1066-198 **Incidence and Characteristics of Ruptured Plaque in Human Femoro-Popliteal Arteries**

Yoichiro Hongo, Ali Hassan, Krishnankutty Sudhir, Paul G. Yock, Peter J. Fitzgerald, Yasuhiro Honda, Stanford University, Stanford, CA

Background: Numerous studies have reported the presence of plaque rupture (PR) at the culprit lesions of acute coronary syndrome. However the incidence and clinical significance of PR in the peripheral circulation have not been well studied.
Methods: Sixty-six patients with intermittent claudication scheduled for elective angioplasty were enrolled per protocol, a femoro-popliteal segment containing one culprit lesion with > 75% stenosis by angiography was selected for IVUS analysis (mean segment length: 11cm). IVUS was performed prior to any intervention. Discontinuity of luminal surface with a cavity within the plaque mass was defined as PR. Incidence and location of PR as well as relationship to clinical risk profiles were evaluated.
Results: Fifty-four PR were observed in 35 (53%) out of 66 segments. Three segments had 3 PR and 12 had 2 PR. Thirty-eight out of 54 PR were observed outside culprit lesions. When patients with or without PR were compared, only incidence of previous myocardial infarction (MI) was significantly higher in patients with PR. (Table)
Conclusion: In the femoro-popliteal circulation, the appearance of PR is common (53%). Higher incidence of prior MI in patients with PR may be reflective of a more generalized vulnerable plaque environment, i.e., "vulnerable patients" with pan-vascular inflammation.

	PR (-) (n=31)	PR (+) (n=35)	P
Sex (M) (%)	65	66	NS
Age	66.2 ± 8.9	66.6 ± 9.8	NS
Stroke (%)	10	14	NS
Myocardial Infarction (%)	19	51	0.007
Hypertension (%)	71	77	NS
Diabetes (%)	36	49	NS
Hyperlipidemia (%)	87	80	NS
Smoking (%)	13	13	NS

ORAL CONTRIBUTIONS

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Advances in Angiogenesis

Monday, March 08, 2004, 9:15 a.m.-10:30 a.m.
Morial Convention Center, Room 265

9:15 a.m.

805-1 **Indirect Imaging of Vascular Endothelial Growth Factor Gene Expression Using a Positron Emission Tomography Reporter Gene**

Joseph C. Wu, Ian Y. Chen, Gobalakrishnan Sundaresan, Yanling Wang, Ronald G. Crystal, Sanjiv S. Gambhir, David Geffen UCLA School of Medicine, Los Angeles, CA, Weill Medical College of Cornell University, New York, NY

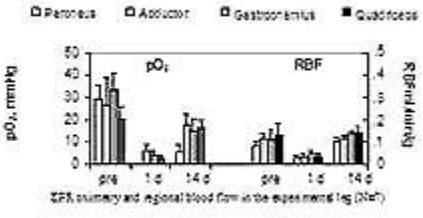
Background: Recent clinical trials with vascular endothelial growth factor (VEGF) have shown inconsistent results. Therefore, understanding the real-time kinetics of gene expression may be crucial. Here, we use micro positron emission tomography (micro-PET) to monitor VEGF by imaging the linked PET reporter gene expression.
Methods: 1) Vector: The adenovirus has two expression cassettes with cytomegalovirus promoters driving $VEGF_{121}$ and herpes simplex virus type 1 mutant thymidine kinase reporter gene (Ad-CMV-$VEGF_{121}$-polyA-CMV-HSV1-sr39tk-polyA). **2) Cell Culture:** Correlation of $VEGF_{121}$ and HSV1-sr39TK was established in transfected rat H9c2 cardiomyoblasts using ELISA and [^{3}H]-penciclovir assays, respectively. **3) *In Vivo* Imaging**: Spraque Dawley rats (n=6) were injected with $1x10^{10}$ pfu via thoracotomy. Control rats (n=6) received Ad-CMV-HSV1-sr39tk, without the linked $VEGF_{121}$. The same animals were scanned for >2 weeks using [^{18}F]-FHBG (2.18±0.29 mCi) as PET reporter probe, followed by a second viral injection at day 30 to assess the efficacy of repeated gene delivery. Data are expressed as mean±s.e.m.
Results: 1) Transfected H9c2 yield robust $VEGF_{121}$ and HSV1-sr39TK expressions: $1x10^{11}$ pfu (6880721 pg/ml and 5.856 %conversion/ug protein/min), 10^{10} (491472 and 0.333), 10^9 (44355 and 0.054), and control (362 and 0.010). Correlation is 0.95. 2) Micro-PET imaging at day 1 (0.311±0.021 %ID/g), 3 (0.270±0.021), 5 (0.248±0.008), 9 (0.115±0.012), and 15 (0.036±0.008) differs significantly from day 17 (0.028±0.017) and control (0.025±0.007) (p<0.05). Repeat injections were imaged at day 2 (0.035±0.008) and 4 (0.026±0.009). Control rats also had similar expression pattern. *In vivo* results were confirmed by *ex vivo* gamma counting, immunohistochemistry, and autoradiography.
Conclusion: This is the *first* cardiac PET study to non-invasively image a linked therapeutic and reporter gene construct. Adenoviral mediated gene expression peaked at day 1-3 and lasted only 2 weeks, with no additional expression derived from repeated injections. Applying this imaging technology to human studies will greatly contribute to the evaluation of safety and efficacy profiles of cardiac gene therapy.

