Twenty-Four Hour Hypothermic Machine Perfusion Preservation of Porcine Pancreas Facilitates Processing for Islet Isolation

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ABSTRACT

Procurement of donor pancreata for islet isolation and transplantation is not yet widely practiced due to concerns about the impact of postmortem ischemia on functional islet yields. Perfusion/preservation technology may help to circumvent ischemic injury as applied in this study of porcine pancreata prior to islet isolation. Pancreata harvested from adult pigs were assigned to 1 of 3 preservation treatment groups: G1, fresh controls, processed immediately with minimum cold ischemia (<1 hour); G2, static cold storage, flushed with cold UW-Viaspan and stored at 2°–4°C for 24 hours; and G3, hypothermic machine perfusion (HMP) on a pulsatile LifePort machine Organ Recovery Systems, Inc., Des Plaines, Ill, United States with KPS1 solution at 4–7°C and low pressure (10 mm Hg) for 24 hours. Islet isolation was then accomplished using conventional methods. Product release criteria were used to assess islet yield and function. Islet yield was markedly different between the treatment groups. There was a statistically significant increased yield in the HMP group over static cold storage in UW-Viaspan ($P < .05$). Functionally, the islets from each experimental group were equivalent and not significantly different from fresh controls (G1). Dithizone staining of islets showed consistently more uniform digestion of pancreata from G3 compared with G1 and G2, with greater separation of the tissue and fewer entrapped islets. HMP for 24 hours was well tolerated, leading to moderate edema but no loss of function of the harvested islets. The edema appeared to aid in enzymatic digestion, producing a greater yield and purity of islets compared with pancreata subjected to 24 hours of static cold storage.

IMPLANTATION of functional islet cells is a potential cure for diabetes, but the availability of high quality islets for transplantation is critical for success. Procurement of donor pancreata for islet isolation and transplantation is not yet widely practiced, due in part to concerns about postmortem ischemia on functional islet yields. Perfusion/preservation technology has had a major impact in circumventing ischemic injury in kidney transplantation.[1–3] Herein we applied this approach to the preservation and procurement of viable islets after hypothermic perfusion preservation of porcine pancreata.

METHODS

Using anesthetized pigs (Domestic Yorkshire, 25–30 kg), pancreata were flushed in situ with cold Lactated Ringer's solution (2 L) prior to excision using a surgical technique that preserved the head, tail, and body of the pancreas together with a section of the duodenum to protect the pancreaticoduodenal arteries. For ex vivo flushing and perfusion, the superior mesenteric artery and celiac trunk were cannulated, and the splenic vessels and all arterial branches on the margin of gastroduodenal, splenic, and hepatic sides of the pancreas were ligated to allow uniform perfusion through the gland with effluent flow through the portal vein. Pancreata were assigned to 1 of 3 experimental groups in which they were processed for islet isolation after the following preservation treatments: G1, fresh controls, processed immediately with minimum cold ischemia (<2 hours; n = 7); G2, static gold storage, flushed with cold UW-Viaspan and stored at 2°–4°C for 24 hours (n = 9), and G3,

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Table 1. Islet Yield and Function Data

Pancreas/Islet Characteristics	G1: Fresh (Untreated Control; N = 7)	G2: Control (Viaspan; N = 9)	G3: HMP (KPS-1; N = 7)
Pancreas weight (g)	114.60 ± 7.16	118.10 ± 5.18	107.10 ± 8.03
Postpreservation edema (%)	—	−2.79 ± 0.71	138 ± 18.6
Digested tissue (%)	82.62 ± 2.62	81.38 ± 1.97	76.13 ± 3.53
Digestion time (s)	757 ± 61	707 ± 39	638 ± 27
Total IEQ	116.894 ± 31,428	74,956.9 ± 16,396	187,857 ± 36,608*
IEQ/(g of digested tissue)	1305 ± 348	784 ± 181†	2397 ± 478
Insulin stimulation index	4.59 ± 1.33	2.45 ± 0.37	2.88 ± 0.44
High-glucose insulin (ng/mL/IEQ)	0.33 ± 0.15	0.20 ± 0.05	0.23 ± 0.08
Insulin content (ng/mL/IEQ)	8.51 ± 3.68	4.75 ± 1.00	11.80 ± 3.79§
Amylase content (μg/mL)	102.13 ± 59.10	13.20 ± 2.14	28.2 ± 7.33
Insulin/amylase (%)	4.71 ± 1.13‡	11.54 ± 1.89	25.53 ± 5.02

