both fibrinogen and CRP.

**ORAL CONTRIBUTIONS**

853 Monocytes, Macrophages, and Plaques I Tuesday, March 19, 2002, 10:30 a.m.-Noon Georgia World Congress Center, Room 254W

10:30 a.m.

853-1 Plaque Inflammation in Atherosclerotic Rabbits Can Be Identified By SPIO; Introducing a Noninvasive Method for Imaging Macrophage Infiltration in Active and Inflamed Vulnerable Plaque
Maziar Azapour, Stu-leo Litovitsky, Alireza Zareba, Ward Casscells, James Willerson, Montee Nazhuang, Center for Vulnerable Plaque Research, University of Texas-Houston and Texas Heart Institute, Houston, Texas.

Background: Super para magnetic iron oxide (SPIO) nanoparticles are magnetic resonance imaging contrast agents with a central core of iron coated by polysaccharides which are currently used for MRI detection of metastatic cancer. After intravenous (IV) injection, these particles are avidly engulfed by circulating monocytes and tissue macrophages. SPIO creates significant darkening in MR images mainly due to its 1'-2 shortening effect. Accumulation of SPIO loaded macrophages in inflammatory foci adds strong imaging of inflammation by MRI. Previously we have shown accumulation of SPIO loaded macrophages in aortic plaques of Apo E deficient mice. Here we report similar finding with slightly different features in nonhuman primates which are currently used for MRI detection of metastatic cancer. After intravenous injection, the aorta was harvested and stained with H&E, Iron and anti macrophage (RAM I I ). Kidneys, liver and spleen were taken and stained with H&E, Iron and rabbit anti macrophage (RAM I I ).

Methods: We injected 10 microg/kg SPIO into 5 rabbits. Two rabbits were sacrificed after 1 week, the aortic arch and thoracic aorta were harvested and stained with H&E, Iron and anti macrophage (RAM I I ). The other 3 rabbits were sacrificed after 4 weeks, the aortic arch and thoracic aorta were harvested and stained with H&E, Iron and anti macrophage (RAM I I ).

Results: At 1 week, SPIO stained macrophages were observed in aortic plaques, but not in the media. Histological examination showed a significant number of SPIO stained macrophages in plaques. At 4 weeks, SPIO stained macrophages were observed in aortic plaques and the media. Histological examination showed a significant number of SPIO stained macrophages in plaques. The number of SPIO stained macrophages was significantly higher in plaques compared to the media.

Conclusion: These findings suggest that SPIO can be used to identify macrophages in atherosclerotic plaques. This is a novel method for imaging macrophage infiltration in vivo. Further studies are needed to determine the clinical implications of this method.

11:00 a.m.

853-2 Role of Circulating Myeloperoxidase Positive Monocytes and Neutrophils In Oclusive Coronary Thrombi

Background: Although the procoagulant potential of activated monocytes is well described, the association between inflammation and thrombus propagation has not been investigated.

Methods: Coronary arteries with acute atheromatous thrombi were studied in longitudinally oriented sections. By immunohistochemistry sections 0.2 cm in length, volumetric analysis of thrombotic lesion degree of occlusion and length of the necrotic core were performed. Thrombi were characterized using antibodies directed against fibrinogen and CD68-labeled anti-tissue factor. Total (vacuolated and non-vacuolated) macrophages were identified by anti-cD68, neutrophils and myeloperoxidase positive macrophages by anti-myeloperoxidase (MPO), and neutrophils by anti-CDS8.

Results: In 69% of thrombi platelet density was greater at the site of plaque disruption while fibrils comprised a larger percentage of the propagated thrombus. Coagulative thrombi were more dense than propagative thrombi (3.4 ± 2.4 vs. 2.8 ± 1.3). There was no difference in underlying diameter narrowing (71 ± 11% vs. 71 ± 20%, respectively or length of necrotic core (8.9 ± 5.1 vs. 9.3 ± 5.6, respectively). Within the clot, oclusal thrombus showed a greater mean density than non-occlusive thrombus of 161 ± 250 positive macrophages/cm² (1.5% ± 2.5% vs. 30% ± 0.5%, p<0.01). MPO+ cells (12.3 ± 7.5% vs. 5 ± 2.7%, p=0.008) and neutrophils (2.9 ± 3.4 vs. 0.36 ± 0.50%, p=0.03). Likewise, the length of the thrombus showed a positive correlation with the intra-clot density of CD68-positive macrophages (ρ=0.004) and MPO-positive cells (ρ=0.04). In the dissected fibrin clot, the correlation of fibrinogen (5.5%) vs. non-coagulative thrombus (0.9%); this association was similar for neutrophils (0.7% vs. 0.4%), but not for total MPO+ cells (13% vs. 26%, respectively). Conclusion: Platelet rich occlusive thrombi are associated with greater density of macrophages and MPO+ cells in the thrombus, and a lesser degree MPO+ cells in the dissected clot. Preplatelet mediators between the thrombus and tissue factor derived from MPO+ cells may contribute to occlusive thrombi.

