1055-152 **Matrix-Immobilized Fibroblast Growth Factor Genes Promoted Angiogenesis in a Porcine Model of Chronic Myocardial Ischemia**

Kun-Lun He, Eva M. Becker, Geng-Hua Yi, Satoshi Mohri, Anguo Gu, Ge-Ping Zhang, Myung J. Lee, Jie Wang, Barbara A. Sosnowski, Glenn F. Pierce, John Doukas, Daniel Burkhoff, Circulatory of Physiology and Cardiology, Columbia University, New York, NY

Background: Direct injections of genes encoding fibroblast growth factor (FGF) have been tested for inducing angiogenesis in ischemic myocardium, but retention of the genes at the injection site may limit its effectiveness. Previous studies demonstrated that matrix-immobilized FGF gene therapy enhances angiogenesis in skeletal muscle wounds. We therefore tested the effects of matrix-immobilized FGF gene therapy on regional angiogenesis in a porcine model of chronic myocardial ischemia model.
Methods: 16 pigs underwent ameroid placement around the left circumflex coronary artery (LCx). At 4 weeks, animals randomly received 15 intra-myocardium injections (0.1ml/each injection, 5×10^{11} viral particles/ml) of matrix-immobilized human adenovirus vectors encoding either FGF2 (n=5) or FGF6 (n=5) genes or a control vector without promoter or transgene insert (n=6) in LCx territory. Myocardial perfusion assessed by color microspheres at rest and during vasdilatory stress (adenosine) was performed before and 4 weeks after treatment. BrdU was injected subcutaneously during follow-up to mark dividing cells.
Results: Resting blood flow showed a small amount of ischemia in the LCX territory which was not influenced by gene therapy. During adenosine stress, however, myocardial blood flow (expressed as a percent flow in the normal, nonischemic zone) was greater in the LCx territory in treated animals compared to controls (epicardial: 77.0±5.1% vs 68.6±3.6%, p<0.05; endocardial: 75.6±2.6% vs 67.5±4.7%, p<0.05) 4 weeks after genes treatment. These benefits were accompanied by histologic evidence of increased BrdU staining using a semi-quantitative score (epicardial: 3.2±0.8 vs 2.1±0.8; mid-myocardial: 3.1±0.9 vs 1.9±1.0; endocardial: 2.7±1.0 vs 1.5±0.8, p<0.05 for each comparison) and overall vascular growth score (epicardial: 2.4±0.6 vs 1.6±0.6; mid-myocardial: 2.4±0.6 vs 1.5±0.5; endocardial: 2.2±0.6 vs 1.5±0.5, p<0.05 for each comparison).
Conclusions: Matrix immobilized FGF genes treatment results in significant increase in myocardial perfusion and vascularity in a porcine model of chronic ischemia. The approach may enhance the effectiveness of gene therapy.

1055-153 **Detection of Unique Factors Involved in Coronary Collateral Growth: Analysis of the Cardiac Interstitial Subproteome**

Nicole L. Lohr, Dorothee Weihrauch, David C. Warltier, William M. Chilian, Medical College of Wisconsin, Milwaukee, WI, Louisiana State University Health Science Center, New Orleans, LA

