Noon

814-5 **elivery of Macromolecules to the Myocardium: An Investigation of Alternative Pathways**

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BACKGROUND: Relatively little is understood concerning how macromolecules (MM) traverse the continuous endothelium of the coronary circulation and gain access to cardiac myocytes (CM). Understanding this process has major implications for gene therapy and molecular therapeutics.
METHODS AND RESULTS: To better understand and exploit selective myocardial uptake of therapeutic MM we used in vitro and in vivo molecular and ultrastructural analysis to investigate uptake by nonspecific cell membrane permeability (NSMP) and specific receptor mediated (RM) transcytotic and internalization pathways. Intracoronary infusion of a hydrophobic homeobox domain resulted in high efficiency traversal of a biologically active fusion peptide across the coronary microvasculature and into the myocyte compartment via the NSMP pathway. After demonstrating feasibility of this NSMP pathway, we investigated ligand specific RM pathways using in vitro and in vivo peptide phage display (PPD) with complimentary M13 and T7 PPD libraries. PPD libraries were either pre-selected for the functional capacity to cross endothelial monolayers in vitro with subsequent in vivo selection for the capacity to traverse the coronary microcirculation and internalize into CM, or were directly selected in vivo. Sequences associated with a RM transcytosis pathway included domains with 85% homology to highly conserved regions of the IgG heavy chain, and domains associated with vesicular transport pathways. Interestingly, sequences associated with the NSMP pathway included a finite sequence conserved in HIV tat protein, a molecule previously demonstrated capable of facilitating MM transport into numerous tissues in vivo. Immunohistochemical analysis demonstrated the ability of selected sequences to internalize phage into cardiac myocytes in vivo.
CONCLUSION: These experiments suggest that specific peptide motifs are capable of facilitating delivery of therapeutic MM to the heart.

ORAL CONTRIBUTIONS

818 Coronary Collateral Circulation and Angiogenis: Clinical

Monday, March 18, 2002, 11:00 a.m.-12:15 p.m.
Georgia World Congress Center, Room 360W

11:00 a.m.

818-1 **Pericardial Levels of the Anti-Angiogenic Factor Endostatin Correlate With Coronary Collateral Development in Patients With Ischemic Heart Disease**

Vipul R. Panchal, Jalees Rehman, Anne T. Nguyen, Keith L. March, *Indiana University, School of Medicine, Indianapolis, Indiana.*

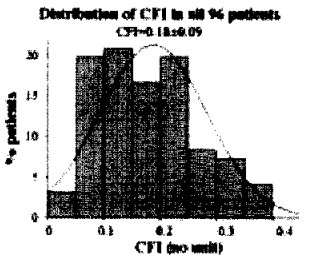
Background: Pericardial fluid analysis may provide a method to assess local factors that mediate pathophysiologic processes in ischemic myocardium. Recently, pro-angiogenic factors have been identified in pericardial fluid of patients with coronary artery disease (CAD). Potent anti-angiogenic factors, such as endostatin, have been shown to inhibit tumor angiogenesis, however, it is not known whether they regulate physiologic angiogenesis in CAD. We hypothesized that patients with poor coronary collateralization would exhibit higher intrapericardial levels of endostatin.
Methods: Human pericardial fluid samples from CAD patients were collected at the time of coronary artery bypass surgery (n=27). The fluids were centrifuged immediately to separate cellular debris, frozen at -70 °C, and subsequently assayed for endostatin by ELISA (lower limit of detection 1.95 ng/ml). Coronary angiograms were reviewed for collateralization by Rentrop's classification and assigned a grade of 0 to 3. Data were analyzed by the Student's t-test expressed as mean ± standard error of mean. Linear regression of means was performed to test whether endostatin levels correlated with the Rentrop grade.
Results: Nineteen patients demonstrated collateral development, whereas coronary collaterals were absent in eight patients. Endostatin levels were significantly higher in grade 0 (n=8) vs. grade 3 (n=5) patients (23.16±4.5 ng/ml vs. 6.82±1.2 ng/ml, p=0.008). There was a significant inverse correlation between endostatin levels and the Rentrop grade of collateral development (r^2=0.94, p=0.03).
Conclusions: Patients with CAD exhibit varying degrees of pericardial endostatin levels. These levels appear to be inversely correlated to the degree of collateral development. Patients without angiographic evidence of collaterals have 3-fold elevated pericardial endostatin levels when compared to those with the highest degree of coronary collateralization. Our results suggest that local pericardial endostatin may modulate endogenous angiogenesis and thereby influence the extent of myocardium at risk in patients with CAD.