11:15 a.m.

853-3 Monocyte-Endothelial Cell Interaction Inhibits Endothelial Cell Apoptosis
Shigemasa Hashimoto, Hiroto Ueba, Masatoshi Kuroki, Yasuhito Maejima, Aki Nabata, Naoko Ikeda, Nobuhiko Kobayashi, Takanori Yatsu, Muneyasu Saito, Masanobu Kawakami, Jichi Medical School, Omiya Medical Center, Saitama, Japan.

Background: Monocyte-endothelial cell (EC) interaction stimulates production of growth factors in EC and may be involved in EC survival. Recent work suggested that monocyte adhesion to EC activates signal transduction events necessary for EC survival. However, the effect of monocyte-EC interaction on EC apoptosis is unclear. In the present study we used human monocyte-derived cell line (THP-1) and human umbilical vein endothelial cells (HUVEC). We investigated the role of monocyte-EC interaction on EC apoptosis.

Methods: We used human monocyte-derived cell line (THP-1) and human umbilical vein endothelial cells (HUVEC) cultured in co-culture system. We measured caspase activity and Bcl-2 activity with apoptosis assay. Production of Bcl-2 and Bax was evaluated by Western blot analysis. Production of Bcl-2 and Bax was measured by morphological findings

Results: Production of Bcl-2 and Bax was measured by morphological findings

Conclusion: The present study suggests that monocyte-EC interaction inhibit EC apoptosis by activating signal transduction events necessary for EC survival. Further investigations are needed to reveal the novel role of cell-cell interaction.
Method: VSMC grown in serum free media for 48hours were co-cultured with monocytes (M) for 72hours, in the presence of M-CSF or IL-10. The apoptotic index was measured using an established apoptosis assay. M-CSF and IL-10 were used at concentrations of 100ng/ml and 100ng/ml to 1ng/ml, respectively. Appropriate controls were set up for all experiments.

Results: IL-13 at physiological concentrations (10ng/ml) did not increase VSMC apoptosis (5.52±2.4%, p>0.05). This was similar to control VSMC with M alone, whereas M-CSF-activated M significantly increased VSMC apoptosis (60.0±3.0%, p<0.005). Supraphysiological doses of IL-13 (500ng and 1ng/ml) did cause an increase in mono- cellular induced killing of VSMC (56.5±3.5% and 59.0±3.6% respectively). However, such effect was shown to be mediated by endogenous production of M-CSF upon IL-13 stimulation. Thus, VSMC co-cultured with M and IL-13 (500ng and 1ng/ml) were pre-treated with an anti-M-CSF neutralizing antibody (1ug/ml). The VSMC apoptosis was nearly abrogated in the presence of the anti M-CSF mAb (17.6±2.1% and 13.6±1.3% p=0.007 and p=0.006 respectively), when compared to VSMC co-cultured with M-CSF activated M alone. A non-specific isotype control (50ug/ml) was used and did not block the effect of IL-13 induced VSMC apoptosis (56.0±2.0%).

Conclusions: M-CSF could function as an important rate limiting cytokine in the process of activated monocyte induced killing of VSMC, suggesting a final common pathway for atherosclerotic plaque destabilization.

11:30 a.m.

1176-5

Arterial Injury in ApeoE -/- and C57BL/6 Wild Type Mice: Evidence for Macrophage Apoptosis as a Mechanism Regulating Tissue-Factor Expression and Platelet Growth

Randalh Hutter, Juan J. Badimion, Bernard V. Sauter, John T. Fallon, Valentin Fuster, Cardiovascular Institute, Mount Sinai Medical Center, New York, New York.