Abbreviation: IEQ, islet equivalent.
*$P < .05$ vs G2: Control group.
†$P < .05$ vs G3: HMP.
‡$P < .05$ vs G3: HMP.
§$P < .05$ vs G2: control group.

hypothermic machine perfusion (HMP), perfused on a pulsatile LifePort machine (Organ Recovery Systems, Inc., Des Plaines, Ill, United States) with KPS1 solution (Organ Recovery Systems, Inc.) at 4°–7°C and low pressure (10 mm Hg) for 24 hours (n = 7). At the end of the perfusion interval, the pancreas was removed from the LifePort perfusion machine and processed for islet isolation. This process involved ductal distension of the gland with liberase (PI) enzyme (Roche, porcine), normothermic digestion, and density gradient purification (Ficoll continuous density gradient on a COBE 2991 [Gambro BCT] cell separator).[4] Standard, accepted product release criteria recently adopted by the major clinical centers were implemented in this study. These tests included islet quantification, islet viability and histology, and functional viability assessment using the glucose-stimulated insulin secretion assay.[5]

RESULTS

Islet Retrieval Data

Progressive edema during continuous hypothermic perfusion of organs is an intrinsic event that is usually restricted by optimizing perfusion media and conditions.[6–8] In this study the measured degree of edema was 0%, −2.8% ± 0.7%, and 138% ± 19% for the 3 groups, respectively (Table 1). Negative edema in G2 was due to the hypertonicity of UW-Viaspan during static cold storage. Islet yield expressed as Islet Equivalents (IEQ) was markedly different between the treatment groups: G1 = 1306 ± 348 IEQ/g; G2 = 784 ± 181 IEQ/g; and G3 = 2397 ± 478 IEQ/g. The increased yield in the HMP group over the static cold storage in UW-Viaspan was statistically significant ($P < .05$). The purity of the islet preparations, measured as the ratio of insulin (from islets) to amylase (from exocrine cells), was as follows: G1 = 4.7% ± 1.1%; G2 = 11.5% ± 2%; and G3 = 25.5% ± 5%. Microscopic examination of the different preparations using Dithizone staining for islets showed consistently more uniform digestion of the pancreata from G3 compared with G1 and G2, with greater separation of the tissue and less entrapped islets. The method of preservation had a significant impact on the extent of digestion time and the amount of free islets released from the digest. Islet sampling during the process of digestion revealed the machine perfused pancreata to show more early free islets and a more homogenous digest, without fragments of exocrine tissue. Tissue digest from both fresh and control group pancreata showed more mantled (incompletely cleaved islets with adherent exocrine tissue) and entrapped islets compared with perfused organs.

The islet retrieval data are summarized in Fig 1, which shows that pancreas perfusion resulted in statistically signifi-

