ELSEVIER

Effects of Pancreas Cold Ischemia on Islet Function and Quality

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ABSTRACT

We used a rat model of pancreas cold preservation to assess its effects on islets. Glands were surgically retrieved and stored in University of Wisconsin (UW) solution for 3 hours (Short) or 18 hours (Long) cold ischemia time (CIT). Islet yield was significantly lower in the Long-CIT than the Short-CIT group, as well as islet recovery after overnight culture ($P < .01$). Islet cell viability after isolation was significantly reduced in the Long-CIT group ($P < .05$). Reversal of diabetes following transplantation of suboptimal islet grafts occurred earlier in the Short-CIT group than the Long-CIT. All animals in the Short-CIT group and 80% in the Long-CIT group achieved euglycemia. Freshly isolated islets showed a significant increase of JNK and p38 ($P < .05$) phosphorylation in Long-CIT compared with Short-CIT. Histopathological assessment of the pancreas showed a significantly higher injury score. Proteomic analysis of pancreatic tissue led to identification of 5 proteins consistently differentially expressed between Short-CIT and Long-CIT. Better understanding of the molecular pathways involved in this phenomenon will be of assistance in defining targeted interventions to improve organ use in the clinical arena.

CURRENT limitations to whole pancreas and islet transplantation include the limited number of organs suitable for transplantation.[1] Several variables contribute to the reduction of islet mass and quality that can be obtained from a donor pancreas.[2] The aim of the present study was to evaluate the molecular mechanisms associated with pancreas cold ischemia that may impact islet quality and function.

MATERIALS AND METHODS

Animals were purchased from Harlan Laboratories (Indianapolis, Ind) and used in compliance of the local Institutional Animal Care and Use Committee. Male Lewis rats were used as pancreas donors. Glands were surgically retrieved and stored in University of Wisconsin (UW) solution for 3 hours (Short) or 18 hours (Long) cold ischemia time (CIT). After cold preservation, islet isolation was performed using Liberase (Roche, Indianapolis, Ind) digestion followed by Euroficoll density purification (Mediatech-Cellgro, Manassas, Va), as previously reported.[3] Islet yields and recovery after overnight culture were measured.[4] Islet cell viability was assessed on dissociated islets using flow cytometry, as described.[4,5] Stress-activated protein kinases were measured using Lumiex technology (BioRad, Hercules, Calif), as described.[6] Islet function was assessed in vivo by transplanting isolated islets into immunodeficient (athymic nu/nu) mice rendered diabetic by streptozotocin (200 mg/kg; Sigma-Aldrich, St. Louis, Mich).[7] Proteomic analysis was performed using 2-DIGE (Applied Biomics; Hayward, Calif, United States).

RESULTS

Islet yield was significantly lower in the Long-CIT (~1.75-fold) than the Short-CIT group ($P < .05$), as well as islet recovery after overnight culture (~1.43-fold; $P < .05$). Islet cell viability after isolation was significantly reduced in the Long-CIT group ($P < .05$; Table 1). Reversal of diabetes following transplantation of suboptimal islet grafts occurred earlier in the Short-CIT group than the Long-CIT group. All animals in the Short-CIT group and 80% in the Long-CIT group achieved euglycemia (not shown). Freshly

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Table 1. Effects of Cold Ischemia of the Pancreas on Islet Yield, Recovery After Culture, and Stress Kinase Phosphorylation

	Short-CIT (n)	Long-CIT (n)	*P*
Islet yield (per donor pancreas)	1515 ± 529 (45)	868 ± 211 (69)	<.05
Islet recovery after culture	82 ± 13 (7)	57 ± 18 (11)	<.01
JNK phosphorylation (fold increase)	1.0 ± 0.4 (5)	1.4 ± 0.9 (5)	<.05
p38 phosphorylation (fold increase)	1.0 ± 0.6 (5)	1.9 ± 0.6 (5)	<.05

isolated islets showed a significant increase of JNK (1.4-fold) and p38 (1.9-fold) phosphorylation in the Long-CIT group compared with the Short-CIT group, respectively ($P < .05$) (Table 1). Histopathological assessment of the pancreas showed significantly increased overall injury score overall in the Long-CIT group than the Short-CIT group (not shown). Preliminary proteomic analysis of pancreatic tissue led to identification of at least 5 proteins consistently differentially expressed between the Short-CIT group and the Long-CIT group (not shown).

DISCUSSION

Cold ischemia of the pancreas significantly affects both islet cell yield and potency (viability and function) paralleled by increased phosphorylation of stress kinases. Better understanding of the molecular pathways involved in this phenomenon will be of assistance in defining targeted interventions to improve organ use in the clinical arena.

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