Published in final edited form as:
Xenotransplantation. 2014 September ; 21(5): 473–479. doi:10.1111/xen.12108.

Magnetic resonance imaging: A tool to monitor and optimize enzyme distribution during porcine pancreas distention for islet isolation

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Abstract

Background—Porcine islet xenotransplantation is emerging as a potential alternative for allogeneic clinical islet transplantation. Optimization of porcine islet isolation in terms of yield and quality is critical for the success and cost effectiveness of this approach. Incomplete pancreas distension and inhomogeneous enzyme distribution have been identified as key factors for limiting viable islet yield per porcine pancreas. The aim of this study was to explore the utility of Magnetic

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Resonance Imaging (MRI) as a tool to investigate the homogeneity of enzyme delivery in porcine pancreata. Traditional and novel methods for enzyme delivery aimed at optimizing enzyme distribution were examined.

Methods—Pancreata were procured from Landrace pigs via *en bloc* viscerectomy. The main pancreatic duct was then cannulated with an 18g winged catheter and MRI performed at 1.5 T. Images were collected before and after ductal infusion of chilled MRI contrast agent (gadolinium) in physiological saline.

Results—Regions of the distal aspect of the splenic lobe and portions of the connecting lobe and bridge exhibited reduced delivery of solution when traditional methods of distension were utilized. Use of alternative methods of delivery (such as selective re-cannulation and distension of identified problem regions) resolved these issues and MRI was successfully utilized as a guide and assessment tool for improved delivery.

Conclusion—Current methods of porcine pancreas distension do not consistently deliver enzyme uniformly or adequately to all regions of the pancreas. Novel methods of enzyme delivery should be investigated and implemented for improved enzyme distribution. MRI serves as a valuable tool to visualize and evaluate the efficacy of current and prospective methods of pancreas distension and enzyme delivery.

Keywords

Diabetes; Distention; Ductal Anatomy; Islet Isolation; Magnetic Resonance Imaging (MRI); Pancreas; Pancreas Divisum; Porcine; Transplantation; Xenotransplantation

Introduction

Porcine islet xenotransplantation is emerging as an attractive alternative to allogeneic clinical islet transplantation as porcine pancreata may be a source of islets capable of meeting the demand (potentially millions of patients with type 1 diabetes). The possibility of islet isolation from porcine pancreata promises a convenient solution to the limited supply of human pancreata (in the thousands per year in the US) by offering the potential for on demand high quantity and quality islets. Porcine islets have been shown to reverse diabetes in a number of small animal models and to maintain normal glucose levels in diabetic non-human primates for more than 100 days [1–4], under the coverage of potent immunosuppression. Clinical translation of porcine islet xenotransplantation will require “clean pigs” maintained in highly specialized and tightly monitored facilities, a costly endeavor [5–7]. This high cost, coupled with current difficulties in consistently obtaining high yields of viable islets from even fully mature animals, highlights the need for further optimization of the porcine islet isolation procedure. The successful intraductal infusion of enzyme solution into the pancreas is known to play an important role in obtaining successful islet isolation outcomes and has been highlighted in this study as an area to be targeted for improvement [8–10].

In this regard, comprehensive knowledge of the anatomy of the porcine pancreatic ductal system is essential for the development of innovative techniques for pancreas preservation and improvements in islet isolation procedures. Due to the complexities of the anatomy of

the ductal system and variations between donors [11–14], complete distension and homogeneous enzyme distribution remain a challenge despite substantial experience in the field. Failure to consistently obtain high viable islet yields from porcine pancreata with traditional enzyme delivery methods may actually be, in part, due to issues associated with incomplete distention and inhomogeneous enzyme distribution.

Depending on the anatomy and individual variations in the pancreatic ductal system, following the division of the pancreas into its three distinct lobes, significant portions of the organ may not be reachable by the enzyme. In this study we utilized Magnetic Resonance Imaging (MRI) to: 1) investigate the delivery, completeness of distension and homogeneity of enzyme distribution by traditionally used methods of enzyme delivery; and 2) compare two novel strategies for enzyme delivery into porcine pancreata and compare them with the traditional method.

