ORAL CONTRIBUTIONS

# 807 Novel Approaches to Cardiovascular Gene Transfer and Targeting

Monday, March 18, 2002, 9:15 a.m.-10:30 a.m.
Georgia World Congress Center, Room 360W

9:15 a.m.

## 807-1 Intraoperative Cell-Cycle Blockade Therapy Using E2F Decoy for Prevention of Early Atherosclerosis in Coronary Bypass Vein Grafts: A Volumetric IVUS Subanalysis From a Prospective Randomized Double Blind Clinical Trial

Mitsuyasu Terashima, Eberhard Grube, Takefumi Takahashi, Hideaki Kaneda, Thomas Felderhoff, Stein Iversen, Paul G. Yock, Yasuhiro Honda, Peter J. Fitzgerald, *Stanford University, Stanford, California, Heart Center Siegburg, Siegburg, Germany.*

**Background:** Previous experimental and peripheral studies of vein grafts have shown that *ex vivo* cell-cycle blockade therapy using E2F Decoy inhibits neointimal hyperplasia and subsequent accelerated atherosclerosis -- the primary factors in late graft failure. The aim of this study was to investigate the safety and efficacy of intraoperative cell-cycle blockade therapy on human coronary saphenous vein bypass grafts (SVG) using volumetric intravascular ultrasound (IVUS).
**Methods:** Decoy oligodeoxynucleotide, which binds and inactivates the pivotal cell-cycle transcription factor E2F, was delivered to the tissue *ex vivo* using a transfection device under completely non-distending conditions. Patients were assigned to either the treatment group (40 µM E2F decoy) or the placebo group (normal saline alone) in a prospective, randomized, and double-blind manner. IVUS was performed on a subset of grafts with automated pullback at 12 months following surgery. On the cross-sectional IVUS image, intimal area was measured as the area of an internal hypoechoic layer between the lumen/intimal border and the external border of hypoechoic layer (EHL). Volumetric IVUS analysis was performed over a 50-mm SVG segment (10 mm to 60 mm from the tip of guiding catheter or SVG orifice) using Simpson's method.
**Results:** Volumetric analysis was available in 65 grafts (35 E2F-decoy-treated : 30 placebo) at 12 months. Intimal volume/50mm and standardized intimal volume index (intimal volume / EHL volume, %) of the E2F group were significantly less than those of the placebo group (intimal volume/50mm: 78.6±45.6 $mm^3$ vs 114.8±78.3 $mm^3$, p=0.024, and standardized intimal volume index: 12.5±6.0% vs 18.4±10.1%, p=0.005). There was no difference in EHL-volume/50mm between the two groups. No adverse IVUS findings were observed in either the E2F or the placebo groups.
**Conclusion:** Volumetric IVUS analysis demonstrated that intraoperative cell-cycle blockade therapy with E2F Decoy significantly suppressed early neointimal hyperplasia of human coronary saphenous vein grafts. This new approach may contribute to reducing long-term morbidity and mortality following primary coronary bypass with saphenous vein grafts.

9:30 a.m.

## 807-2 A Tissue Engineered Stent for Cell-Based Vascular Gene Transfer

Carmelo J. Panetta, Katsumi Miyauchi, David Berry, Robert D. Simari, David R. Holmes, Robert S. Schwartz, Noel M. Caplice, *Mayo Foundation and Clinic, Rochester, Minnesota, Juntendo University, Tokyo, Japan.*

