272A ABSTRACTS - Vascular Disease, Hypertension, and Prevention
JACC March 19, 2003

Coronary Endothelial Function: The Impact of Aging In a Matched Study Group With Normal Coronary Arteries

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Background: Coronary endothelial vasodilator function is attenuated with aging. It is unknown whether this finding is simply related to greater prevalences of risk factors and/or subclinical atherosclerosis among the elderly or alternatively to a direct or indirect effect of aging. Methods: Using graded coronary inusions of acetylcholine (ACH), endothelial function was tested in 25 patients between the ages of 20 and 45 years (defined as young) and in 25 matched patients aged 60 or older (defined as old). All patients had angiographic normal coronary arteries and were matched for gender, race, body mass index (BMI), indexed left ventricular mass (LVMH), low density lipoprotein cholesterol (LDL-C), and mean arterial pressure. Results: The study included 14 women and 11 men in each of the young and old groups. Age was 38.8 +/- 1.2 years in the young group and 64.6 +/- 0.7 in the old. Both groups were moderately overweight but not obese. Both were hypertensive patients with ischemic heart disease and 78% were current smokers. ACh dose was 15.9 ± 0.06 μmol/kg in both groups. Dose response curves relating % increase in coronary blood flow (CBF) to ACh dose were significantly attenuated among the elderly subjects (p<0.013 by ANOVA) despite similar BMI, LVMH, LDL-C, and blood pressure. Peak increase in CBF after ACh was 228 +/- 18% among the young and 162 +/- 23% among the old (p=0.03). Peak increase in CBF after ACh was completely reverted by vitamin C. Oxidative stress was suggested to play a major role in the deleterious effects of homocysteine on the endothelium of coronary vessels. Conclusion: The marker of oxidative stress, FORM, had a significant correlation with CIMT (r=0.42, p=0.01). There was also a strong correlation between FORM and HsCRP (r=0.72, p<0.001). The subjects were also stratified based on their FORM results into those w/ FORM ≥ 391 and those < 391 Carr Units. The results of the various markers of vascular health are shown below.

<table>
<thead>
<tr>
<th>FORM ≥ 391 (Carr Units)</th>
<th>FORM &lt; 391 (Carr Units)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD (mg/kg)</td>
<td>1.16 (+/−2.6)</td>
<td>6.06 (+/−2.3)</td>
</tr>
<tr>
<td>CFVR (%)</td>
<td>0.57 (+/−0.10)</td>
<td>0.66 (+/−0.14)</td>
</tr>
<tr>
<td>HsCRP (mg/l)</td>
<td>7.07 (+/−2.5)</td>
<td>4.93 (+/−2.3)</td>
</tr>
</tbody>
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Conclusion: The marker of oxidative stress, FORM, predicts individuals with elevated HsCRP, abnormal endothelial function, and early atherosclerosis. This suggests that it can be used as a marker of overall vascular health in humans.

1131-143 Comparison Between the Flow Velocity: Pressure Gradient Relation and the Coronary and Fractional Flow Reserve in the Assessment of Coronary Stenoses

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Background. Recently, we assessed the feasibility and reproducibility of the flow velocity: pressure gradient (v-dp) relation. This index includes simultaneous measurement of both the diastolic flow velocity(v) and pressure gradient (dp), giving a comprehensive description of the hemodynamics of a coronary stenosis. The aim of the study was to calculate the value of the v-dp relation with the coronary flow velocity reserve (CFVR) and fractional flow reserve (FFR) in the assessment of the severity of a coronary stenosis.

Methods. In all patients a MIBI SPECT or dobutamine stress echocardiogram was performed to select patients with a coronary stenosis ≥50% in a major coronary vessel. The fitted optimal cut-off point for the v-dp relation was 0.50 for both CFVR and FFR. The cut-off points 0.70 and 0.85 were used for sensitivity, specificity, and degree of agreement (kappa) were compared for the three indices.

Results. The v-dp relation had a higher sensitivity but a lower specificity compared to the FFR. Overall, the v-dp relation was significantly (p<0.0001) better than the FFR or CFVR to assess the significance of coronary stenoses.

Conclusion. The v-dp relation more accurately predicts the significance of coronary stenoses compared to the CFVR or the FFR.