9:30 a.m.

805-2 **Repeated Noninvasive Measurement of pO_2 by a Novel Electron Paramagnetic Resonance Method in a Rabbit Model of Hindlimb Ischemia**

Karen L. Moodie, Oleg Y. Grinberg, Huagang Hou, Jane Tomaszewski, Stalina A. Grinberg, Eugene Demidenko, Bruce J. Friedman, Harold M. Swartz, Mark J. Post, Dartmouth Medical School, Hanover, NH, Maastricht University, Maastricht, The Netherlands

Background: Current non-invasive methods to assess blood flow and local oxygen tension in tissue are limited by high variability, low sensitivity or small penetration depth. This has been an obstacle to the evaluation of strategies to reverse severe limb ischemia.
Methods: EPR pO_2 measurements (using previously injected Char) were obtained pre, 1 day and 14 days after femoral artery ligation. Char is inert but its EPR spectrum changes in response to oxygen. Regional blood flow (RBF) was measured with microspheres (15 µm BioPal) injected into the left ventricle.
Results: Regional blood flow measurements indicated similar pre and post ligation flows in the control hind limb, significantly reduced flow in the ligated leg immediately post ligation with normal flow restored by two weeks. EPR oximetry was able to differentiate the pO_2 values in the muscles examined and additionally found significantly reduced pO_2 values pre and post ligation in the treated leg with pO_2 returning to just 55% of baseline by two weeks. Using a mixed model of linear regression analysis, the correlation coefficient of pO_2 and RBF was r=0.42, p<0.01.
Conclusion. EPR oximetry has proven applicable for repeated non-invasive pO_2 measurement in the rabbit model of hind limb ischemia. Additionally, results indicate that blood flow and pO_2 show a linear correlation immediately post ischemia. This technique will be a valuable addition to hindlimb ischemia models and will help in understanding the physiologic relation between oxygenation and RBF.



EPR oximetry and regional blood flow in the experimental leg (Mean)

9:45 a.m.

805-3 **The Modulating Role of CD44 During Arteriogenesis: An Experimental and a Clinical Approach**

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

Introduction and hypothesis: Recently we have shown that CD44 is strongly expressed during arteriogenesis in mice and that the absence of this receptor strongly inhibits this process. In the current study we have further explored the mechanisms through which CD44 controls arteriogenesis. Perivascular leukocytes as well as FGF-2 and PDGF-B expression were determined in CD44 null and wildtype mice. In addition, we determined CD44 expression on activated monocytes in patients with coronary artery disease.
Methods: Using immunohistochemistry, the protein expression of FGF-2 and PDGF-B was determined in proliferating collateral arteries (day 7 after femoral artery ligation) from CD44 null and C57BL/6J wildtype mice. mRNA content of the aforementioned growth factors was then determined with quantitative RT-PCR of laser micro-dissected collateral arteries. Perivascular leukocytes were identified with CD11b. In patients, the expression of CD44 was measured on activated monocytes and correlated to the pressure derived collateral flow index.
Results: In control mice, both FGF-2 and PDGF-B were expressed strongly in the vessel wall of collateral arteries. The expression of these growth factors was almost abolished in the CD44 null mice. In contrast, on the mRNA level, no reduction was found (mRNA ratio of CD44 to wildtype, FGF-2: 3.4, PDGF-B: 2.9). The number of perivascular leukocytes was significantly reduced in CD44 null mice (wildtype: 29% ± 12% vs. CD44 null: 18% ± 7% CD11b positive cells/square, P<0.01). FACS-analysis showed a significantly lower CD44 expression on stimulated monocytes from patients with a poor collateralization (CFI=<0.25) as compared to patients with a good collateralization (CFI>0.25)(poor collateralization: 1764±572 vs. good collateralization: 2817±1029, arbitrary units, P<0.05).
Conclusion: This study shows that CD44 is required for leukocyte extravasation and the maintenance of FGF-2 and PDGF-B protein expression during collateral artery growth. Moreover, a strong CD44 expression on stimulated monocytes is related to a well developed collateral network in patients.