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LETTER

The Pancreas as an Islet Transplantation Site. Confirmation in a Syngeneic Rodent and Canine Autotransplant Model

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Summary

Context The availability of islet transplantation is limited by both the number of donor pancreata and the number of islets required for successful transplantation. There is evidence that the liver presents a less than optimal environment for islets that contributes to short- and long-term beta cell destruction or failure.

Objective It is our hypothesis that the pancreas is a suitable transplant site and may require fewer islets than standard sites such as the liver or kidney, and could lead to improvements in transplantation outcomes.

Methods To test this hypothesis both a rodent and a canine model were used. Syngeneic rat islets were transplanted to the pancreas, liver, or kidney of Lewis rats. Fasting blood glucose levels were compared for three months as an index of islet function. Dogs received an islet autotransplant to a pancreatic remnant. Insulin and glucose concentrations were followed for six months.

Results In the rat, normoglycemia was maintained with 600 islets transplanted in the pancreas in contrast to the liver (3,200 islets) or kidney (1,000-2,000 islets). Dogs remained normoglycemic after receiving an intra-pancreatic islet transplant (mean 7,640±3,600 islets). There was no evidence of pancreatitis or nutritional deficiency in either species.

Conclusions The pancreas should be considered as an islet transplant site. The pancreas is the native milieu for islets, and offers the advantage of requiring fewer islets than other conventional sites, thereby increasing the possibility that one donor pancreas may serve one or more recipients.

Islet transplantation as a treatment for diabetes received significant attention when Shapiro and colleagues reported insulin independence or marked improvement in seven type I patients who received allogeneic islet transplants and a steroid free immunosuppression regimen [1]. Despite successes, multiple donor pancreata and transplants were required to achieve a sufficient functional islet mass [2]. The fact that multiple donors and several transplant procedures are required to achieve a sufficient surviving functional islet mass, demonstrates the importance of developing alternative methods to ensure the functional survival of islets sufficient to prevent diabetes using a smaller numbers of islets, thereby allowing one pancreas to serve the needs of one recipient.

It is accepted that a portion of non-immune graft failure may be the result of the implantation of a suboptimal number of islets [3, 4] as well as a function of the quality of the islets transplanted. The isolation process is

traumatic and exposes pancreatic islets to exogenous digestive enzymes and by-products which may be internalized by the islet cells. Islets exhibit a time dependent loss of insulin secretory ability from exposure to collagenase that is paralleled by an increased activation of apoptotic pathways and beta cell loss.

Transplanted islets are placed at further risk of failure by chronic metabolic stress which may be exacerbated by immunosuppression. We have previously suggested that organs often used as transplant sites, e.g. the liver or kidney, present markedly different environments for the transplanted islet including decreased interstitial tissue fluid oxygen availability and angiogenic responses which may affect islet survival [5, 6, 7]. Other factors, such as a self-limited transaminitis [8], chronic hypoxia, and local elevated postprandial glucose concentrations in the liver may contribute to beta cell loss. The pancreas was tested as a transplant site because the pancreas provides the liver with insulin directly through the portal vein and has a high angiogenic potential [5, 7], as well as a high tissue fluid oxygen content. Because of these potential advantages, the pancreatic site may allow the use of fewer islets to prevent diabetes in the recipient. We performed a study in a syngeneic rodent model in order to eliminate the confounding effects of immunosuppression and in a large animal autotransplant model as proof of principal. All animal procedures were approved by the institutional animal studies review board as required [9].