**Background**: Cell based gene transfer using a stent platform would provide significant advantages in terms of site-specific gene expression in the vasculature. The current study presents a novel stent design that allows stable in vivo transgene expression over a 4 week period in the vasculature.
**Methods:** A fiber matrix mesh was attached to Wiktor stent and coated with fibronectin prior to seeding with autologous porcine smooth muscle cells (SMC), which were stably transduced with a plasmid encoding green fluorescence protein (GFP). Cells were grown to confluence in the mesh-stent prior to deployment in the porcine coronary artery. Four animals were studied, each receiving one autologous seeded mesh-stent. After four weeks, quantitative coronary angiography (QCA) was performed on each animal prior to sacrifice. The coronary containing the mesh-stent was removed, a section was plastic embedded for hemotoxylin and eosin stain and the other section for fluorescent microscopy. The cells were removed from the vessel, and the number of GFP expressing cells were counted using flow cytometry.
**Results:** Fibronectin coating of the mesh allowed 20 fold greater seeding of cells compared no coating ($4.9 \times 10^5$ +/- $0.4 \times 10^5$ vs. $0.2 \times 10^5$ +/- $0.03 \times 10^5$ cells/$cm^2$ mesh; <0.001, n=6). Stable in vivo GFP expression within the mesh stent was demonstrated four weeks after implantation in the porcine coronary artery by fluorescent microscopy and flow cytometry. No significant change in GFP positive cell number within the stent occurred in vivo when compared to pre-insertion analysis of a companion cell seeded mesh-stent ($5.8 \times 10^6$ +/- $1.5 \times 10^6$ vs. $5.3 \times 10^6$ +/- $0.7 \times 10^6$ cells/$cm^2$ mesh; p=0.3). QCA revealed a maximal mean percent stenosis of 13.5 +/- 5.9 % (n=4) and histologic analysis revealed no apparent inflammatory infiltrate in the stented segment.
**Conclusions:** This is the first in vivo study to show stable cell-based gene transfer of large numbers of genetically engineered cells using a stent platform. These results have implications for gene therapy where long term delivery of transgene is a desirable therapeutic option.

9:45 a.m.

## 807-3 A New Approach for Inhibition of Neointimal Lesion Formation by Targeting Endothelin Expression Through CAAT/Enhancer Binding Protein Decoy Oligonucleotide

Ute Kelkenberg, Andreas H. Wagner, Jasmin Sarhaddar, Markus Hecker, Heiko E. von der Leyen, *Hannover Medical School, Hannover, Germany, University of Göttingen, Göttingen, Germany.*

**Background**: Deformation-induced synthesis of endothelin-1 (ET-1) may play a pivotal role in restenosis following percutaneous transluminal angioplasty. Thus, inhibition of ET-1-synthesis may be a potential promising therapeutic approach to treat restenosis. Since effective inhibitors of preproendothelin 1 processing to ET-1 are not available, we employed decoy oligonucleotides (dODN) to evaluate the effect of genetically engineered inhibition of ET-1 expression *in vivo*.
**Methods:** We analysed carotid arteries of hypercholesterolemic rabbits after balloon injury and incubation with therapeutic decoy (CAAT/enhancer binding protein [C/EBP] consensus dODN [10 µM, 30 min] or control decoys (C/EBP mutant dODN or buffer; n=7/group). Efficiency of decoy uptake was demonstrated using Texas-red labelled decoys.
**Results:** 2 days after transduction, C/EBP consensus decoy application resulted in significant reduction of C/EBP activity (electrophoretic mobility shift assay) and ET-1 expression (immunohistochemistry). 28 days post balloon injury we saw a significant reduction of intimal thickening (computerized morphometry; intima/media-ratio: 1.13 ± 0.22 [C/EBP consensus dODN] vs. 1.75 ± 0.45 [controls]; mean ± SEM, n = 7, p = 0.003). Macrophages were found throughout the intima of untreated and mutant decoy-dODN-transfected arteries corresponding to areas of macroscopic plaque. In the C/EBP consensus decoy-treated animals, only isolated macrophages in areas of small neointimal accumulation and no foam cells were observed (macrophage-positive area: 10.4 % ± 4.6 [C/EBP consensus dODN] vs. 17.2 % ± 4.9 [controls]; n = 7, p = 0.04).
**Conclusion:** These data indicate that intravascular application of a therapeutic decoy inhibiting C/EBP activity may be a feasible, safe, and effective method to reduce restenosis following balloon injury, especially under hypercholesterolemic conditions.

10:00 a.m.

## 807-4 Applicability of a Highly Efficient Electro-Gene Transfer Approach for the Human Heart

Luyi Sen, Guanggen Cui, Jack Judy, Hillel Laks, *UCLA Medical Center/UCLA School of Medicine, Los Angeles, California.*

