1051-131 **Combined Treatment With Epsilon Protein Kinase C Activator and Delta Protein Kinase C Inhibitor Ameliorates Ischemia Reperfusion Injury With Prolonged Ischemia in Rat Cardiac Allografts**

Masashi Tanaka, Raya D. Terry, G. Kimia Mokhtari, E. Grant Hoyt, Tomoyoshi Koyanagi, Koichi Inagaki, Theo Kofidis, Daria Mochly-Rosen, Robert C. Robbins, Stanford University, Stanford, CA

Inhibition of delta protein kinase C (δPKC) and activation of epsilon PKC (εPKC) have both been shown to limit damage from ischemia and reperfusion (I/R). Studies have pertained to ischemia or myocardial infarction in particular and have been conducted in cell cultures, isolated rat heart models, or porcine models. However, whether these isozymes can ameliorate I/R injury in the transplanted heart has not been determined. We investigated the ability of PKC regulator to reduce I/R injury with prolonged ischemia in rat cardiac allografts. Methods: Hearts of PVG rat ($RT1^c$) were heterotopically transplanted into ACI rats ($RT1^a$). After cardioplegic arrest of the donor heart, the ascending aorta was ligated, and 2 mL of εPKC activator (ψεRACK, 1.5 nmol) solution was then injected (antegrade coronary injection). Hearts were procured and submerged in the same drug solution (0.5 μM) for 30 or 120 min at 4°C. Before reperfusion, 1 ml of δPKC inhibitor (δV1-1, 30 nmol) solution was injected into the recipient IVC. Control animals were treated with normal saline. Grafts were procured after 4 h of reperfusion (n = 6 each group) and analyzed for super oxide generation by the spin-trapping method; for myeloperoxidase (MPO) activity, TNF-α, IL-1β, and MCP-1 production by ELISA; and for apoptosis by TUNEL and by caspase-2, -3, -8, and -9 activities. Results: With 30-min ischemia, MPO activity, TNF-α production, and caspase-9 activity decreased significantly in the PKC regulator-treated group. With 120 min-ischemia, MPO activity, TNF-α, IL-1β, MCP-1 production, cardiomyocyte apoptosis, and caspase-2, -3 and -9 activities decreased significantly in the PKC regulator-treated group. Super oxide generation and caspase-8 activity did not differ significantly between the two groups with 30-min or 120-min ischemia. Conclusions: Combined treatment with εPKC activator and δPKC inhibitor attenuates the I/R injury that occurs with prolonged ischemia but does not suppress generation of super oxide. This treatment inhibits the mitochondrial pathway on the induction of apoptosis. These peptides may be useful in clinical transplantation for organ preservation and prevention of I/R injury.

1051-132 **S-Nitric Oxide-Human Serum Albumin, a Novel Intravenous Nitric Oxide-Donor, Minimizes I/R Injury After Four Hours of Ischemia in an Orthotopic Heart Transplant Model in the Pig**

Roman Gottardi, Tomas Szerafin, Severin Semsroth, Harald Gasser, Seth Hallstroem, Rainald Seitelberger, Ernst Wolner, Bruno K. Podesser, University of Vienna, Vienna, Austria

