Title

Improved Islet Yield From UW Solution Preserved Pancreas With Dimethyl Fumarate Supplementation.

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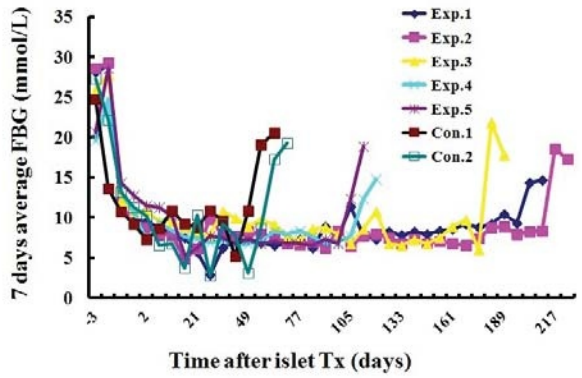

Figure 1. Results of islet transplantation (Fasting blood glucose were monitored twice a week).

In contrast, two recipients in control group were only 39 and 58 days, respectively. IVGTT results showed islet grafts were fully functional in both groups. Functional islets in bone marrow cavity were detected up to 225 days after transplantation by DTZ stain.

Conclusions: The operation of islets engrafted into bone marrow cavity is easier than into liver. The allogeneic islets in bone marrow cavity can survive and keep function for longer period than in liver in diabetic rhesus monkeys. The bone marrow cavity is a better site for islet transplantation.

Abstract# C1719

A Novel Microfluidic-Based Array For High-Resolution High-Content Imaging of Islets Before Transplant. M. Nourmohammadzadeh,[1,2] J. Mendoza-Elias,[1,2] L. Zeng,[1] Q. Wang,[1,2] Z. Li,[1,2] Y. Xing,[1,2] J. Oberholzer,[1,2] Y. Wang.[1] *[1]Surgery, University of Illinois at Chicago, Chicago, IL; [2]Bioengineering, University of Illinois at Chicago, Chicago, IL.*

Background: In order to facilitate in vitro study of pancreatic islets before the transplant, a microfluidic chip was designed to trap individual islet in an array format which allows us to study physiology and pathophysiology of islets in high-resolution fashion for high-content imaging.

METHODS: (1) A microfluidic array was designed based on hydrodynamic principle for pressure drop in a micro channel. (2) Fluid flow simulation performed using COMSOL to verify design principle and minimal shear stress on islet cells. (3) A thin PDMS membrane was integrated as an oxygen controller.

RESULTS: (1) Flow simulation verified the design principle, showing maximum velocity in trapping sites with minimum shear stress. (2) The device is capable of trapping up to 200 islets individually in an array format with 99% efficiency. (3)Both loading/trapping islets and fluid flow control were performed using gravity based flow control which eliminated the need for pumps. (4) The oxygen controller can provide fast oxygen microenvironment (less than 30 s) and varying and consistent oxygen concentrations and profiles. (5) As a result of having one layer design and using a very thin glass substrate,the device allowed multiparametric fluorescent and confocal imaging of islet cellular and subcellular metabolic activity and ion signaling such as mitochondrial energetics, ROS levels, calcium influx, and redox activity for high resolution high-content imaging purposes.

CONCLUSION: We designed, fabricated, and validated a novel microfluidic platform that allows trapping individual islets with high efficiency. Device also allowed for co-culturing islets and hypoxia studies, showing an improvement over conventional hypoxia chambers. The device allowed high-resolution imaging of islet using fluorescence and confocal microscopy and high-content multiparametric imaging of key insulin stimulator-secretion coupling factors. This work demonstrates the feasibility of array-based cellomics analysis.

Abstract# C1720

Improved Islet Yield From UW Solution Preserved Pancreas With Dimethyl Fumarate Supplementation. S. Li,[1] L. Robles,[1] C. Takasu,[1] K. Vo,[1] M. Takasu,[1] C. Foster,[1] N. Vaziri,[2] M. Stamos,[1] H. Ichii.[1] *[1]Department of Surgery, University of California, Irvine, Orange, CA; [2]Department of Medicine, University of California, Irvine, Orange, CA.*

Background: One of the major obstacles in clinical islet transplantation is the insufficient number of viable islets derived from preserved pancreas. During pancreas preservation and islet isolation, islets suffer from hypoxia and oxidant stress. In responses to oxidative stress, Nuclear factor erythroid-derived 2 factor (Nrf2) promotes expression of genes encoding antioxidant and detoxifying enzyme. Dimethyl fumarate, a novel Nrf2 activator, is also an intermediate in the citric acid cycle used by cells to produce energy in the form of ATP. We hypothesized that supplementation of preservation solution with DMF may improve islet yield by protecting islet during pancreas preservation.

Method: 8-week old male Sprague Dawley rats were used as pancreas donors. The common bile duct was cannulated and the pancreas was removed en bloc with duodenum and spleen and preserved in UW (UW group) or UW + DMF, 50uM (DMF group) at 4°C for 24 hours after flushing by same preservation solution via aorta. After 24 hour cold storage, the ADP/ATP ratio and expression of antioxidant proteins in pancreatic tissue were evaluated. Pancreas was then digested by collagenase and isolated rat islets were examined by assessing islet yield and cure rates after transplantation to diabetic nude mice.