Methods

Rat Model

Male Lewis rats were used throughout the study in a syngeneic islet transplant model. Diabetes was induced in islet recipients by the intravenous injection of streptozocin (65 mg/kg body weight). Islets were isolated, from donor animals by collagenase digestion followed by ficol density gradient centrifugation as previously described [10]. Islets were washed in CMRL 1066 culture media and sorted for purity. Islets were held overnight prior to transplantation. Prior to creating the transplant pocket, a Clark-type glass oxygen micro-electrode was placed beneath the organ capsule in order to measure tissue fluid oxygen concentrations at each site as described by Carlsson *et al*. [11]. Two determinations were made at each respective site in each islet recipient. A single elongated pocket (2-2.5 cm) was made between the respective organ capsule and parenchyma to receive the islets. The respective organ capsule was gently grasped with forceps and a small incision was made through the capsule with a micro-scalpel. The pocket was formed by a microspatula advanced along the length of the organ. Islets of approximately equal size (200-250 micrometers diameter) were hand-picked for transplantation. Islet recipients received 600, 1,000, 2,000, or 3,200 islets as transplants to the liver, kidney, or pancreas. The islets were introduced into the pocket by expulsion from an elongated micropipette tip. After transplantation, samples for fasting blood glucose were obtained at weekly intervals for up to three months. Blood glucose measurements were performed with a commercial glucometer. Because of the difficulty in obtaining a sample sufficient for the measurement of blood glucose and insulin, samples for insulin analysis were obtained at the end of the study from the isolated perfused organ [12] or after an acute increase in fasting blood glucose indicative of islet failure. Pancreata were harvested and processed for histological study by standard methods using paraformaldehyde fixation and paraffin embedment. Sections were immunostained for insulin with fluorescein isothiocyanate (FITC) fluorescent antibody.

Canine Model

Seven mongrel dogs, three male, four female, weighing 23±3 kg were used as subjects in an islet autotransplant study. Partial pancreatect-omy was performed as previously described [13]. The head and tail of the pancreas were removed and processed for islet isolation. The pancreatic remnant consisted of about 25-30%

Table 1. Comparison of fasting blood glucose and insulin values from the rat (mean±SEM).

	Fasting blood glucose (mmol/L)	Perfusate insulin (pmol/L)
Normal pancreas (No. 15)	6.1±0.2	265±30
Diabetic control (No. 10)	11.9±0.8 [b]	66±5 [b]
Renal site (No. 15)	7.3±0.6 [ac]	30±5 [bd]
Intrapancreatic site (No. 15)	4.5±0.4 [bd]	155±15 [ad]
Liver site (No. 15)	-	-

1,000 islets were transplanted to the kidney and pancreas. Organs were harvested after three weeks for perfusion.
[a] P<0.05, [b] P<0.01 *vs*. normal pancreas; [c] P<0.05, [d] P<0.01 *vs*. diabetic control (ANOVA)

of the intact pancreas. Care was taken to preserve the pancreatic vasculature to the remnant and the pancreatic duct. As a control, a subset of five dogs had a partial (50%) pancreatectomy without islet replacement in order to achieve an islet content similar to the animals receiving an islet transplant. In each procedure, the edges of the remaining remnant were clamped and compressed by a mattress suture line parallel to the cut edges. No bleeding or leakage was observed.

Islets were isolated by a modification of an automated method [14]. A mean of 169,640±71,890 were isolated with a purity of 87.9±4.5%. A mean of 7,640±3,600 islets per kg body weight was transplanted to the donor pancreas remnant immediately after isolation. Islets were placed at random into the donor pancreas into 4-6 sites by expulsion from a cannula. The injection continued as the cannula was withdrawn in order to increase the distribution of islets within the pancreatic remnant. No bleeding or leakage was observed from the puncture sites. Dogs received insulin sufficient to maintain fasting blood glucose for 3-5 days. Thereafter no further treatment was required. All dogs had free access to water and a standard laboratory diet. Samples for fasting blood glucose, insulin, and amylase/lipase concentrations were obtained at weekly intervals for six months as a determination of islet and exocrine pancreatic function. Pancreata were harvested at the end of the study and were process for histological examination. Tissues were formaldehyde fixed and embedded in paraffin. Sections were immunostained for insulin and were counterstained with toluidine blue.

