Published in final edited form as:

Transplant Proc. 2010 December ; 42(10): 4209–4212. doi:10.1016/j.transproceed.2010.09.138.

Surgical Protocol Involving the Infusion of Paramagnetic Microparticles for Preferential Incorporation within Porcine Islets

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Abstract

Introduction—Despite significant advances, widespread applicability of islet cell transplantation (ICT) remains elusive. Refinement of current islet isolation protocols may improve ICT outcomes. Islet purification by magnetic separation (MS) has shown early promise. However, surgical protocols must be optimized to maximize the incorporation of paramagnetic microparticles (MP) within a greater number of islets. The objective of this study is to explore the impact of MP concentration and infusion method on optimizing MP incorporation within islets.

Methods—Five porcine pancreata were procured from donors following cardiac death. Splenic lobes were isolated and infused with varying concentrations of MP (8, 16 and 32×10^8 MP/L of cold preservation solution) and using one of two delivery techniques (hanging bag versus hand-syringe). Following procurement and infusion, pancreata were stored at 0–4°C during transportation (< 1 hour), fixed in 10% buffered formalin and examined by standard MRI and histopathology.

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Results—T2*-weighted MRI illustrated homogeneous distribution of MP in all experimental splenic lobes. In addition, histologic analysis confirmed that MP were primarily located within the microvasculature of islets (82–85%), with few MP present in acinar tissue (15–18%), with an average of 5–7 MP per islet (within a 5 μ m thick section). The highest MP incorporation was achieved at a concentration of 16×10^8 MP/L using the hand-syringe technique.

Conclusion—This preliminary study suggests that optimization of a surgical protocol, MP concentrations and applied infusion pressures may enable more uniform distribution of MP in the porcine pancreas and better control of MP incorporation within islets. These results may have implications in maximizing the efficacy of islet purification by MS.

Introduction

Islet cell transplantation (ICT) has shown promise in the treatment of type 1 diabetes (T1D), but widespread applicability has not yet been achieved. Among the challenges limiting outcome is the inability to recover a sufficient amount of viable islets from a donor pancreas (1–4). Islet isolation techniques involving purification by magnetic separation (MS) have exhibited promise in obviating some of these challenges (5–10). Early studies in rodents have revealed the potential of using MS in conjunction with an intravascular infusion of paramagnetic microparticles (MP) to preferentially label the islets (7). Distribution of MP throughout the entire human pancreas appears to be reproducible, but purification of this larger volume of tissue (following digestion) has thus far been limiting (9). Quadrupole magnetic sorting (QMS) was primarily developed for single cell suspensions, but may represent the most promising technique for the MS of islets from acinar tissue, particularly in larger-sized organs. However, unlike the MP distribution achieved in human organs (9), the first attempts at infusing MP into porcine pancreata have resulted in poor distribution (10). With porcine xeno-ICT having moved to the forefront of the field (11) and MS having shown considerable promise in improving islet isolation and purification over currently-used protocols (5–10), improving MP distribution throughout the porcine pancreas and the fraction of total islets labeled with MP represent two objectives for the successful application of QMS technology to porcine islet purification. New insight into the anatomical variability in porcine pancreatic vascular and ductal anatomy may provide an opportunity for improved surgical techniques accompanying MP infusion. Additionally, systematic investigation of selected surgical parameters may enable optimization of MP distribution and preferential incorporation of MP within porcine islets. The objective of this preliminary study was to explore the impact of the MP concentration in the perfusate and the infusion method used (either hanging bag or hand-syringe) during pancreas procurement on MP distribution and preferential islet labeling.

Methods

Porcine pancreas procurement and MP infusion

All procedures involving animals were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Minnesota. The porcine pancreas procurement was performed by standard protocol used at the Schulze Diabetes Institute and has been described elsewhere (11,12). Briefly, 5 pancreata were harvested from adult Landrace pigs following cardiac death using an *en bloc* technique. The posterior aorta was identified, longitudinally divided and both the celiac trunk (CT) and superior mesenteric artery (SMA) were cannulated. Distal splenic, gastric and hepatic vessels were clamped and 1 L of cold preservation solution (CPS, cold storage/purification stock solution containing 2% Pentastarch, Mediatech, Inc, Herndon, VA) was flushed into both the CT and SMA, simultaneously. During the flush, the main pancreatic duct was identified and cannulated just proximal to its insertion into the duodenum. Approximately 60 mL of CPS was then

