healthy study participants were analyzed by whole-blood aggregometry. Platelet aggregation was determined by the increase in impedance across paired electrodes in response to the aggregatory agents collagen and ADP, respectively. For CV patients, these data were regarded 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 well as by ASA and clopidogrel. While decrease in collagen-induced platelet aggregation was significant with captopril (24%; P=0.02) and ramipril (29%; P=0.03), an adverse increase was seen with enalapril (11%; P<0.05). Following collagen induction, platelet aggregation decreased by 23% (P<0.01) with ASA and with ASA/clopidogrel by 35% (P=0.04). After ADP induction, inhibition with ASA/clopidogrel 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 antithrombotic 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 findings provide 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 antithrombotic effect is not due to direct interaction between ACE inhibitors and thrombocytes.

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/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 components may reduce ox-LDL-mediated expression of adhesion molecules.

Materials & Methods: Cultured human coronary artery endothelial cells (fourth generation) were incubated with ox-LDL (40 μg/ml) for 24 hrs. Parallel groups of cells were pretreated 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 adherence of monocytes to the cultured endothelail cells, and inhibited the activity of PKB. Both EPA and DHA decreased ox-LDL-induced upregulation of P-selectin and ICAM-1 expression and adherence of monocytes, and increased the activity of PKB (all P<0.02 vs. ox-LDL alone, n=6). 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 attenuate the ox-LDL-induced expression of adhesion molecules and the adherence of monocytes to the endothelial cells. These effect of EPA and DHA are mediated by modulation of PKB activity. These effects of EPA/DHA may underlie the anti-atherosclerotic effects of fish/fish oils.

1132-121 Angiotensin Receptor Type 1 (AT_1) Independent Growth Effects of Intracellular Angiotensin II (Ang II) in Cardiac Myocytes

Rajesh Kumar, Sandhya Sanghi, Kenneth M. Baker, David E. Dostal, The Texas A & M University System Health Science Center, Temple, TX

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 intracrine role for Ang II has been postulated; however, no direct growth related evidence supports this postulate. We present evidence of growth promoting effects of intracellular Ang II in rat neonatal cardiac myocytes. Others and we have previously demonstrated AT_1 like intracellular Ang II binding sites on hepatocyte nuclear envelopes.

Methods: Neonatal rat ventricular myocytes were infected with the Tet-off adenovirus coding for Ang II peptide (Ad_Ang II). The effect on growth was studied by ^{3}H-Leucine incorporation after 72 h of infection in the presence and absence of the cell permeable and impermeable Ang II receptor antagonists, losartan and candesartan, respectively. Intracellular and extracellular Ang II levels were measured by a competitive ELISA assay.

Results: In myocytes infected with Ad_Ang II (72 h), the levels of intracellular Ang II were increased by 72.4 % (99 ± 6 vs 58 ± 15 pM) compared to control virus (Ad_Cont). An increase of 132 % (378 ± 70 vs 162 ± 39 pM) in extracellular Ang II levels was also observed, possibly due to a positive-feedback loop. In Ad_Ang II treated myocytes, there was a 63.7 ± 39.6 % increase in ^{3}H-Leucine incorporation, which was **not** blocked by losartan and candesartan, indicating that this effect was mediated through mechanisms independent of an AT_1 like plasma membrane or nuclear receptor. Conditioned medium obtained from Ang II expressing cells stimulated growth in naive cells by 11.1 ± 3.5 %, which was completely blocked by losartan and candesartan.

Conclusion: Intracellular Ang II promotes cardiac myocyte growth through mechanisms independent of plasma membrane AT_1 receptor activation and secreted growth factors. This observation may represent a second level of control for Ang II mediated growth and provides a potentially important target for therapeutic inten ention.