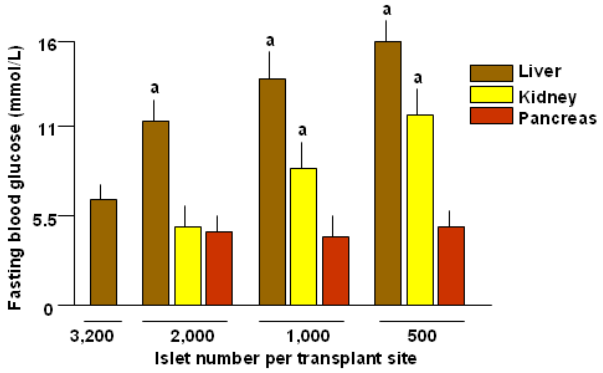

Figure 1. Fasting blood glucose levels per transplant site *versus* islet number in the rat.

Fasting blood glucose levels are dependent upon the number of islets transplanted to the specific transplant site. Data are expressed as mean±SEM per group from the day of transplant to islet failure or the end of the three month study period.
[a] P<0.001 versus respective pancreatic site per group (ANOVA; No. 10 in each group).

Statistics

Statistical analyses were performed using ANOVA. Data are expressed as mean±SEM.

Results

Rat Model

Glucose testing was begun five days after transplantation. As shown in Figure 1, normal fasting blood glucose levels were obtained at the pancreatic site with 600 islets whereas 3,200 islets were required in the liver and over 1,000 were required in the kidney. These observations suggest that diabetes may be prevented by a range of islet numbers depending upon the transplant site. Blood glucose and insulin values differed between the transplant sites despite the presence of an equal number of islets in the transplant in each subset of islets (Table 1, Figure 1). Rats

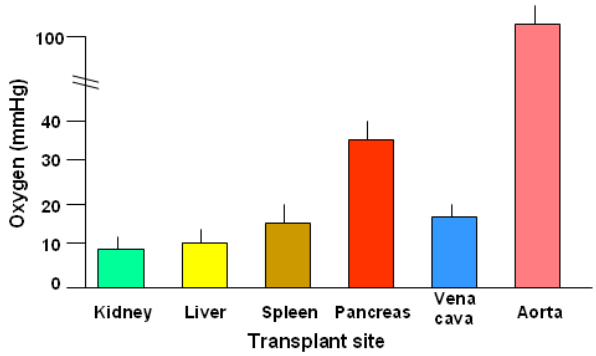

Figure 2. Tissue fluid oxygen availability.

The liver and kidney present a low oxygen environment as a potential islet transplant site. Oxygen availability in the pancreas is much higher that in the liver, thereby presenting a more favorable environment for islet survival. Data are expressed as mean±SEM. (No. 10 per site).

receiving a pancreatic site islet transplant were normoglycemic within one week post transplant and remained normoglycemic throughout the study, whereas graft failure occurred within 2-3 weeks in the liver and renal sites dependent upon the number of islets transplanted (Table 2). The difference in islet performance and survival between sites may be related in part to the variation in tissue fluid oxygen concentrations between sites (Figure 2). The pancreatic site has more available tissue fluid oxygen than the other sites tested. Although it is known that islets may regenerate in rodents, no regeneration of islets was noted, as native islets remained beta cell deficient after streptozocin treatment (Figure 3). The transplanted islets were robust and well vascularized (Figure 3).