11:15 a.m.

818-2 **Is There Collateral Flow in Normal Coronary Arteries?**

Kerstin Wustmann, Tilmann Pohl, Stephan Zbinden, Stefan Windecker, Franz R. Eberli, Bernhard Meier, Christian Seiler, *Cardiovascular Center Bern, University Hospital Bern, Bern, Switzerland.*

Background: Anatomic studies have inconsistently described the human coronary circulation to be with or without anastomoses between different vascular regions. So far, coronary collateral flow has not been determined in vivo among patients (pts) with angiographically normal coronary arteries.
Methods: In 96 pts (61±11 years, men/women 67/29) undergoing coronary angiography for chest pain, collateral flow index (CFI, no unit) was measured in vessels without stenoses. Angiographically, 53 pts had entirely normal coronary arteries, 43 pts presented with a stenosis in another vessel than that undergoing CFI measurement. CFI expressing collateral flow relative to normal antegrade flow was determined by intracoronary (i.c.) wedge pressure measurements via sensor-tipped PTCA guidewires distal to the balloon-occluded coronary artery.
Results: Observed frequencies of CFI:



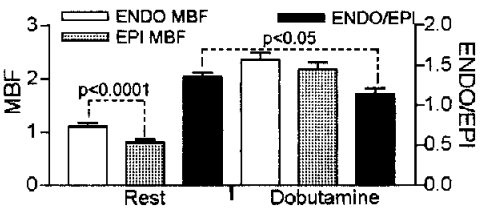
In the two groups, differences in CFI (0.19±0.10 vs. 0.17±0.07), absence of anglna pectoris (11/53 vs. 10/43) and pathological i.c. ECG-changes (6/47 vs. 2/41) during vessel occlusion were not significant (ns). CFI in the LAD/LCX/RCA was 0.18±0.09/0.19±0.09/0.16±0.08 (ns), respectively.
Conclusions: These findings suggest, that there are preexisting, functionally conductive collateral vessels even in pts with entirely normal coronary arteries. This is in contradiction to common knowledge indicating that coronary anastomoses develop de novo in a myocardial area jeopardized by ischemia.

11:30 a.m.

818-3 **Measurement of Absolute Subendocardial and Subepicardial Blood Flow in Normal Humans**

Masood A. Khan, Ornella Rimoldi, Roger J C. Hall, Paolo G. Camici, *MRC Clinical Sciences Centre, Hammersmith Hospital, London, United Kingdom, National Heart & Lung Institute, Faculty of Medicine, Imperial College, London, United Kingdom.*

Background: Experimental studies have shown that myocardial ischemia is more severe in the subendocardial (ENDO) than subepicardial (EPI) layer. Technical limitations have prevented the measurement of blood flow (MBF) in these layers in normal humans. We report the first measurement of ENDO and EPI MBF in normal humans using a high sensitivity PET scanner.
Methods: Seven healthy, male volunteers (age 48 ± 3 years) were screened to confirm the absence of cardiac disease. An EXACT 3D PET scanner (reconstructed resolution 6.7mm Full-Width Half-Maximum) was used with ^{15}O labelled water as a tracer. Scans were done at rest and during dobutamine (maximum 36 ± 5 µg/kg/min) stress. During analysis, endocardial and epicardial borders were traced on short axis images of the left ventricle (LV). The LV wall was divided into equal inner and outer halves and MBF (ml/min/g) computed using a mono-compartmental model that includes correction for spill-over from blood and surrounding tissue as well as for partial volume. MBF data are mean ± SEM.
Results: Septal thickness was 12 ± 2mm and posterior wall 9 ± 1mm. Heart rate and blood pressure were 59 ± 6 and 117/70 and 121 ± 17 and 149/69 at rest and during stress respectively. Rest ENDO MBF was higher than EPI MBF; during stress, ENDO and EPI MBF were similar with an ENDO/ EPI ratio close to unity.



Conclusion: These preliminary results indicate that in line with animal studies resting ENDO MBF is higher than EPI MBF but that transmural MBF distribution is uniform during metabolic vasodilation.