1132-122 Genetic Determinants of Left Ventricular Mass: The Role of Beta-1 Adrenoceptor Variation in Normal, Athletic, and Disease Populations

Tony Stanton, John R. Payne, Hugh E. Montgomery, Graham Watt, Anna F. Dominiczak, Alan G. Jardine, John M. Connell, University of Glasgow, Glasgow, United Kingdom, University College London, London, United Kingdom

Background: Left ventricular (LV) mass is an extremely strong predictor of cardiovascular morbidity and mortality in the general population. The beta-1 adrenoceptor (B1AR) is a key cell surface signalling protein expressed in the heart. As beta-blocker therapy is cardioprotective and myocyte growth is stimulated by receptor agonists, it is possible that variation in the receptor gene may affect LV mass.The gene of the B1AR has two known functional polymorphisms; one of these confers an amino acid change at position 389 from glycine (G) to arginine (C). The arginine-form of the receptor (C) has been demonstrated to have far greater activity when bound by agonist and also increased GTP binding. **Methods & Results:** We therefore examined the relationship between this variation in the B1AR gene and the effect on LV mass in 3 different populations.The first comprised 2280 healthy individuals with ECG-derived LV mass. No significant difference was found between LV mass and B1AR genotype. The second population comprised 207 healthy males who underwent a 10 week cardiovascular training programme. LV mass was determined by MRI at the start and end of the training period. No difference was found for the increase in LV mass when analysed for B1AR genotype. The third population comprised 249 patients with renal disease with echocardiographically determined LV mass. There was a highly significant difference in LV mass between the GG group when compared to the other 2 groups, CG:GG p<0.05 and CC:GG p<0.01. There were no significant difference in blood pressure measurments, ejection fraction or age between genotypes.Omitting those on renal replacement therapy, beta-blockers or ACE inhibitors did not diminish the strength of the association.

Conclusion: Genetic variation at the B1AR locus is of greater importance in defining LV mass in pathological circumstances.

1132-123 Effects of Vascular Endothelial Growth Factor on Proliferation and Differentiation of Embryonic Stem Cells

Yu Chen, Yinke Yang, Qingen Ke, Jamal S. Rana, James P. Morgan, Yong-Fu Xiao, Harvard Medical School, Boston, MA

Our previous study showed that compared to transplantation of embryonic stem cells (ESCs) alone in infarcted hearts, improvement of cardiac function was significantly greater in post-infarcted mice transplanted with ESCs overexpressing vascular endothelial growth factor (VEGF). Accumulated evidence demonstrates that VEGF affects embryonic development and stimulates vascular growth. In the present study, we investigated the effects of VEGF, in vitro, on ESC proliferation and differentiation in the presence or absence of leukemia inhibitory factor (LIF). ESC proliferation was measured by the MTT method (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 numbers of cells were significantly increased by 45 ± 5% (n = 7) and by 79 ± 7% (n = 7) at 6 and 11 days in culture, respectively. Similar results were observed in the experiments of ESCs transfected with VEGF cDNA (phVEGF165). In addition, the hanging drops method was used to evaluate differentiation of ESCs cultured in the absence of LIF. The portion of ESCs that differentiated to cardiac α-myosin heavy chain (α-MHC) positive cells was sorted by flow cytometry. Compared to VEGF-untreated ESCs, 20 ng/ml VEGF increased the number of α-MHC positive cells by 84 ± 8% (n = 3) at 11 days in culture. In ESCs transfected with VEGF cDNA, the number of α-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 α-MHC protein was significantly increased in ESCs treated with 20 ng/ml VEGF or in ESCs transfected with VEGF-cDNA. Our data demonstrate that VEGF did not affect proliferation of ESCs cultured in the presence of LIF, but stimulated 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 α-MHC positive cells is probably important for future cell therapy to regenerate injured myocardium.

1132-124 Location of Mutation in the KCNQ1 Gene Does Not Influence Outcome of Long QT Syndrome Patients

Wojciech Zareba, Arthur J. Moss, Gloria Sheu, 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 syncrome (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 KCNQ1 gene and cardiac events in LQT1 patients.

Methods. Study population consisted of 216 LQT1 patients with KCNQ1 gene mutation. Demographic, clinical, and follow-up information was compared among patients with different location of KCNQ1 mutations defined as: pre-pore region including N-terminus (1-