1131-144 Experimental Hyperhomocysteinemia Rapidly Impairs Coronary Flow Velocity Reserve in Healthy Adults

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Background: Moderate elevations in plasma homocysteine concentrations are associated with ischemic heart disease. We tested the hypothesis that experimental hyperhomocysteinemia could impair the coronary endothelial function by increasing superoxide production. Methods: We studied 11 healthy volunteers 23.4 ± 0.9 years of age on average. Coronary flow velocity was measured by transthoracic-Doppler echocardiography performed. v and dp were measured at the proximal left anterior descending artery (p=0.04, 0.04, respectively) in spite of an elevation of the plasma homocysteine level (from 11.7 ± 6.4 mmol/l to 30.6 ± 8.6 mmol/l, p<0.0001), while the averaged diastolic peak velocity under hyperoxic conditions (ADP+Hyp) and CFR were decreased significantly (from 77.2 ± 11.4 cm/sec and 4.1 ± 0.7 to 75.5 ± 11.2 cm/sec and 3.5 ± 0.5, p=0.024 and 0.026, respectively). With simultaneous administration of vitamin C, however, ADP+Hyp and CFR did not decrease (from 84.4 ± 20.3 cm/sec and 3.9 ± 0.8 to 83.3 ± 21.0 cm/sec and 3.9 ± 0.7, N.S. and N.S., respectively). In spite of an elevation of the plasma homocysteine level (from 11.7 ± 6.4 mmol/l to 30.6 ± 8.6 mmol/l, p<0.001), moreover, there was a significant negative correlation between plasma homocysteine level and CFR (r=-0.542, p=0.0082). Conclusion: Elevating plasma homocysteine concentration induced an acute impairment of coronary vessel resistance, and this effect was completely reverted by vitamin C. Oxidative stress was suggested to play a major role in the deleterious effects of homocysteine on the endothelial function of coronary vessels.

1131-145 Differential Antiplatelet Effects of Angiotensin Converting Enzyme Inhibitors: Ex Vivo and In Vitro Studies Using Whole Blood Aggregrometry

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Background: Increasing evidence suggests that angiotensin converting enzyme (ACE) inhibitors have anti-thrombotic effects. Therefore, we sought to evaluate the coagulative ex vivo activity of cardiovascular (CV) patients grouped for treatment with either captopril, enalapril, or losartan. All agreeing with other ASAs and non of these medications and, in addition, the in vivo effects of these ACE inhibitors on platelet aggregation of healthy study participants. Methods: Blood samples from 303 CV patients and from 10
healthy study participants were analyzed by whole-blood agglutination. Platelet aggregation was determined by the increase in impedance across paired electrodes in response to the agonist agents collagen and ADP, respectively. For CV patients, therapy was required for medication. Blood samples from healthy study participants were studied after addition of ACE inhibitors in different dosage titrations. Results: As the central finding, platelet aggregation was attenuated ex vivo by captopril and ramipril as shown in the figure. While treatment of collagen-induced platelet aggregation was significant with captopril (24%: P<0.01) and ramipril (29%; P<0.01), an adverse increase was seen with enalapril (11%: P<0.05). Following collagen induction, platelet aggregation decreased by 25% (P<0.01) with ACE and with AngII/angiotensin by 50% (P<0.04). After ADP induction, inhibition with AngII/angiotensin was 85% (P<0.01), with ASA 19% (P=0.03) and with captopril there was a trend of inhibition (27%; P=0.14); no significant antimicrobial effect was seen with ramipril or enalapril. In vitro, there was no significant change of platelet aggregation after addition of specific ACE inhibitors. Conclusions: Our finding suggests the direct evidence for ACE inhibitors to decrease platelet aggregation ex vivo. A differential anti-aggregatory profile of ACE inhibitors may explain different effects on CV events as observed in large clinical trials. Failure of effects in vitro suggests that the antimicrobial effect is not due to direct interaction between ACE inhibitors and thrombocytes.

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POSTER SESSION

1132 Molecular Cardiology

Monday, March 31, 2003, 3:00 p.m.-5:00 p.m. McCormick Place, Hall A Presentation Hour: 4:00 p.m.-5:00 p.m.

1132-120 Fish Oil Attenuates Ox-Low-Density Lipoprotein Induced Expression of Adhesion Molecules in Human Coronary Artery Endothelial Cells

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Background: A number of studies have suggested anti-atherosclerotic effects of fish oil. Uptake of oxidized low-density lipoprotein (ox-LDL) by endothelial cells is an early step in atherogenesis. Ox-LDL upregulates expression of adhesion molecules, such as P-selectin and intracellular adhesion molecule-1 (ICAM-1). We hypothesized that fish oil may reduce ox-LDL-mediated expression of adhesion molecules.