BACKGROUND: In clinical heart transplantation the ischemic period tolerated by the heart is still limited. Prolonged ischemia is associated with increased risk of acute and chronic rejection. Depletion of nitric oxide (NO) is a major contributor to ischemia/reperfusion (I/R) injury. We hypothesize that NO-substitution with S-Nitroso Human Serum Albumin (S-NO-HSA), a new physiologic NO-donor, could bridge NO-starvation during I/R and improve hemodynamic outcome in a model of orthotopic heart transplantation in the pig.
METHODS: Donor pigs (n=19; 37,1±6,1 kg) were monitored and randomized to treatment with (1μmol/kg/h) S-NO-HSA (n=11) or HSA (n=8; control) one hour prior to explantation. Hearts were harvested and stored using Bretschneider solution with addition of 10μmol/L S-NO-HSA or HSA respectively (4°C for 4 hours). Recipient pigs (n=19; 36.5±5,9 kg) were monitored and put on cardiopulmonary bypass (CPB). Donor hearts were implanted followed by 60 min of controlled reperfusion before subsequent weaning from CPB (epinephrine 0,4 μ/kg/h) and a follow up of two hours. Thirty min prior to estimated aortic declamping, infusion with S-NO-HSA or HSA respectively started for 60 min (0,1 μmol/kg/h).
RESULTS: At the end of S-NO-HSA infusion in donors , a decrease in heartrate, mean arterial pressure, left ventricular systolic pressure (LVP sys) and Wedge pressure compared to control was observed. During the first 15min of reperfusion, coronary flow/cardiac output per kg bodyweight was significantly higher in the S-NO-HSA versus the HSA group ($p<0.01$). During the entire follow up there was a lower wedge pressure in the treatment group that reached significance after 60 min ($p<0.05$). LVP sys was significantly higher in the treatment group 30 min after weaning from CPB ($p<0.05$) and became highly significant after 75 min ($p<0.01$).
CONCLUSIONS: In a pig model of orthotopic heart transplantation with 4 hours of ischemia S-NO-HSA significantly minimizes I/R injury and improves organ function as shown by an improvement of myocardial perfusion during early reperfusion, a lower wedge pressure, indicating a better diastolic function, and a better systolic function after weaning from CPB in the S-NO-HSA group.

1051-133 **Localized Combinatorial Interleukin-4 and Interleukin-10 Gene Therapy Readjusts the Balance of Endogenous Th1/Th2 Cytokines and Induces the Tolerance of Cardiac Allografs**

Hiroshi Furukawa, Kiyohiro Oshima, Thomas C. Tung, Hyde Russell, Guanggen Cui, Hillel Laks, Luyi Sen, UCLA Medical Center/UCLA School of Medicine, Los Angeles, CA

The balance of Th1/Th2 cytokine plays an important role in the allograft tolerance, however, its operating mechanism remains unknown. Using a functional cervical heterotopic rabbit heart transplant model, we tested the hypothesis that liposome-mediated *ex vivo* intracoronary interleukin-4 (IL-4) and IL-10 combined gene therapy may induce localized IL-4 and IL-10 over expression that may synergistically regulates endogenous Th1/Th2 expression, which may contributes the allograft tolerance. The mean survival of cardiac allograft was prolonged from 7± 1 days in Control Group (CG) to 14±3 days in IL-4 gene therapy group (IL-4G), 28± 7 days in IL-10 gene therapy Group (IL-10G) and 135± 20 days in IL-4 and IL-10 combined gene therapy Group (IL-4&10G). The transgene and exogenous protein expression in IL-4&10G reached the peak in postoperative day (POD) 5-8, and slowly reduced thereafter. The rejection score in IL-4&10G (n=15) was significantly lower (2.2± 0.2, $p<0.05$) than that of CG (3.6± 0.2, n=15) and IL-10G (2.7± 0.3, n=15) in POD3-6, and 2.0± 0.0 in POD>31. In single cytokine gene therapy caused an excessive localized exogenous cytokine gene and protein expression which significantly downregulated the expression of the same endogenous cytokine, IL-4 or IL-10 gene ($p<0.01$), but in a certain extent upregulated the other Th2 cytokine genes expression ($p<0.01$). In IL-4&10G, endogenous IL-4 and IL-10 gene expression was decreased in the early stage, then increased at POD 18-20 and remained in a high level for a long period of time. The expression of Th1 cytokine genes, IL-1β and TNF-α , were significantly decreased by the synergistic inhibition of IL-4 and IL-10, and maintained in the low level in the late stage. IFN-γ gene expression level was increased in IL-4G, decreased in IL-10G. The slightly decreased IFN-γ gene expression level observed in the combined gene therapy group that could be resulted from antagonistic immunoregulatory effects of two cytokines. We conclude that localized overexpression of exogenous IL-4 and IL-10 induced by ex vivo gene transfer could act synergistically for readjusting the balance of endogenous Th1/Th2 cytokines that may contribute to the allograft tolerance.