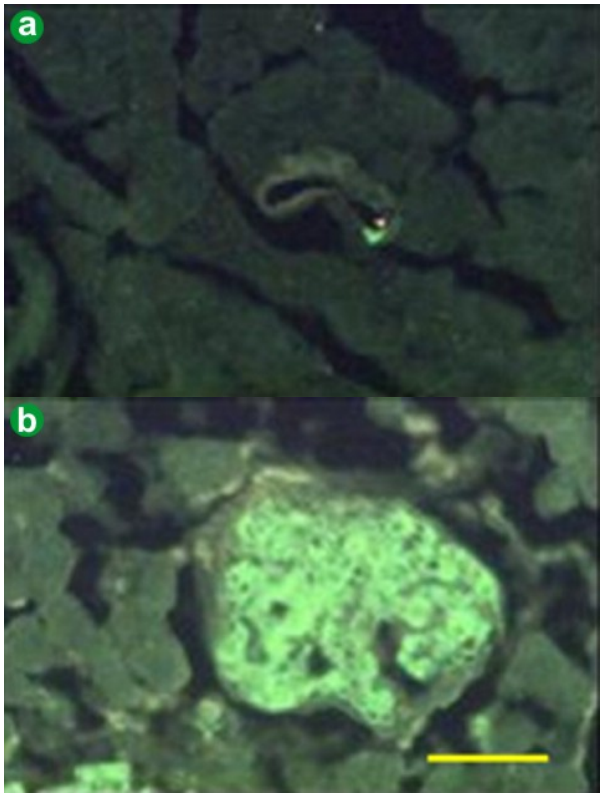

Figure 3. Comparison of native *in-situ* and transplanted rat islets in streptozocin-diabetic rat pancreas. Beta cells were immunostained for the presence of insulin. **a.** Few beta cells remain in the native *in-situ* islet. The islet architecture is abnormal as the beta cells have been destroyed by streptozocin. The islet is mostly composed of alpha and delta cells. **b.** The transplanted islet remains robust and is well populated by beta cells. Scale bar 50 µm.

Table 2. Rat survival versus number of islets.

Weeks post transplant	**1**	**3**	**5**	**7**	**9**	**11**	**13**	**15**
Islets transplanted: 0								
- Diabetic control	8/10	0/10						
Islets transplanted: 600								
- Liver	8/10	0/10						
- Kidney	15/15	13/15	0/15					
- Pancreas	15/15	15/15	15/15	15/15	15/15	15/15	15/15	15/15
Islets transplanted: 1,000								
- Liver	9/10	0/10						
- Kidney	15/15	15/15	15/15	14/15	14/15	13/15	12/15	10/15
- Pancreas	15/15	15/15	15/15	15/15	15/15	15/15	15/15	15/15
Islets transplanted: 2,000								
- Liver	10/10	8/10	0/10					
- Kidney	15/15	15/15	15/15	15/15	15/15	15/15	15/15	15/15
- Pancreas	15/15	15/15	15/15	15/15	15/15	15/15	15/15	15/15
Islets transplanted: 3,200								
- Liver	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10

Canine Model

Dogs receiving an intra-pancreatic islet transplant maintained fasting blood glucose in the normal range throughout the six month study. Dogs that underwent partial pancreatectomy without islet replacement had significantly less insulin and higher fasting blood glucose. Data were obtained through the study period are presented in Table 3. There was no evidence of pancreatitis, malnutrition , or digestive insufficiency during the study as suggested by amylase values which remained within the normal range through out the study (Table 3). The transplanted islets were well vascularized and well populated with beta cells (Figure 4).

In both the rodent and canine models islet failure was considered to have occurred if fasting blood glucose was elevated above 11 mmol/L for three consecutive days. Those animals were euthanized under surgical levels of anesthesia.

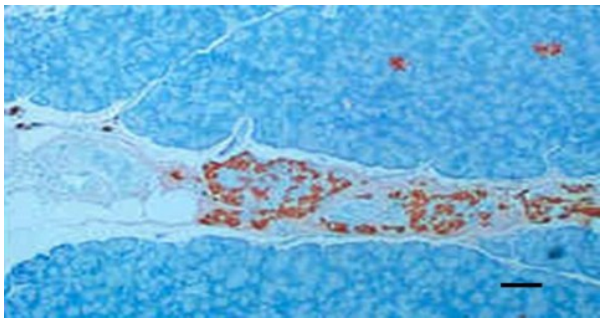

Figure 4. Canine islets transplanted into the canine pancreas. The islets were immunostained for insulin as a marker for islet beta cells. The islets are well populated by beta cells and are well vascularized. Scale bar 50 μm.