Materials and Methods

Six female Landrace non-heart beating donor pigs aged between 6 to 24 months with mean weight of 452 ± 99 lb (average $\pm$ standard deviation, range 248 to 680 lb) were subjected to pancreas resection. All animals were operated on according to the rules and regulations of the Institutional Animal Care and Use Committee of the University of Minnesota.

Procurement

Animals were heparinized, euthanized by sodium pentobarbital overdose, and, following the cessation of heart rhythm, completely bled out and eviscerated. All intra-abdominal organs were procured *en bloc* onto a procurement table where the pancreas was dissected free of the other organs, as previously described [11].

MRI

Following procurement, the pancreata were placed on ice and transported to a 1.5 T magnet (MagneX Scientific, Abingdon, UK) using an Apollo spectrometer (Tecmag, Houston, TX) for MRI. Pancreata were placed into the magnet, as illustrated in Figure 1. Briefly: after division and cannulation, pancreata or separated lobes were placed into the MRI system and baseline images were acquired. A circulating water bath chiller and heat exchanger were used to maintain cold temperatures throughout the infusion and imaging process. Syringes attached to pre-primed lines running into the bore of the magnet were then used to manually infuse each pre-cannulated ductal bed with 5 mM gadolinium contrast solution (Magnevist®, Bayer Healthcare, Berlin, Germany), and post-infusion images were taken to determine the extent of contrast distribution. The dosing of the contrast was selected based on prior experience to maximize the contrast for this magnet. Temperatures were maintained from 4–8 °C with a specially designed water jacket running to a refrigerated water bath. Imaging was performed using a Gradient Echo Imaging sequence with a 90° flip angle, an echo time (TE) of 14 ms and a repetition time (TR) of 20 ms. Images spanned a field of view of 30 cm $\times$ 30 cm at a resolution of 256 $\times$ 256 from 5 mm slices, each image had 4 acquisitions averaged to ensure clarity.

Pancreas Distention

Based on the pancreatic ductal anatomy and the different types of ductal networks described in our previous work [11], the following 3 methods of ductal infusion were investigated twice for each method: **Method 1**, division of the pancreas into its 3 distinct lobes followed by antegrade infusion of the main duct of the splenic lobe, the main duct of the connecting lobe, and the main duct of the duodenal lobe (traditional method); **Method 2**, division of the duodenal lobe from the rest of the pancreas leaving the bridge between connecting and splenic lobes intact followed by antegrade infusion into the pancreatic lobes via cannulation of the main duct of the duodenal lobe, the main duct of the splenic lobe, and the main duct of the connecting lobe; **Method 3**, cannulation of the main pancreatic duct at the papilla of an in-tact pancreas, and infusion of the total amount of contrast solution. Examples of each of these methods are illustrated in Figure 2. Pancreata were infused with up to a total of 0.6 cc of Lactated Ringer's solution, doped with 5 mM gadolinium contrast agent (Magnevist®, Bayer Healthcare, Berlin, Germany), per gram of pancreas. For **Method 2**, 33% of the combined weight of the splenic and connecting lobes was assigned to the connecting lobe and 67% to the splenic lobe for purposes of contrast solution allocation.

Results

Six pancreata were investigated for this study. Pancreata were randomly assigned a distention technique with 2 investigated for each of the methods. Using MRI we were able to detect differences in enzyme distribution during distention. In 2 cases where the duodenal lobe was separated from the rest of the pancreas, as is done in **Methods 1 and 2**, we observed reduced or no delivery of contrast solution to regions of the duodenal lobe (Figure 3). Additionally, we observed a lack of delivery of contrast solution to a large portion of the connecting lobe in one case infused using the traditional **Method 1** (Figure 4A). All other cases demonstrated a relatively homogeneous delivery of contrast solution throughout all regions of the pancreas.