Background: Angiogenic and arteriogenic gene therapy in the heart has yet to be realized, despite the fact that a well developed coronary collateral vasculature has tremendous therapeutic benefit. One complication of past therapeutic angiogenic approaches is that a single factor was used, and perhaps this is the reason for failure; namely,other critical factors were overlooked. Accordingly, our goal was to detect unique factors involved in coronary collateralization. **Methods:** We utilized a canine model of collateral development (repetitive ischemia [RI]: one 2 min occlusion/hour, 8/day, for 21 days after which collateral flow equals that in the normal zone) in which we collected myocardial interstitial fluid (MIF) using an intramyocardial catheter. Since angiogenesis relies on the interaction of proteins in the extracellular environment, our focus was to analyze the MIF for a subproteome of blood borne and/or secreted factors associated with collateral growth via comparison between RI and Sham groups (without RI). Briefly, 30µl of MIF was electrophoresed (2-D; pH gradient, 4.0-7.0; 18% polyacrylamide gel for optimal separation of proteins under 25 kDa). **Results:** Spot analysis revealed approximately 72 proteins present in MIF of the experimental group but not in MIF of the sham group 7 days after initiation of RI. We selected 1 distinct protein and identified it using trypsin digestion in conjunction with MALDI-TOF mass spectroscopy. The protein, with an approximate molecular weight of 20 kDa and a pI of 5.5, exhibits significant homology to the human Fc gamma receptor I, which is expressed by macrophages and neutrophils. Presence of this protein implies inflammatory cell recruitment to the ischemic zone. Perhaps future therapeutic angiogenic approaches should consider amplification of such endogenous mechanisms that would stimulate monocyte and polymorphonucleocyte migration to facilitate collateral vessel formation. **Conclusion:** These data demonstrate the utility of identifying a subproteome of interstitial factors within the normal sequelae of ischemic heart disease and compensatory collateral growth.

1055-154 **Arteriogenesis Is Reduced in CD44 Knockout Mice**

Niels van Royen, Imo Hoefer, Michiel Voskuil, Ronald van der Neut, Christoph Bode, Ivo R. Buschmann, Steven Pals, Jan J. Piek, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, University of Freiburg, Freiburg, Germany

Introduction and hypothesis: The CD44 receptor mediates the transformation of inactive TGF-ß into active TGF-ß. Moreover, CD44 mediates the presentation of bFGF by macrophages to the FGF receptor-1 on adjacent cells. Since both active TGF-ß as well as b-FGF have profound pro-arteriogenic effects we hypothesized that CD44 mediates the arteriogenic response upon arterial occlusion. We tested this hypothesis in a model of femoral artery ligation in CD44 -/- mice. Methods: The right femoral artery of CD44 knockout mice as well as their matching controls (C57/bl6) were occluded with a double ligation, just distal from the ligamentum inguinalis. Seven days after ligation, immunohistochemistry was performed to detect CD44 expression. In addition, the distal abdominal aorta was cannulated and both hindlimbs were perfused with a physiological buffer solution at 4 different pressure levels (ranging from 70 to 100 mmHg). At each pressure level a differently coloured microsphere was infused. Maximal vasodilation was achieved using adenosine (1mg/kg/min). Microspheres were quantified via FACS-analysis and flow was calculated for each muscle sample. Data are expressed as percentage of normal maximal perfusion. Results: In control mice a strongly increased staining for CD44 was observed in proliferating collateral arteries as identified by KI67. 7 days after femoral artery ligation C57/bl6 control mice showed a 55% ± 7% restoration of normal flow. For CD44 -/- mice a 23% ± 4% flow restoration was observed (p < 0.01). Conclusion: This study, for the first time has shown the role of CD44 during arteriogenesis. During natural arteriogenesis the expression of CD44 is upregulated in growing collateral arteries. Moreover, the absence of this receptor reduces significantly the natural restoration of flow upon femoral artery occlusion.

POSTER SESSION

1056 Lipids: Genetic Regulation and Post-Translational Modification

Sunday, March 30, 2003, 3:00 p.m.-5:00 p.m.
McCormick Place, Hall A
Presentation Hour: 4:00 p.m.-5:00 p.m.

