1154-81 **Relations of Left Ventricular Hypertrophy to Markers of Systemic Inflammation and Microangiopathy in Adults With Type II Diabetes: The Strong Heart Study**

Vittorio Palmieri, Russell P. Tracy, Mary J. Roman, Lyle Best, Aldo Celentano, Giovanni de Simone, David C. Robbins, Richard B. Fabsitz, Barbara V. Howard, Richard B. Devereux, *Weill Medical College of Cornell University, New York, New York.*

Background: Left ventricular (LV) hypertrophy (H) is a marker of preclinical 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 Indian adults with diabetes (WHO criteria; 60±7 yrs/old, 52% with hypertension (HTN)] without clinically overt CVD. LVM index >49.2 g/$m^{2.7}$ in men or >46.7 g/$m^{2.7}$ in women defined LVH. Urinary albumin/creatinine (UACR) ≥30 mg/g defined microangiopathy.
Results: LVH (n=422) was associated with higher levels of fibrinogen (410 vs 371 mg/dl), CRP (9.44 vs 6.88 mg/L) and UCRA (1,412 vs 456 mg/g, all p<0.01). In a multiple regression analysis adjusted for gender, body mass index (BMI), systolic blood pressure (SBP), HTN, Doppler stroke volume (SV) and microangiopathy, LVM index was associated with fibrinogen (β=0.08, p<0.01), but not with CRP (multiple R=0.52, p<0.001); CRP 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 R=0.50, p<0.001). After controlling for age, gender, SBP and HTN, log-UACR was more closely related to fibrinogen (partial r=0.47, p<0.001) than to CRP (partial r=0.05, p=NS). BMI was related to both fibrinogen (partial r=0.13) and CRP (partial r=0.23, both p<0.01), independent of age, gender, SBP, HTN and Log-UACR.
Conclusions: In a population-based cohort of adults with diabetes, but without clinical CVD, fibrinogen was higher with LVH independently of microangiopathy. The association of LVH to higher fibrinogen and microangiopathy obscured the univariate association between LVH and CRP. Higher fibrinogen in the presence of LVH suggests higher blood viscosity and prothrombotic tendency associated with increased LVM. Fibrinogen was more closely related to albuminuria than CRP. Obesity, a strong stimulus to LVH and source of proinflammatory cytokines, was independently associated with both fibrinogen and CRP.

ORAL CONTRIBUTIONS

853 Monocytes, Macrophages, and Plaque 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**

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Background: Super para magnetic iron oxide (SPIO) nanoparticles are magnetic resonance imaging contrast agents with a central core of iron oxide 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 T-2 shortening effect. Accumulation of SPIO loaded macrophages in inflammatory foci enables 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 atherosclerotic plaques of hereditary hypercholestrolemic Watanabe rabbits.
Methods: Three hereditary hypercholestrolemic Watanabe rabbits (>36 months) were injected IV 1mmol/kg SPIO. Then the animals were sacrificed at days 5 and 10 after injection. Multiple samples from aortic root to abdominal aorta, as well as heart, lung, kidneys, liver and spleen were taken and stained with H&E, Iron and rabbit anti macrophage antibody (RAM11).
Results: SPIO particles were abundantly found in macrophages in reticuloendothelial system, liver, spleen and lungs. In the aorta, the presence of SPIO was mostly restricted to macrophages and foam cells in the sub-endothelial areas of the atherosclerotic plaques suggesting only newly recruited macrophages. No difference in the distribution pattern was detected between days 5 and 10. Areas of plaque hemorrhage were minimal in these animals.. The cap overlying the macrophages was always thin. Iron-laden monocytes were also seen in the lumen, suggesting a relatively easy transport across the vessel endothelium.
Conclusion: 1) The SPIO nano-particles offer a unique and physiologic opportunity to trace macrophage infiltration into atherosclerotic plaque. 2) SPIO can be imaged non-invasively by MRI enabling detection of plaque inflammation, a well-known feature of vulnerable plaques. 3) Since SPIO is clinically available, human clinical trials are feasible and greatly warranted.

10:45 a.m.

853-2 **Role of Circulating Myeloperoxidase Positive Monocytes and Neutrophils in Occlusive Coronary Thrombi**

Allen P. Burke, Frank D. Kolodgie, Andrew Farb, Deena Weber, Renu Virmani, *Armed Forces Institute of Pathology, Washington, Dist. of Columbia.*