Results: The islet yield (IEQ) in DMF group was significantly higher than UW group (969.3 ± 273.7 vs 412 ± 80.8; respectively, $P<0.05$). The cure rate after transplantation in DMF group (57.1%) was similar with UW group (66.7%, $P>0.05$). Likewise the percentage of viable and 8-OHDG positive cells in the isolated islets were comparable in the two groups. After 24 hour preservation, the ADP/ATP ratio of pancreas in DMF group was 4-fold lower than in the UW group. Enhanced expressions of HO-1 and NQO1 of pancreas were detected in DMF group.

Conclusion: UW supplementation with DMF significantly improved the islet yield compared with UW cold storage alone. The islet function and viability were comparable in both groups. Thus DMF may protect islets and reduce islet loss during preservation and isolation procedure by enhancing the citric acid cycle and antioxidant defense system.

Abstract# C1721

Human α1-Antitrypsin Blocks Injury-Induced Inflammation By High Mobility Group Box-1 (HMGB1) in Pancreatic Islets. D. Ochayon, M. Mizrahi, G. Shahaf, E. Lewis. *Department of Clinical Biochemistry and Pharmacology, Ben Gurion University of the Negev, Beer-Sheva, Israel.*

Introduction. The extracellular form of high mobility group box 1 (HMGB1) is involved in pathologies such as cellular injury and autoimmunity. Indeed, upon binding to immune-related receptors, HMGB1 promotes an inflammatory response, such that might take part in pancreatic islet destruction during both autoimmune diabetes and islet transplant rejection. Specifically, circulating HMGB1 levels were found to be elevated in patients with type 1 diabetes, and increased release of HMGB1 was found to correlate with injury degree and poor transplantation outcomes in islet allografting. The anti-inflammatory and immune-modulatory acute-phase protein α1-antitrypsin (AAT) promotes islet survival in both transplantation and autoimmune diabetes models. While AAT binds to damage-released proteins, such as HSP70 and gp96, its protective mechanism of action is yet unknown. **Aim.** Examine whether the protective activity of AAT is related to direct neutralization of extracellular HMGB1. **Methods.** Peritoneal macrophages and primary mouse islets were evaluated for inflammatory responses under stimulation with recombinant HMGB1 (rHMGB1) in the presence/absence of clinical-grade human AAT (Glassia, Kamada Ltd., Israel). Islet function was evaluated by insulin release. The effect of AAT on CpG-induced surface expression of HMGB1 was evaluated. AAT:HMGB1 binding was determined by direct ELISA. **Results.** Inducible surface expression of HMGB1 was reduced by AAT in peritoneal macrophages. Accordingly, in all tested cell cultures, AAT diminished rHMGB1-induced inflammatory responses (e.g., in HMGB1-stimulated islets, IL-1β release was reduced 2-fold, IL-6 2.5–fold and IL-1Ra was elevated 1.8–fold by added AAT; in these samples, inflammation-disrupted insulin release was restored). HMGB1-inducible TLR2 and CD40 surface expression were diminished by AAT. Finally, rHMGB1 directly bound AAT in a concentration-dependent manner. **Conclusion.** Our findings suggest that AAT is a naturally-occurring regulator of inflammatory responses mediated by extracellular HMGB1, such that preclude outcomes of islet transplantation. AAT may therefore be considered for therapy of cell damage-mediated pathologies in a clinically-safe manner.

Abstract# C1722

Eupatilin Induces Heat-Shock Protein-70 and Glutathione Expression, Attenuates Ischemic Damage and Apoptosis and in Mouse Islets. H. Jang, S. Kim, M. Oh, J. Lee, K. Kang, D. Han. *Surgery, University of Ulsan Gangneung Asan Hospital, Gangneug, Gangwon, Korea, Republic of; Anesthesia, University of Ulsan Gangneung Asan Hospital, Gangneug, Gangwon, Korea, Republic of; Surgery, University of Ulsan Gangneung Asan Hospital, Gangneug, Gangwon, Korea, Republic of; Natural Medicine Center, Korea Institute of Science and Technology (KIST), Gangneung, Gangneung, Gangwon, Korea, Republic of; Natural Medicine Center, Korea Institute of Science and Technology (KIST), Gangneung, Gangwon, Korea, Republic of; Surgery, University of Ulsan Asan Medical Center, Seoul, Korea, Republic of.*

The islet transplant is believed to be an attractive approach for cure of diabetes mellitus. Heat-shock protein (HSP70), which plays a vital role in cellular protection in various tissues subjected to stress. Eupatilin (5,7-dihydroxy-3,4,6-trimethoxyflavone), a pharmacologically active flavone derived from the Artemisia plant species, has been reported to have anti-oxidant, anti-inflammatory activities and expression HSP. We performed this study to examine the hypothesis that preoperative eupatilin administration induces HSP70 before islet transplant attenuating ischemic damage to mouse islets.Balb/c mice were randomly divided into two groups according to the treatment of eupatilin after isolation, cultured in medium with or