Electroporation is a technique involving the application of short duration, high intensity electric field pulses to cells. It is commonly used for in vitro gene transfection of cell lines and primary cultures, but limited work has been reported in small animal organs and tissue. Recently, we have established a novel electroporation system for ex vivo gene transfer in large animal and human hearts. This device consisted of two electrode arrays directly in contact with the endocardium and epicardium in order to achieve uniform electroporation-enhanced gene transfer to the entire human heart. Using our recently developed rabbit heterotopic functional heart transplant model, we compared the efficiency of ex vivo intracoronary recombinant human interleukine 10 (IL-10) gene transfer, mediated by either electroporation (EP), liposome (LP) or adenovirus (AD). In EP group, the burst of electric pulses (pulse length 5 ms, number of pulse 10, burst-interval 2 min) was applied to the heart during and after ex vivo intracoronary gene infusion for 20 minutes. With 10mV/cm electric field strength, transgene was homogeneously transferred into the whole rabbit heart in 3-10 minutes. A significant increase in IL-10 mRNA level was observed 2 hours and reached a peak 3 days after gene transfer. In EP group, the transgene expression in the donor left ventricular myocardium was more than 5 times higher compared with that in the LP group, and 1.25 fold higher than that in AD group (n=8, p<0.01). The increase in transgene expression was paralleled with IL-10 protein expression. The distribution of the electroporation-mediated transgene expression was much more uniform than that in liposome-mediated gene transfer and adenovirus-mediated gene transfer. The hemodynamic and electrophysiologic parameters recorded from the donor heart were the same in EP group and the control group 6 days after transplantation. The arrhythmogenic effect was highest in AD group, less in LP group, and none in EP group. These results suggest that this new electroporation-mediated gene transfer is highly efficient and no significant cardiac adverse effect and is potentially applicable for ex vivo or in vivo gene delivery in human heart.

10:15 a.m.

## 807-5 Ultrasound in Conjunction With an Ultrasonic-Reflective Transfection Agent Enhances Gene Delivery to Cells

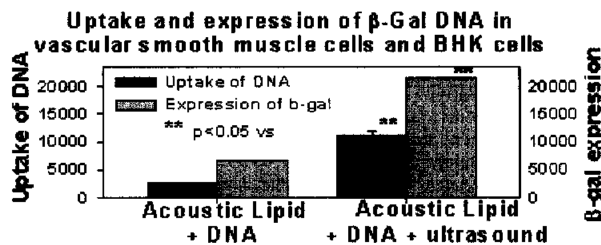
Shaoling Huang, David D. McPherson, Robert C. MacDonald, *Northwestern University, Chicago and Evanston, Illinois.*

Cationic liposomes have been developed that both provide ultrasonic enhancement and gene delivery to a target site. Low level ultrasound may enhance transfection, while retaining acoustic properties.
**Methods**: Liposomes consisting of cationic phospholipid (O-ethyldimyristoylphosphatidylcholine; EDMPC) and cholesterol were prepared according to a procedure previously described for anionic lipids and involving dispersion of the lipids in mannitol solution, lyophilization and rehydration. The liposomes were combined with DNA to form lipoplexes, which only slightly diminished ultrasound reflectivity. Cultured cells in 6-well plates were exposed to 1-Mhz ultrasound (0.5 W/$cm^2$, 30 sec) in the presence of the acoustic lipoplexes. Quantification of transfection efficiency was by -galactosidase expression and that of DNA uptake by fluorescence of YOYO-labeled -galactosidase plasmid (-gal DNA).
**Results**: Uptake of DNA into vascular smooth muscle cells and transfection of BHK cells

Hypertension, Vascular Disease, and Prevention

were each strongly enhanced (up to 5 fold) by ultrasound exposure. (** p<0.05 vs. acoustic lipid + DNA)

**Conclusions**: Ultrasound in conjunction with ultrasound-reflective lipoplexes improves gene transfer. Acoustic liposomes conjugated to antibodies have independently been shown to target to vascular disease sites in vivo; thus, using ultrasound, it becomes feasible to both identify a disease site and activate a therapeutic agent in situ.




ORAL CONTRIBUTIONS

## 814 Cardiac Gene Expression

Monday, March 18, 2002, 11:00 a.m.-12:15 p.m.
Georgia World Congress Center, Room 160W

11:00 a.m.

### 814-1 Bradycardia and Abnormal Calcium Handling in Transgenic Mice Overexpressing Juntin

Myeong-Chan Cho, Jin-Sook Kwon, So-Young Choi, Tae-Jin Youn, Dong-Woon Kim, Chang-Soo Hong, Do-Han Kim, *Chungbuk National University Hospital, Cheongju, South Korea, Kwangju Institute of Science and Technology, Kwangju, South Korea.*

Background; Junctin is integral membrane protein that co-localize with the ryanodine receptor (RyR), calsequestrin and triadin at the junctional SR membrane in cardiac and skeletal muscle.

Methods; To investigate physiological role of junctin in excitation-contraction coupling, transgenic mice overexpressing junctin were developed.