Discussion

Allogeneic islet transplantation offers great promise for patients who suffer from type I diabetes. Despite remarkable progress, the results five years following the introduction of the Edmonton protocol are disappointing as patients still require multiple islet infusions owing to high islet failure rates with high graft failure rates [1, 2]. Several strategies to enhance islet cell function are currently under investigation and include alternative sources of cells [15, 16, 17], improved harvesting and islet isolation techniques [3, 18, 19, 20], and the modulation of immunologic interactions between the recipient and grafted tissue in allogeneic transplantation [3, 21]. Among such strategies, alternative methods of implantation should also be an area of consideration. A change in the site of implantation from the liver could have an immediate positive effect on islet cell function and would likely offer an improved metabolic environment for islets than does the current hepatic-portal environment.

The method of transplanting islets into the portal vein likely causes major risks to islets that ar e deleterious to cell survival. Islets lodge in the portal venous bed immediately following transplantation where they receive portal blood before neovascularization in the hepatic parenchyma 2-3 weeks post-transplantation [22, 23]. The hepatic site has high concentrations of toxic metabolites and cytokines by virtue of its metabolic functions of detoxification and reprocessing as well as the proximity of lymphocytes and macrophages to the implanted islets. Moreover, Moberg *et al.* [24] demonstrated an inflammatory blood reaction involving the expression of tissue factors within minutes post-transplantation, posing an imminent danger to islets, as it has been demonstrated that beta cells selectively express tissue factor

Table 3. Comparison of fasting blood glucose and insulin values from canine islet recipients six months after islet transplantation (mean±SEM).

	Fasting blood glucose (mmol/L)	Plasma insulin (pmol/L)	Serum amylase (U/L)
Normal dog (No. 15)	5.3±0.16	54±3.6	700±90
Subtotal pancreatectomy (No. 5)	7.2±0.6 [a]	9.7±2.8 [b]	397±83 [b]
Subtotal pancreatectomy with intra-pancreatic islet replacement (No. 7)	5.8±0.77 [c]	50±5.0 [d]	588±152

[a] P<0.05, [b] P<0.001 *vs*. normal dog; [c] P<0.005, [d] P<0.001 *vs*. subtotal pancreatectomy (ANOVA)

after isolation. The method of islet transplantation may have an effect upon islet survival and function as well as the transplant site. Islets transplanted to the liver and spleen employ venous infusion into the respective organ. Other sites such as the pancreas and renal capsule use injection or implantation into a subcapsular pouch. Whether these various methods have an effect upon the islets is not known. A direct comparison by vascular embolism into the pancreas or kidney is difficult methodologically and may result in vascular and organ damage. The development of local infarction and the release of tissue factors may favor physical implantation, and attendant inflammation, although vascular infusion places the islets into the oxygen bearing vascular system. These differences may from the basis for future research.

Insulin release is primarily regulated by changes in circulating glucose concentrations. Islets in their native site of the pancreas are normally regulated by arterial concentrations of glucose that range from 4.5-7 mmol/L. Glucose levels in the portal environment are much higher however, and can exceed 11 mmol/L following meals [25, 26], thereby exposing islets to chronic supraphysiological levels of hyperglycemia that may be toxic to islets [27, 28, 29, 30, 31, 32]. Chronic exposure to hyperglycemia, occurring with progressive failure of beta-cells and glucose intolerance, or due to the location of the islet graft in the hepatic-portal environment, can lead to beta-cell death.