Materials & Methods: Cultured human coronary artery endothelial cells (fourth generation) were incubated with ox-LDL (40µg/ml) for 24 hrs. Paralleled groups of cells were pre-treated with docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA) (10 and 50 µM), the two major components of fish oil, for overnight before incubation with ox-LDL. Another group of cells was treated with the protein kinase B (PKB) inhibitor wortmannin (100 nM) along with DHA (50 µM).

Results: Ox-LDL markedly increased the expression of P-selectin and ICAM-1 (both protein and mRNA) in HCAEcs, enhanced the adhesion of monocytes to the cultured endothelial cells, and inhibited the activity of PKB. Both EPA and DHA decreased ox-LDL-induced upregulation of P-selectin and ICAM-1 expression and adhesion of monocytes, and increased the activity of PKB in a dose-dependent manner (P<0.05). The effects of 50 µM concentration were more pronounced than the effects of 10 µM (P<0.05). Importantly, the PKB inhibitor wortmannin attenuated the effects of DHA (P<0.05).

Conclusions: The present study shows that both EPA and DHA antagonize the ox-LDL-induced expression of adhesion molecules and increase the adhesion of monocytes to the endothelial cells. These effects of EPA/DHA may underlie the anti-atherosclerotic effects of fish oil.

1132-121 Angiotensin Receptor Type 1 (AT1) Independent Growth Effects of Intracellular Angiotensin II (Ang II) in Cardiac Myocytes

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Background: Cardiac remodeling and growth can be elicited through autocrine and paracrine actions of Ang II, via binding to the AT_1 plasma membrane receptor. An intracellular role for Ang II has also been elucidated; however, the intracellular events are not yet well defined. The ability of intracellular Ang II levels to modulate PKB activity is unknown. The effects of ox-Ang II may underlie the anti-atherosclerotic effects of fish oil.

Methods: Whole cell myocytes were infected with the Tet-off adenovirus coding for Ang II peptide (Ad_Ang II). The effect on growth was studied by [3H]-Leucine incorporation in the presence of LIF, the mitogen (Cell proliferation kit I, sigma). Compared to the control group, 20 ng/ml VEGF did not affect proliferation of ESCs cultured in the presence of LIF (1000 units/ml) at 6 and 11 days. However, in the absence of LIF, 20 ng/ml VEGF significantly enhanced ESC proliferation. The number of ESCs was significantly increased by 76% (n = 7) at 6 and 11 days in culture, respectively. Similar results were observed in the experiments of ESCs transfected with VEGF cDNA (pVEGF165). In addition, the hanging drop method was used to evaluate differentiation of ESCs cultured in the absence of LIF. The portion of ESCs that differentiated to cardiac a-meromyosin heavy chain (a-MHC) positive cells was sorted by flow cytometry. Compared to VEGF-untreated ESCs, 20 ng/ml VEGF increased the number of a-MHC positive cells by 6±8% (n = 3) at 11 days in culture. In ESCs transfected with VEGF cDNA, the number of a-MHC positive cells was increased by 52 ± 7% (n = 3) at 11 days in culture. Moreover, Western blot analysis further confirmed that in the absence of LIF, the amount of a-MHC protein was significantly increased in ESCs treated with 20 ng/ml VEGF or in ESCs transfected with VEGF-cDNA. Our data demonstrated that VEGF did not affect proliferation and differentiation of ESCs cultured in the absence of LIF. The information of the VEGF-induced significant increase in differentiation of ESCs to cardiac a-MHC positive cells is probably important for future cell therapy to regenerate injured myocardium.

1132-122 Location of Mutation in the KCNQ1 Gene Does Not Influence Outcome of Long QT Syndrome Patients

Wochez Zabka, Arthur J. Moss, Gloria Sheue, Elizabeth S. Kaufman, Jennifer L. Robinson, Mark L. Andrews, Elizabeth Carroll, for International LQTS Registry, University of Rochester, Rochester, NY

Background. Recent data showed that long QT syndrome (LQTS) patients with mutations in the pore region of HERG (LQT2) gene have significantly higher risk of cardiac events than patients with mutations in non-pore region. The aim of this study was to determine whether there is an association between location of mutations in the LQTS gene and cardiac events in LQTS patients.

Conclusion: Study population consisted of 216 LQTS patients with KCNQ1 gene mutation. Demographic, clinical, and follow-up information was compared among patients with different location of KCNQ1 mutations defined as: pre-pro region including N-terminus (1-