Results; Transgenic (TG) mice exhibited 14-fold higher levels of junctin in myocardium and survived into adulthood, but developed cardiac hypertrophy of variable severity from mild increase in mass to generalized cardiac hypertrophy with early death. Junctin TG mice had a normal LV end-diastolic dimension and systolic function on echocardiography. In two transgenic lines (Lines 122 and 129) having higher junctin expression, RV failure with marked RA and RV dilatation developed and subsequently resulted into early death. In junctin TG mice, ANF expression was increased in all cardiac chambers. Microscopic examination of the TG heart revealed general cardiac hypertrophy with increased collagen contents and thrombus in both atria. Ultrastructural analysis demonstrated irregular Z-band intervals, loss of mitochondrial membrane or cisternae. The HR (375±51/min) of the TG mice was less than that (440±61/min) of their littermates (p<0.01). Bradycardia with intermittent ventricular premature beats was observed in the young TG mice. In TG heart, the expression levels of triadin and ryanodine receptor were significantly decreased and protein level of dihydropyridine receptor was increased without significant changes in other E-C coupling proteins. The capacitance of TG cardiomyocytes (313.8±15.2pF) was similar to that (324.6±16.7pF) of wild-type. $Ca^{2+}$ release from SR in single cardiomyocytes did not appear to be altered in TG mice. On the other hand, L-type $Ca^{2+}$ current density in the ventricular cardiomyocyte was significantly increased, which could cause the observed prolonged action potential durations and bradycardia.

Conclusion; These results suggest that regulation of junctin expression must play an important role in proper heart function.

11:15 a.m.

### 814-2 Gene Therapy Using a Novel Method of Delivery: Continuous Perimuscular Infiltration

Ariel Roguin, Samy Nitecki, Irit Rubinstein, Edmond Sabo, Zaid A. Abassi, Meira Frank, Nina S. Levy, Orit Lache, Aharon Hoffman, Andrew P. Levy, *Rambam Medical Center, Haifa, Israel, Technion - Israel Institute of Technology, Haifa, Israel.*

**Introduction:** Gene therapy with angiogenic cytokines that induce the development of collaterals in ischemic tissue are usually given by a single intra-arterial or intra-muscular (IM) injection. Naked DNA has no immunological side effects however its infectivity is usually low. Our aim was to evaluate the usefulness of a new method for gene therapy - continuous infiltration of naked-DNA and compare it with IM in augmenting collateral formation and tissue perfusion in a mouse unilateral ischemic hindlimb model.

**Methods:** The left iliac artery of C57 mice was ligated in two places and cut. An osmotic infusion pump containing a placebo or VEGF in different concentrations was implanted intra-abdominaly and the outlet tube from the pump fenestrated and tunneled into the left quadriceps muscle. This novel system delivers perimusculary the tested drug in a constant rate of 1 μL/hour for 7 days. Ischemic (left)/ normal (right) limb blood flow was measured using a laser Doppler blood flow meter and was monitored every 3-days.

**Results:** The flow restoration using continuous infiltration was faster and more complete than with IM injection (p<0.05). For the continuous infiltration group; auto-amputation due to severe ischemia was observed only in the placebo group (33% Vs. 0%). VEGF resulted in a faster and more complete restoration of blood flow. Furthermore, the restoration of blood flow by VEGF was dose dependent. By day 7 the blood flow was almost complete for the group treated with 500 μg VEGF, while in the placebo group the flow returned to normal only after 21 days (p<0.05). Vessel density using smooth muscle actin (arteriogenesis) and factor VIII (angiogenesis) were higher in the VEGF continuous infiltration group. PCR demonstrated the plasmid infiltrated only in the ischemic leg and in the liver

**Conclusions:** Our results demonstrate that using a continuous perimuscular infiltration for 7-days of naked-DNA encoding VEGF via an osmotic pump is associated with muscle and limb salvage due to the rapid and successful restoration of blood flow. The flow rate restoration was faster and more comlete than the traditional IM method. This method could serve as another option for gene therapy delivery.

11:30 a.m.