slowly infused over two minutes by hand-syringe into the main pancreatic duct. Following the flush, the pancreas was excised and divided into the combined connecting/duodenal lobes (control) and splenic lobe (experimental), all while taking care to preserve native vasculature. Varying concentrations of paramagnetic MP (4.5 μ m diameter, Dynabead M450, Invitrogen, Carlsbad, CA) suspended in 1 L of CPS were then infused into the splenic lobe only, via the CT and SMA by either hanging bag (gravity) or hand-syringe (applied pressure) infusion. Suspension concentrations of 8, 16 or 32 ($\times 10^8$) MP per L of CPS were used. Infusion of MP suspension was immediately followed by a 1 L flush with CPS. All lobes were then submerged into CPS and stored at 0–4°C for transportation from the procurement facility (< 1 hour). The splenic lobe was fixed with 180 mL of 10% buffered formalin injected via the CT and SMA and then all lobes were completely immersed in formalin.

MRI

Following complete formalin fixation (≥ 24 hours), pancreata underwent MRI. MRI was performed at 1.5 T with an APOLLO spectrometer (Tecmag Inc., Houston, TX, USA) and a custom-built 16-leg low-pass birdcage resonator (24 cm diameter, 20 cm in length). Images were obtained with a T2*-weighted true three-dimensional gradient echo sequence with one dimension frequency-encoded and the other two dimensions phase-encoded. A nonselective pulse was used to nutate the nuclei approximately 25 degrees during the scan. Typical acquisition parameters included a 9.6 msec acquisition time, 6.5 msec echo time, 120 msec repetition time, and a 20 cm $\times$ 10 cm $\times$ 5 cm field of view.

Histopathology

After MRI, biopsies were collected from several regions of the pancreas, embedded in paraffin and sectioned at 4 μ m. Sections were examined using hematoxylin and eosin (H/E) and insulin immunohistochemical stains. Sections were evaluated by an experienced histopathologist (T.D.O.) to estimate fraction of MP incorporation within islet and acinar tissues.

Results

T2*-weighted MRI illustrated a uniform distribution of hypointense regions, indicating the presence of MP throughout the experimental splenic lobe in all organs (Figure 1). Histologic analysis confirms that MP were found predominantly within islet microvasculature, with very few present in surrounding acinar tissue (Figure 2). Using varying MP concentrations and either hanging bag or hand-syringe infusion techniques has enabled MP incorporation in up to 90% of islets, as estimated by histology based on fraction of islets containing at least one MP. In the histologic sections examined, MP were preferentially distributed within islets (82–85%) rather than acinar tissue (15–18%), with an average of 5–7 MP per islet (within a 5 μ m thick section). The highest MP incorporation within islets was achieved using the hand-syringe infusion technique with a concentration of 16×10^8 MP per liter of CPS.

Discussion

Islet cells endure significant stresses under current isolation protocols and many islets are either never recovered during isolation (4,13) or are lost via a number of cell death processes (13–17). Currently, few institutions are able to treat T1D with islets from a single donor pancreas (1,2). Consequently, if more viable islets can be isolated per pancreas, there may be an opportunity to reduce the frequency of ICT requiring multiple donors or to treat multiple patients with one donor (18). Additionally, with porcine xeno-ICT providing much hope (11), improving the porcine islet isolation process has become a worthwhile endeavor.

MS is a technique that may allow for isolation of superior quality islets. Using magnetic forces to preferentially separate islets from the acinar tissue of the pancreas following enzymatic digestion is attractive for several reasons. It may eliminate the use of continuous density gradient centrifugation, which imparts prolonged exposure to the harsh proteolytic components of digestion, toxic substances and mechanical stresses (4,13). MS may also shorten isolation time and thereby minimize ischemia to islets, which are known to be particularly sensitive to hypoxia (19–22). The number of MP required to achieve purification by QMS has been determined analytically based on fluid dynamics treatments and is largely a function of islet size and starting position in the fluid flow field (10,23). Islets that reach the QMS for purification are restricted to 500 μm in diameter and smaller by the mesh screen size used in the Ricordi chamber. All islets containing at least 10 MP will have sufficient mobility for QMS purification. Purification of islets having less than 10 MP is a function of size of the islet and relative starting position in the QMS magnetic field. When considering the 3-dimensional shape of a spherical islet, our data indicates that labeled islets will likely contain greater than 10 MP, on average.