1051-134 **Downregulation of Connexin 43 Expression in Chronic Cardiac Allograft Rejection**

Lathasree Polavaram, Daniel Zaghi, Guanggen Cui, Hardeep Singh Ahluwalia, Luyi Sen, UCLA Medical Center/UCLA School of Medicine, Los Angeles, CA

Down-regulation of the major gap-junctional protein of the ventricular myocytes, Connexin43 (Cx43), has previously been reported in heart failure due to ischemic dilated Cardiomyopathy (ICM) and idiopathic dilated cardiomyopathy (DCM). However, whether it is also altered in chronic cardiac allograft rejection remains unknown. In this study, left ventricle samples from 40 patients with dilated cardiomyopathy and end-stage heart failure underwent heart transplantation were analyzed for Cx43 and Cx40 using Western blot analysis. Fourteen patients had DCM, 12 had ICM and 14 had chronic cardiac allograft rejection underwent re-transplantation (CAR). Actin antibody was used as an internal control. Left ventricular samples from 6 donor hearts were used as normal control (NLC). Cx43 expression level was significantly decreased in CAR group (0.68±0.31) compared with that in NLC (1.14±0.49, p=0.019). The down-regulation of Cx43 was also observed in ICM (0.73±0.36, p=0.057). In DCM, Cx43 expression level was only slightly decreased (0.85±0.32, p=0.1205). Cx40 expression was slightly increased in CAR, ICM and DCM compared with that in NLC (p=0.14, p=0.14 and p=0.22 respectively). All the 3 groups showed increased Pulmonary Capillary Wedge Pressure (DCM: 19.92±10.82, ICM: 22.88±10.34, CAR: 18±7), Right Atrial Pressure (DCM: 8.5±3.17, ICM: 10.11±5.77, CAR: 13.7±5.5). ICM and DCM in addition had increased Left Ventricular End Diastolic Diameter (LVEDD) and decreased Left Ventricular Ejection Fraction (LVEF). The decrease of LVEDD and LVEF in CAR was only mild to moderate. There is no significant correlation between Cx43 and LVEDD and LVEF (p>0.05). In conclusion, these results suggest that the altered connexin expression in ventricular tissue may be related to the inflammation in ischemic cardiomyopathy and CAR. The down-regulation of Cx43 might be responsible for the alteration of electrical excitation and conduction in myocardium, but may not play a major role in the hemodynamics in both ICM and CAR.

POSTER SESSION

1068 Mechanisms in Heart Failure: Myocytes, Myoblasts, and Stem Cells

Monday, March 08, 2004, 9:00 a.m.-11:00 a.m.
Morial Convention Center, Hall G
Presentation Hour: 9:00 a.m.-10:00 a.m.

1068-121 **Impact of Myocardial Viability Assessment on the Beneficial Effect of Cardiac Resynchronization Therapy in Patients With Ischemic Cardiomyopathy**

Jacopo Dalle Mule, Renzo Perelli, Marco Mazzella, Felice Martinelli, Alessandro Proclemer, Virginia Scarabeo, Maria Liberata Di Sipio, City Hospital Pieve di Cadore Cortina Auronzo, Pieve di Cadore Cortina, Italy, Ospedale San Martino, Belluno, Italy

Background we hypothesized that imaging with $Thallium^{201}$ imaging for viability could help identify patients with ischemic left ventricular (LV) systolic dysfunction under optimized medical treatment and dyssynchrony who benefit from cardiac resynchronization therapy (CRT)
Methods: we assessed viability status in 45 consecutive patients (age 64 ±7 y.o.) with LV EF <= 35%, QRS >= 120 ms, NYHA class 3 or 4, using SPECT $Thallium^{201}$ stress, early and 24-h redistribution imaging with a 20-segment model before CRT with biventricular pacing (BiV), measured EF, inter- and intra-ventricular synchrony conduction delay with gated radionuclide ventriculography , LV End Diastolic Volume Indices (LVEDVI) with echocardiography before and 6 months after BiV
Results:
Patients with <= 5 scarred segments on $Thallium^{201}$ imaging (Group A) demonstrated improvement of LVEF at 6 months (from 29 ±5% to 36 ±5%, $p<0.0001$), 21/23 (91%) had an EF increase >5 units, NYHA class (from 3.3 ±0.5 to 2.5 ±0.5, $p<0.0001$), reverse remodelling (LVEDVI decreased from 99 ±17 ml/m^2 to 86 ±16 ml/m^2, $p<0.0001$).
Conversely, patients with >5 scarred segments (Group B) failed to improve in LVEF (30