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 luminal thrombi were studied in longitudinally oriented histologic sections 2.5 cm in length. Morphometric analysis of thrombus length, degree of occlusion and length of the necrotic core were performed. Thrombi were characterized using antibodies directed against fibrin II and CD61-platelet antigen. Total (vacuolated and non-vacuolated) macrophages were identified by anti-CD68, neutrophils and non-vacuolated macrophages by anti-myeloperoxidase (MPO), and neutrophils by anti-CD66.
Results: In 82% of thrombi platelet density was greater at the site of plaque disruption while fibrin comprised a larger percentage of the propagated thrombus. Occlusive thrombi were longer (8.3 ± 4.2 mm) than non-occlusive thrombi (2.9 ± 2.4 mm, p=.01). There was no difference in underlying diameter luminal narrowing (71 ± 11% vs. 71 ± 20%, respectively) or length of necrotic core (8.9 ± 5.1 vs. 9.3 ± 6.6, respectively). Within the clot, occlusive thrombi showed a greater mean density than non-occlusive thrombi of CD68-positive macrophages (15.7 ± 12.5% vs. 3.0 ± 2.7%, p=0.05), MPO+ cells (12.2 ± 7.5% vs. 5 ± 2.7%, p=.006), and neutrophils (2.9 ± 3.4 vs. 0.36 ± 0.50%, p=.03). Likewise, the length of the thrombus showed a positive correlation with the intra-clot density of CD68-positive macrophages (p=0.004) and MPO-positive cells (p=0.04). In the disrupted fibrous cap, the density of MPO+ cells was greater in occlusive (5.5%) vs. non-occlusive thrombi (0.9%); this association was similar for neutrophils (0.7% vs. 0.4%), but not for total CD68+ macrophages (13% vs. 20%, respectively).
Conclusion: Platelet-rich occlusive thrombi are associated with greater density of macrophages and MPO+ cells in the thrombus, and to a lesser degree MPO+ cells in the disrupted cap. Proinflammatory mediators within the thrombus and tissue factor derived from MPO+ cells may contribute to occlusive thrombi.

11:00 a.m.

853-3 **Monocyte-Endothelial Cell Interaction Inhibits Endothelial Cell Apoptosis**

Shigemasa Hashimoto, Hiroto Ueba, Masatoshi Kuroki, Yasuhiro Maejima, Aoi Nabata, Naoko Ikeda, Nobuhiko Kobayashi, Takanori Yasu, Muneyasu Saito, Masanobu Kawakami, *Jichi Medical School, Omiya Medical Center, Saitama, Japan.*

Backgroud: 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) to investigate the effect of monocyte-EC interaction on apoptosis of HUVEC. **Method and Results:** THP-1 were stimulated with phorbol 12-myristate 13-acetate to adhere to HUVEC and HUVEC were cultured in serum-free medium for 12 h with or without THP-1. Apoptosis of HUVEC was examined by morphological findings and caspase-3 activity assay. Production of Bcl-2 and Bax was evaluated by Western blot. Caspase-3 activity of HUVEC co-cultured with THP-1 was significantly inhibited compared to HUVEC without THP-1 (60% inhibition, n=5; p<0.05). Fluorescence microscopical findings of apoptosis were also inhibited in HUVEC with THP-1 , using FITC-conjugated permeable caspase inhibitor VAD-FMK. Production of Bcl-2 increased 2.3±0.4-fold in HUVEC with THP-1 compared to HUVEC without THP-1 (n=5; p<0.05), whereas Bax production was unchanged. However these findings were not shown in HUVEC cultured in conditioned-medium derived from direct cocolture nor in separated coculture. Western blot anakysis disclosed that ERK1/2 and p38 MAPK were phosphorylated in HUVEC with THP-1, however, a MEK inhibitor PD98059 and a p38 MAPK inhibitor SB203580 had no effect on caspase-3 activity in HUVEC with or without THP-1. To investigate of molecular mechanism of apotosis inhibition , we also assessed other pathway but revealed no effect of Src inhibitor pp1, PI3-kinase inhibitor LY294002, PKC inhibitor Staurosporine and Calphostin C , and NOS inhibitor LNAME.**Conclusion:** These data demonstrate that THP-1-HUVEC direct contact inhibits apoptosis of HUVEC and Bcl-2 may be involved in a signaling pathway necessary for EC survival mediated by monocyte-EC interaction. Further examinations are needed to reveal mechanism of this novel role of cell-to-cell interaction.

11:15 a.m.

853-4 **Monocyte-Induced Killing of Vascular Smooth Muscle Cells: Is M-CSF the Final Common Pathway?**

Sanjay S. Vasudevan, Puvi N. Seshiah, Dean Kereiakes, Pascal J. Goldschmidt-Clermont, *Duke University Medical Center, Durham, North Carolina.*

Background: Macrophage Colony Stimulating Factor (M-CSF) is an important hematopoietic growth factor for the survival and proliferation of macrophages. A higher concentration of M-CSF has been associated with poor outcome in patients with coronary artery disease. We have shown that M-CSF is needed to reconstitute the killing of vascular smooth muscle cell (VSMC) by monocyte-derived macrophages, as is observed in vulnerable plaques. We have now hypothesized that the effect of M-CSF in promoting the apoptosis of VSMC, upon interacting with macrophages, is specific.