Several alternative sites of islet implantation have been considered and include transplantation beneath the kidney capsule, peritoneal cavity, testes, the spleen, omentum and pancreas [33, 34, 35]. Some success has been reported using the pancreatic site [34], however undifferentiated fetal beta cells were used. Although the source and maturity of the islets was different, the latter study and our present report agree that the pancreas is a suitable islet transplant site. The kidney capsule is useful for research purposes, but is not a viable option in humans because of inefficient vascular innervation in primates, the propensity of the capsule to develop fibrosis, and because the subcapsular space allows for limited nutrient diffusion [28, 36, 37, 38]. The spleen has been suggested and is currently a site used in large animal models [39, 40], but poses a risk for hemorrhage [41]. The liver was chosen as the current clinical transplant site for allogeneic islets because of historic experience in using the liver in autologous islet transplantation [1] and easy surgical access. The omentum is a potential site because of its angiogenic properties [34, 42] and the pancreas has been proposed with the rationale that as this is the native site of beta cells and has adequate oxygen availability as well as requisite factors for more normal revascularization and reinnervation [5, 6, 43]. In addition, there is evidence supporting the positive milieu of the pancreas that would confer advantages to the short and long-term survival of islets post-implantation. The site of islet transplantation is important in several respects other than direct blood flow. We have noted a difference in angiogenic potential, or sensitivity to angiogenic growth factors, fibroblast growth factor and vascular endothelial growth factor, between potential islet transplant sites [5, 6, 7]. Regulated insulin secretion and islet survival post-implantation relies on rapid islet revascularization [5, 42, 44, 45]. The interactions between the transplanted islet and the local recipient tissues have not been previously considered as contributing to islet survival and function, but are likely to be very important. In support of this concept, we have shown that vascular endothelial growth factor and nerve growth factor enhance the revascularization and reinnervation of transplanted islets [6]. It has been noted that transplanted islets lack the normal microvascular pattern [23, 44, 45, 46] which may account in part for delayed and abnormal insulin secretion from transplanted islets. It is important to note that islets transplanted to the pancreas appear anatomically more normal and have a vascular supply present in the islet core in contrast to islets transplanted elsewhere (Figure 3) [22, 45, 46]. It is logical to conclude that factors required to support

islet cells would be in higher concentrations in the pancreas than in other tissues and may contribute to an islet friendly environment. The toxic chemical milieu of the transplant site is also important to consider. In addition, radiological and histological changes can be demonstrated in the areas surrounding transplanted islets in the liver, but do not occur in the pancreatic site. These changes have been interpreted as periportal glycogen accumulation in liver biopsies, although, the clinical significance remains to be elucidated. The present report has also noted differences in oxygen availability between sites, thus confirming the report by Carlsson *et al*. [11]. The pancreas has the highest tissue fluid oxygen content compared to the liver and kidney. Isolated islets are avascular and must rely upon the diffusion of oxygen for survival prior to revascularization. The presence of an increased oxygen concentration in the surrounding tissue fluid is therefore important to ensure the survival of islet cells because the revascularized islets cannot receive more oxygen than the organ in which they reside. The combination of direct hepatic insulin supply, local tissue factors, and oxygen content suggest that the pancreas is an islet favorable transplant site, requiring fewer islets to prevent diabetes in the recipient.

The results shown in the present report suggest that the pancreas is a successful islet transplant site. The results also demonstrate that fewer islets are required to reverse diabetes at the pancreatic site as compared to the liver or kidney sites, thereby reducing or possibly eliminating the need for multiple donor pancreata and multiple transplants. The reduction in the number of islets required at the pancreatic site may be the result of a number of factors. The pancreatic site provides undiluted insulin directly to the liver, thereby allowing the use of fewer islets to regulate glucose synthesis and secretion. The latter result may be in part due to the more efficient insulin supply to the liver and in part from the suppression of glucagon from the native beta cell deficient islets remaining in the pancreas through the contribution of insulin via the pancreatic tissue fluid surrounding islets or though the vasculature after islet revascularization [13]. The proposed superiority of first pass portal insulin supply to the liver rather than systemic insulin is supported by our results and by an animal model that used vascular shunting between islet bearing kidneys and the portal vein [47] to regulate blood glucose.

In conclusion, the present report supports the recommendation that the pancreas should be considered as an alternative implantation site and that an otherwise minimal or suboptimal number of free or immunoisolated islets, if transplanted elsewhere, may be sufficient to prevent hyperglycemia in the recipient.

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