### 814-3 Distinct Expression Patterns of Connexins in the Chambers and Conduction System of the Human Fetal Heart

Riyaz A. Kaba, Steven R. Coppen, Emmanuel Dupont, Jeremy N. Skepper, Suzy Elneil, Magdi H. Yacoub, Stephen Rothery, Nicholas J. Severs, *National Heart & Lung Institute, Imperial College, London, United Kingdom, University of Cambridge, Cambridge, United Kingdom.*

Background: Gap junctions have been shown to play an important role in the structural and electrophysiological development of the mouse heart. This study was undertaken to determine the specific expression pattern of three connexin isotypes, connexins 40, 43 and 45, in the atria and ventricles of the 9-week human fetal heart. Methods: Five hearts of 9-week old fetuses obtained from surgical termination of pregnancy were rapidly frozen for examination by immunocytochemistry and Western blotting. Confocal microscopy was employed to study the distribution of immunolabeled connexins 40, 43 and 45 in the atria and ventricles. Double labeling techniques were applied to examine the spatial relationship of expression of pairs of connexin isotypes. Hearts were also analyzed by Western blotting for connexins 43 and 40 after separating the atria and the ventricles. Results: The predominant connexin in the 9-week human fetal atrium was connexin40, which was co-expressed with lower levels of connexin45. Connexin43 (the major human adult cardiac connexin) was virtually undetectable in the atria. In the ventricles, by contrast, connexin43 predominated while connexin45 was found at similar levels to those in the atria. Connexin40 was far less abundant in the ventricles than in the atria. Western blot analysis of connexins 43 and 40 confirmed the results obtained by the corresponding immunoconfocal studies. In regions of the early ventricular conduction system, from the commencement of the bundle branches through to the trabecular layer, all three connexins were prominently expressed. Conclusions: Our findings show that different regions (atria, ventricles and central conduction system) of the early human fetal heart display discrete connexin profiles. Enhanced connexin expression acts as a marker of the forming ventricular conduction system as early as 9-weeks in the human fetus. The distinct pattern of connexin expression between the fetus and adult emphasizes the dynamic nature of gap junction expression during morphogenesis of the human heart.

11:45 a.m.

### 814-4 Platelet GPIa (G873/873A) and GPIIbIIIa (leu33/33pro, PIA1/PIA2) Glycoprotein Receptor Polymorphisms: An Analysis of Functional Effects and Association With Risk of Premature Myocardial Infarction

Ravi K. Singh, Hashmik Patel, Peter Braund, Julian Stribling, Pat Desouza, Suzanne Cheng, Alison H. Goodall, Nilesh J. Samani, *University of Leicester, Leicester, United Kingdom, Roche Molecular Systems, Alameda, California.*

**Background** There is controversy about the role of platelet glycoprotein receptor polymorphisms, GPIa (G873/873A) and GPIIbIIIa (leu33/33pro), in increasing risk of MI. The purpose of this study was to examine functional correlates of the two polymorphisms and investigate their role in determining risk of premature MI (<50 years), a situation in which any increased thrombotic tendency is likely to be most manifest.

**Methods** 200 cases with a validated premature MI (mean event age 42.7±5.4 years) and 200 age, sex and smoking status matched controls were studied. All cases were stable and at least 3 months beyond their thrombotic event. DNA was genotyped for the GPIa (G873/873A) and GPIIbIIIa (leu33/33pro) polymorphisms by PCR. Flow cytometric analysis using fresh citrated whole blood was used to quantify GPIa and GPIIbIIIa receptor expression and fibrinogen binding to the GPIIbIIIa receptor in response to agonist stimulation (ADP and thrombin) using CD49b, CD41 and anti-fibrinogen monoclonal antibodies respectively. Platelet volume, count and plasma fibrinogen were also measured.

**Results** The GPIa polymorphism demonstrated a gene dosage effect on the expression of the receptor on the platelet surface in both cases and controls (mean florescence index G/G vs G/A vs A/A = 3.77±0.18, 5.05±0.23, 5.86±0.45 and 3.98±0.25, 5.27±0.27, 6.13±0.25 respectively, all p-values < 0.01). However, there were no significant differences between cases and controls for genotype distributions for either the GPIa (G/G vs G/A vs A/A = 37, 43, 20 and 41, 43, 17% respectively, p = 0.68) or GPIIbIIIa (leu/leu vs leu/pro vs pro/pro = 71, 27, 1 and 68, 30, 2 respectively, p= 0.76) polymorphisms. The GPIIbIIIa polymorphism did not influence either receptor expression or fibrinogen binding, even taking into account platelet volume, count and fibrinogen level.

**Conclusions** The GPIa (G873/873A) polymorphism has a significant gene dosage influence on the expression of the receptor on the platelet surface. However, this does not lead to an increased risk of premature MI in subjects carrying the 873A allele. The GPIIb/IIIa (leu33/pro33) polymorphism does not modify either expression or activity of the receptor or influence risk of early MI.