Shenkman and colleagues published results of preferential porcine islet labeling via MP infusion prior to separation by QMS (10). They demonstrated that paramagnetic MP were preferentially sequestered within the islet microvasculature. Islets undergoing MS from acinar and ductal tissue during isolation did not exhibit diminished viability. Despite being able to illustrate the potential applicability of QMS to porcine islet purification, they noted an inhomogeneous distribution of MP throughout the pancreas as well as variable islet yields. Specifically, they described a narrow cylindrical region of infused MP extending from the splenic lobe into the duodenal lobe with the highest concentrations in the region most proximal to the point of infusion at the CT. The inhomogeneous MP distribution in the porcine pancreas may have been due to the use of a donation after cardiac death model with poor flushing. Comprehensive characterization of the complex vascular and ductal anatomy of the porcine pancreas (12) and improvements in the procurement and flushing techniques (24) have created a new opportunity to re-visit this problem. With a better understanding of porcine anatomy, surgical procurement and MP infusion techniques were substantially improved. In this preliminary study, we were able to show an improvement in the distribution of MP throughout the experimental splenic lobe, as shown by MRI and histology, by adjusting the MP concentration and infusion pressure. Despite a limited number of organs studied, we have observed improvements in the distribution of MP by using a manual hand-syringe infusion. Higher infusion pressures may allow for more homogeneous distribution of MP throughout the porcine pancreas and improved sequestration of MP within islet microvasculature. These early studies suggest that optimization of selected surgical procurement parameters may allow for better control of MP incorporation within islets. It is important to note that only splenic lobes were infused with MP via the CT and SMA in this study, whereas entire pancreata were infused with MP via the CT in the study published by Shenkman (10). Division of the pancreas and concomitant infusion of MP via both the CT and SMA may have enabled better distribution of MP throughout the splenic lobes.

Future studies will involve further optimization of surgical protocol, MP concentration and infusion pressures and techniques, including the use of machine perfusion and heart-beating porcine donors. It is conceivable that these techniques may be extended and optimized for use with human pancreata. In the past, QMS equipment had been largely used for single cell suspensions and was not initially developed to accommodate larger particles or cell aggregates (such as islets) or large suspension volumes, as is the case following the digestion of human or porcine pancreata. New QMS equipment has been developed that is capable of processing larger particles and volumes (8). Future QMS studies will include the use of this new equipment. Additionally, methods for rapid and quantitative assessment of

bead incorporation in islets as opposed to acinar tissue are needed for optimizing bead infusion protocols. Histology is a useful tool but is neither rapid nor quantitative, especially for large organs. A new technique involving particle-tracking velocimetry may enable real time quantitative assessment of bead incorporation into islets versus acinar tissue in the raw pancreas digest and could be proven extremely valuable in optimizing the process.

Acknowledgments

Research funding provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (2R44DK072647-02A1), the Schott Foundation, the Carol Olson Memorial Diabetes Research Fund and the Iacocca Foundation.

Abbreviations

CPS	Cold preservation solution
CT	Celiac trunk
H/E	Hematoxylin and eosin
ICT	Islet cell transplantation
MP	Paramagnetic microparticle(s)
MS	Magnetic separation
QMS	Quadrupole magnetic sorting
SMA	Superior mesenteric artery
T1D	Type 1 diabetes

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Figure 1. T2*-weighted MRI of the control connecting/duodenal lobe (above) and the experimental splenic lobe (below), in which infused MP resulted in well-distributed hypointense regions. MP were infused into the splenic lobe at a concentration of 16×10^8 MP/L of cold preservation solution using the hand-syringe technique.

Figure 2.

Representative low and high magnification micrographs of an islet located in the experimental splenic lobe (distal region), illustrating minimal accumulation of MP in the acinar tissue (Fig. 1A, H/E) and significant accumulation within the islet (Fig. 1B, H/E). Representative high-magnification micrograph showing preferential seeding of MP within capillaries of an islet located in the proximal splenic lobe, near the site of infusion at the celiac trunk (Fig. 1C, insulin). Insets in both high magnification images further illustrate presence of MP specifically within capillaries (outlined by dotted black lines). Histological samples were taken from a splenic lobe infused with 16×10^8 MP/L of cold preservation solution using the hand-syringe technique.