Background: Macrophages undergoing apoptosis are considered contributing to progression of atherosclerotic lesions. Tissue factor is a key cell-mediated activator of extrinsic coagulation cascade and can induce thrombotic formation.

Methods: In this study, we examined the role of macrophage apoptosis on plaque growth by comparing neointima formation in ApeoE -/- (n=10) and C57BL/6 wild type mice (n=23) in a model of femoral arterial denudation injury.

Results: Arterial injury resulted in significantly increased neointima formation in ApeoE -/- mice (-10 weeks) compared to wild type mice (4.8±1.2 mm² x 10^-2 vs. 0.73±0.03 mm² x 10^-2, p<0.01). Apoptotic macrophages and foam cells as detected by propidium iodide expression, characteristic morphology and positive staining for MOMA-2 were found only in lesions of ApeoE -/- mice and accounted for up to 33% of 6% of intimal cells at 4 weeks after arterial injury compared to less than 0.5% of apoptotic cells in neointima of wild type mice (p<0.01). Importantly, neointima size significantly correlated with the content of apoptotic macropaghes in neointima (r=-0.64, P<0.01). In addition, apoptotic macrophages were directly associated with increased cellular tissue factor expression (n=0.57, P<0.01) as well as reduced alpha-actin expression (n=0.75, P<0.01) in neointima of ApeoE -/- mice.

Conclusions: Apoptosis of macrophages and enhanced expression of cellular tissue factor in neointima correlated with increased plaque growth and change of neointima to an unstable plaque-like phenotype. These findings point to an important role of programmed cell death in modulating arterial lesion biology and controlling thrombogenic properties of intimal lesions following arterial injury.

11:45 a.m.

1185-3

Asymmetric Dimethylarginine Stimulates the Expression of Matrix Metalloproteinase-9 in Human Monocytes via Tumor Necrosis Factor-a

Eun-Myung Jang, Sangyoul Jeon, Jong-Eui Park, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea. Center for Clinical Research, Samsung Biomedical Research Institute, South Korea.

Background: Asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of nitric oxide synthase, has recently been reported as an atherogenic molecule. ADMA induces the endothelial dysfunction and influences the monocyte adhesion to the endothelial cells. We tried to investigate whether ADMA induces the release of proinflammatory cytokines and the expression of matrix metalloproteinase-9 (MMP-9).

Methods: Human mononuclear cell line, THP-1 was incubated with ADMA (0.1-10 μM) in a time-dependent manner and supernatants were harvested for the detection of proinflammatory cytokines by ELISA. The expression of MMP-9 by ADMA was determined by gelatin-zymography and northern hybridization.

Results: The expression of MMP-9 by ADMA was determined by gelatin-zymography and northern hybridization. Inhibition of nitric oxide production augments the increase in CSA-induced O_2^- production compared to HUVEC alone (p=0.04). Furthermore, there was a synergistic response to L-NMMA and CSA with an 85±10% increase in O_2^- production compared to HUVEC alone (p=0.04). Superoxide production was measured (n=4) by lucigenin-dedived chemiluminescence (5 μM).

Conclusion: CSA administration resulted in a significant increase in SBP from 113±5 mmHg (mean± SEM) pretreatment to 184±2 mmHg on day 12 (p<0.001). In contrast, co-administration of Temol with Temol alone completely inhibited the CSA-induced increase in SBP (111±2 mmHg on day 12). No effect of Temol alone had any effect on SBP. Induction of HUVEC with CSA resulted in a 135±1% increase in O_2^- production compared to HUVEC alone (p<0.05). Incubation of HUVEC with the endothelial nitric oxide synthase inhibitor L-NAME, L-NMMA, 1 mmol/L resulted in an 11.5% decrease in O_2^- production (p<0.04). Furthermore, there was a synergistic response to L-NAME and CSA with an 86% increase in O_2^- production compared to HUVEC alone (p<0.001). Treatment with 1 mmol/L completely inhibited the increase in O_2^- both in CSA and CSA+L-NMMA treated cells.

Conclusions: CSA treatment increases blood pressure and endothelial cell O_2^- production. Inhibition of nitric oxide production augments the increase in CSA-induced O_2^- production, suggesting that excess O_2^- may scavenge nitric oxide and decrease its bioavailability.