Discussion

Variations in the anatomy of the pancreatic ductal system may be a major factor affecting successful islet isolation from porcine pancreata. Presently, these variations are not considered prior to distention and this can leave regions within the pancreas with reduced enzyme delivery. Inefficient or limited enzyme delivery is regarded as a major cause of incomplete tissue digestion in some cases, which contributes to reduced islet yields. As such, new techniques must be developed to maximize delivery for all anatomical variants, or the distention should be actively monitored with a tool similar to the one discussed here to ensure optimal and efficient enzyme delivery and distribution. Pancreatic anatomy varies from donor to donor [15] and previous studies [16–18] have demonstrated the importance of pig strain on islet isolation outcome. In our study we used Landrace pigs as they are considered to be highly suitable donors for islet isolation and xenotransplantation [18–20] since isolations with this breed can yield large numbers of islets compared to other breeds. A previous study from our laboratory on adult Landrace pig pancreas anatomy [11] suggested that the current methods of enzyme delivery may limit the uniform delivery of enzyme

throughout the pancreas when atypical anatomical variations of the ductal network are observed. While visual assessment of pancreatic distention can give some idea of gross delivery, it is difficult to determine or quantitate the extent of delivery within the pancreas since only the superficial aspect can be observed. The MRI method proposed allows for clear visualization of the internal ductal system and can be used to appreciate the efficacy and homogeneity of enzyme delivery throughout and within the entire pancreas.

This study aimed to demonstrate that MRI can be utilized as a tool to evaluate the efficacy of enzyme delivery throughout the pancreatic ductal system, and that it can be used as a cost-effective method to screen and evaluate novel strategies for enzyme infusion with the goal of optimizing enzyme delivery and improve islet isolation outcomes. Three methods of delivery (Figure 2) including the presently used method (**Method 1**) were investigated, and imaged to evaluate distention efficacy and enzyme delivery. As demonstrated in Figure 3, methods of enzyme delivery that involved removal of the duodenal lobe from the rest of the pancreas can result in reduced or even total lack of delivery to significant portions of the lobe. Additionally, it was demonstrated that for pancreata with an atypical ductal anatomy, severing the bridge prior to enzyme infusion can prevent delivery to significant regions of either the connecting lobe (Figure 4B, Type 'A' ductal anatomy) or the splenic lobe (Type 'B' anatomy) [12–14, 21–23]. The data presented in this study further suggests that traditional methods of porcine pancreas distention may not facilitate reliable and homogeneous delivery of enzyme throughout the 3 lobes of the pancreas. The most likely cause of the incomplete distention of the pancreatic duct is the severing and mishandling of key ductal networks required for the delivery of the enzyme. One proposed way to avoid disrupting these ductal networks is to avoid segmenting the pancreas prior to distention (**Method 3**). All pancreata investigated with this method exhibited a uniform delivery of contrast medium throughout the organ. Therefore, minimizing the manipulation of the ductal network prior to distention may facilitate optimal distribution of the enzyme.

Prior knowledge of the pancreatic ductal system and the variable anatomy could be used to determine the most effective distention method for each pancreas, and would allow for disqualification of poorly distended pancreata before proceeding with the costly islet isolation process. Further development of this technique may enable prospective and real-time visualization of ductal network distention by MRI. If this could be developed as a prospective tool, it would enable internal visualization to ensure that all regions are adequately infused with digestive enzyme, and could improve the use of targeted local parenchymal enzyme injection to rescue a portion of inadequately distended tissue. Knowing the variable ductal distribution, and avoiding disruption the ductal networks caused by segmenting the pancreas, may make the distention process more efficient and effective, which may intern improve islet isolation outcomes.

The imaging technique described here for internal visualization of the porcine pancreatic ductal system may also be useful for human pancreata. One major example where MRI could offer value is for cases of pancreas divisum, where there is a lack of a connection between the main and the accessory pancreatic ducts. This condition afflicts 10–22% of all pancreata harvested for islet isolation [24]. It is anticipated that this anatomical variation could affect the distention of significant parts of the pancreas. This may be especially