1056-118 **Does the -514T Gene Promoter Variant of Hepatic Lipase Predict Increased High-Density Lipoprotein Levels and Reduced Risk of Angiographic Coronary Artery Disease?**

Bryant M. Whiting, Jeffrey L. Anderson, Cory A. King, Jason McKinney, Joseph B. Muhlestein, Kaisorn Chaichana, Tami L. Bair, Sandra P. Reyna, Benjamin D. Horne, John F. Carlquist, LDS Hospital, Salt Lake City, UT, University of Utah, Salt Lake City, UT

Background: Hepatic lipase (HL) is a key enzyme in lipoprotein metabolism and reverse cholesterol transport. HL activity increases the density and atherogenicity of LDL particles and decreases HDL. The variant T allele of a common polymorphism in the promoter region of the HL gene (C-514T) is associated with decreased HL activity. Carriers of the variant -514T allele might be at lower risk for coronary artery disease (CAD), but clinical studies are limited and conflicting. Our objective was to evaluate C-514T as a protective factor for CAD in a large, prospectively ascertained population, and confirm its reported effect on plasma lipids.
Methods: To assess the risk of the C-514T HL polymorphism for CAD (≥70% stenosis), we prospectively genotyped 3,868 consenting patients undergoing diagnostic coronary angiography. Genotyping employed PCR amplification of the promoter region and single probe hybridization with melting curve analysis using the SNP Odyssey Analyzer (Idaho Technology).
Results: Overall genotype frequency was: CC, 58.5%; CT, 36.0%; TT, 5.5%. Plasma LDL did not differ by genotype: CC, 112.4 mg/dL; CT, 110.9 mg/dL; TT, 106.4 mg/dL (p=NS). However, the TT genotype was associated with significantly higher plasma HDL: CC, 38.2 mg/dL; CT, 39.8 mg/dL; TT, 43.2 mg/dL (p<0.001). Allele frequencies were similar among patients with significant CAD (n=2,696) and angiographically normal controls (n=1,172): C allele, 0.76 vs 0.77, respectively; T allele, 0.24 vs 0.23, respectively. Similarly, no difference in diagnosis of CAD was observed by genotype: CC, 69%; CT, 71%; TT, 68% (p=NS).
Conclusions: This large, prospective study confirmed a beneficial effect of the HL C-514T polymorphism on plasma lipids (increased HDL) but failed to find a postulated protective association between the HL C-514T polymorphism and the presence of angiographically defined CAD. The reason for the discrepancy between intermediate phenotype (lipids) and clinical disease (CAD prevalence) is uncertain, but it suggests the presence of genetically-linked effect modifiers that deserve further exploration.

1056-119 **Effects of Fenofibrate on Transcription of DNA Variants at 5 Untranslated Region of Human Apolipoprotein A-I Gene**

Akira Matsunaga, Hideya Nimura, Kazuo Owaki, Kazue Yonemura, Keijiro Saku, School of Medicine, Fukuoka University, Fukuoka, Japan

Previously, we demonstrated that point mutations at nucleotide position -27 of the putative TATA box signal sequence and G to A substitution at position –75 of apolipoprotein (apo) A-I gene, and were accompanied with decrease in expression of apoA-I (Arterioscler Thromb Vasc Biol 19:348-355, 1999). Here we report on the effects of hypolipidemic drug, fenofibrate on the variant of apoA-I promoter elements. We cloned the 5' region of the apoA-I gene (-333 to +119 bp) with wild type (-27A/-75G), and variant haplotypes (-27A/-75A, -27C/-75G, and –27C/-75A) into a pGL3-luciferase reporter gene basic vector, and transfected the constructed vectors into HepG2 cells. The plasmid, pSV-beta-galactosidase control vector was included in each transfection to normalize the transfection efficiency. The transcriptional levels of variant promoters -27A/-75A, -27C/-75G, and –27C/-75A, assessed by luciferase assay, were 39, 42 and 24% compared with those of wild type (-27A/-75G). Fenofibrate (0-200mM) increased the transcriptional levels of wild type and mutant promoter elements in a dose-dependent manner. Relative transcriptional levels of wild type (-27A/-75G), -27A/-75A, -27C/-75G, and –27C/-75A in HepG2 cells treated with fenofibrate (200mM) increased 186,135, 141, and 113%, respectively. These results suggest that fenofibrate increase the transcription of the variant apoA-I promoter elements, -27 TATA box and –75 G/A substitutions.