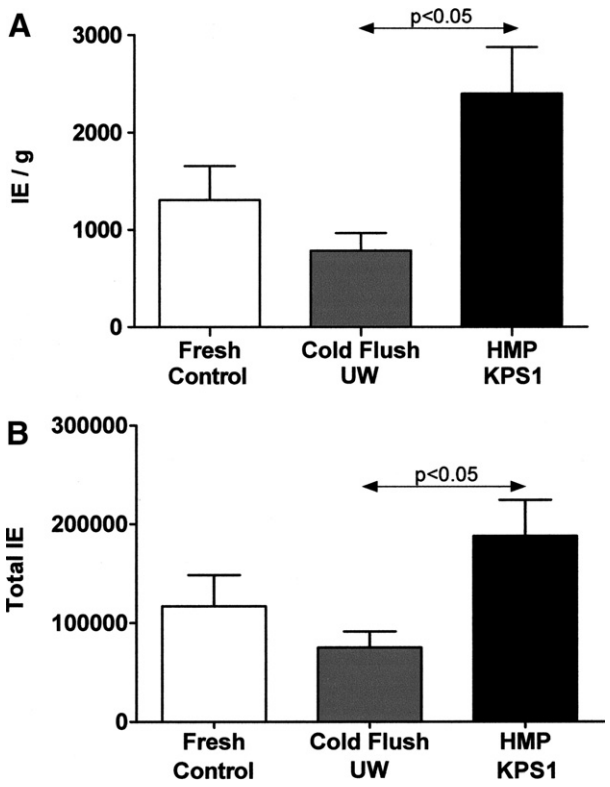

Fig 1. Islet retrieval data (mean ± SEM) expressed as both IEQ per unit weight of digested pancreas (**A**) and total islet yield per pancreas (**B**). Group 1, fresh control pancreata (N = 7); group 2, preservation controls cold stored in UW solution (N = 9); group 3, HMP with KPS1 solution (N = 7).

cantly higher IE/g of digested tissue compared with the fresh and control groups. Machine perfusion allowed the remnant blood to be washed off and also, based on the amount of water accumulation (edema), provided a disrupted extracellular space without a negative impact on the ductal distension and islet viability. These features ultimately helped more to rapidly free islets. A correlation between edema and digestion time seemed to exist: 638 ± 26 seconds and 138% ± 19% edema for HMP and 757 ± 61 seconds and 707 ± 38 seconds and no edema for fresh (G1) and cold stored in UW (G2), respectively. The greater the edema the shorter the digestion time.

Islet Integrity

Table 1 summarizes the data for islet function in terms of insulin content and the ability to respond to a secretory challenge, which was expressed as the Stimulation Index determined by comparing the insulin released during sequential exposure to low (2 mmol/L, nonstimulatory) versus high (20 mmol/L, stimulatory) glucose concentrations. Functionally, the islets from each experimental group were equivalent and not significantly different from the controls (G1) with insulin stimulation indices of G1 = 4.6 ± 1.3, G2 = 2.5 ± 0.4, and G3 = 2.9 ± 0.4. However, insulin content (ng/mL/IEQ) was different between the treatment groups with the highest insulin content in islets harvested from HMP (G3) = 11.8 ± 3.8 compared with G1 = 8.5 ± 3.7 and G2 = 4.8 ± 1.1 for fresh controls and static cold storage, respectively. The mean insulin content of islets isolated from perfused pancreata (G3) was significantly higher than that of the UW/Viaspan cold-stored control group (G2) but not significantly different from the mean values from fresh tissue (G1). Moreover, the stimulation indices showed that the insulin secretory function of the islets isolated from perfused pancreata was not compromised when compared with the control groups.

Histology Results

The basic structure of perfused pancreata was well preserved, showing the normal conformation of secretory cells with an abundance of secretory granules concentrated at the apical pole of the pyramidal cells. By contrast, a moderate degree of cellular disruption and degranulation was observed among samples processed from pancreata cold stored for 24 hours in UW-Viaspan.

DISCUSSION

Ever since the first experimental attempts to ameliorate type 1 diabetes by transplantation of allograft donor islets, the field has been challenged by the need for improved methods of retrieving islets from donor pancreata. During the course of these studies, we discovered that the technique of hypothermic machine perfusion preservation resulted in a greater yield of islets than either fresh, or static cold-stored pancreata. In fact, we observed a 1.6–1.8 times greater yield over the fresh group of pancreata and an approximately 3-fold greater amount than for pancreata preserved in UW Viapsan.

We concluded that 24 hours of HMP was well tolerated, leading to moderate edema but no loss of function of the harvested islets. On the contrary, the edema appeared to aid in the subsequent disruption of the gland during enzymatic digestion, producing a greater yield and purity of islets compared with pancreata subjected to 24 hours of static cold storage in UW-Viaspan. Further research is needed to determine whether this phenomenon is peculiar to the porcine model, or whether the same salutary effects of hypothermic machine perfusion can be achieved with human organs.

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