important in clinical islet auto-transplantation [25] for patients with chronic pancreatitis, as pancreas divisum is reported to be associated with chronic pancreatitis [24]. Kin et al. used the traditional technique for distention, and compared isolation outcomes from normal versus visually identified pancreas divisum cases [24]. They observed no visual differences in distention quality, and found no statistical differences in islet yields between the 2 ductal variants, although mean islet yield was lower for pancreas divisum. Application of this imaging tool would enable the direct visualization of enzyme distribution for each of these cases and may offer an explanation for the lower islet yields observed for pancreas divisum. Furthermore, MRI could help detect regions with reduced enzyme delivery surrounding the accessory pancreatic duct due to narrowing. If these regions are not consistently and homogeneously distended, different paths for distention could be tested and optimized to potentially improve human islet isolation. This technique can facilitate the visualization of possible ductal anomalies or narrowing, the recognition of which could make the process of pancreas distention and islet isolation more effective.

Conclusions

Presently utilized methods for enzyme delivery through ductal distension in pig pancreata are incapable of delivering the enzyme consistently and homogeneously to all pancreatic regions given the variability in porcine pancreas anatomy. Visual assessment of pancreatic distention cannot accurately assess whether enzyme is effectively delivered throughout the entire pancreas. MRI is a valuable tool that can help improve the understanding of the variable pancreatic ductal anatomies, and may help overcome the difficulties associated with incomplete distention and inhomogeneous enzyme distribution for islet isolation. This imaging method can be used to help ensure uniform enzyme distribution in both porcine and human pancreata, and to evaluate and optimize new distention methods that might ensure adequate distention of all regions despite anatomical variations. The presented MRI based method should be further developed into a prospective and quantitative tool for visualization of enzyme distribution during pancreas distention for islet isolation. This would enable anatomy specific modifications to distention protocols for each individual pancreas, and may increase the consistency and success of certain islet isolations.

Acknowledgments

We thank William G. Purvis and Jennifer P. Kitzmann for graphical and editorial assistance and Samuel A. Stein for technical assistance with MRI imaging. This work was supported by the NIH, National Institute of Diabetes and Digestive and Kidney Diseases Grant Numbers R01DK068717 and R44 DK070400, as well as The Carol Olson Memorial Diabetes Research Fund, The Iacocca Foundation, and The Schott Foundation.

Abbreviations

cc	cubic centimeter
MRI	magnetic resonance imaging
T	Tesla
TR	repetition time

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Figure 1.

Illustration of the experimental setup for ductal infusion in a 1.5 T magnet for MRI. (Right Side) After division and cannulation, pancreata or separated lobes were placed into the MRI system and baseline images were acquired. A circulating water bath chiller and heat exchanger were used to maintain cold temperatures throughout the infusion and imaging process. (Left) Syringes attached to pre-primed lines running into the bore of the magnet were then used to manually infuse each lobe or section of the pancreas with the gadolinium contrast solution, and post-infusion images were taken to determine the extent of contrast distribution.

Figure 2.

Pictures depicting the different cannulation and infusion methods: (A) Traditional method separating the pancreas into 3 distinct lobes with antegrade infusion of the main ducts (**Method 1**); (B) Separation of the duodenal lobe with antegrade infusion into divided pancreas via cannulation of the main ducts with the bridge left intact (**Method 2**); and (C) Cannulation of main pancreatic duct at the papilla and infusion of total amount of contrast (**Method 3**).

Figure 3.

MRI of pancreata before and after infusion with contrast solution for each of the 3 methods investigated. Areas of the pancreas that were well distended and infused with contrast solution appear as intense white regions in the post-infusion images. When the duodenal lobe was separated, occasionally regions with no delivery or reduced delivery (outlined in green) were observed in the duodenal lobe.

Figure 4.

(A) MRI of a porcine pancreas with an uncommon variant ductal anatomy infused using **Method 1**. (B) Illustration of the common types of porcine pancreata ductal anatomy observed by Ferrer-Fabrega et al. in 2008 highlighting type 'A' that is shown by the MRI image. For this pancreas, areas of the connecting lobe outlined in green did not receive any contrast medium (white) during infusion because the ductal system in part of the connecting lobe drains through the bridge and out through the splenic lobe ductal system. **Method 1** will not effectively distend this portion of the connecting lobe in these pancreata.