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FOCUS: TRANSLATIONAL MEDICINE

Islet Transplantation in Type I Diabetes Mellitus

Ryan M. Jamiolkowski[a], Lucie Y. Guo[a], Yun Rose Li[a], Sydney M. Shaffer[a], Ali Naji[b*]

[a]Medical Scientist Training Program, Perelman School of Medicine at University of Pennsylvania, Philadelphia, Pennsylvania; [b]University of Pennsylvania Medical Center, Transplantation Department, Philadelphia, Pennsylvania

For most patients with type I diabetes, insulin therapy and glucose monitoring are sufficient to maintain glycemic control. However, hypoglycemia is a potentially lethal side effect of insulin treatment in patients who are glycemically labile or have hypoglycemia-associated autonomic failure [1]. For those patients, an alternative therapy is beta cell replacement via pancreas or islet transplantation. Pancreas transplants using cadaveric donor organs reduce insulin dependence but carry risks involved in major surgery and chronic immunosuppression. Islet transplantation, in which islets are isolated from donor pancreases and intravenously infused, require no surgery and can utilize islets isolated from pancreases unsuitable for whole organ transplantation. However, islet transplantation also requires immunosuppression, and standard steroid regimens may be toxic to beta cells [2]. The 2000 Edmonton Trial demonstrated the first long-term successful islet transplantation by using a glucocorticoid-free immunosuppressive regimen (sirolimus and tacrolimus). The Clinical Islet Transplantation (CIT†) Consortium seeks to improve upon the Edmonton Protocol by using anti-thymocyte globulin (ATG) and TNFα antagonist (etanercept). The trials currently in progress, in addition to research efforts to find new sources of islet cells, reflect enormous potential for islet transplantation in treatment of type I diabetes.

*To whom all correspondence should be addressed: Ali Naji, MD, PhD, Professor of Surgery, University of Pennsylvania Medical Center, 3400 Spruce Street, 1 Founders, Transplantation Department, Philadelphia, PA 19104; Tele: 215-662-2066; Fax: 215-615-4900; Email: Ali.Naji@uphs.upenn.edu.

†Abbreviations: CIT, Clinical Islet Transplantation; ATG, anti-thymocyte globulin; DCCT, Diabetes Control and Complications Trial; SPK, simultaneous kidney-pancreas; PAK, pancreas-after-kidney; PTA, pancreas transplant alone; CITR, Collaborative Islet Transplant Registry; NIH, National Institutes of Health; FDA, Food and Drug Administration; iPSCs, induced pluripotent stem cells.

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WHEN INSULIN ISN'T ENOUGH: THE NEED FOR ALTERNATIVE THERAPIES IN TYPE I DIABETES

Type I diabetes mellitus is a chronic autoimmune disease resulting from selective destruction of insulin-producing beta cells in the islets of Langerhans [3]. The absolute deficiency of insulin results in a wide spectrum of metabolic dysfunction, particularly impaired glucose homeostasis. The discovery of insulin in 1922 was a monumental achievement, transforming type I diabetes mellitus from a death sentence to a manageable, chronic condition. The Diabetes Control and Complications Trial (DCCT) confirmed that tight glucose control prevents or even reverses long-term complications of type 1 diabetes [4]. However, insulin therapy itself can be life-threatening, as an overdose can result in severe hypoglycemia. In fact, tight glucose control is associated with more frequent episodes of hypoglycemia, especially in patients during acute illnesses [5]. Many patients experience wide excursions in plasma glucose levels that lead to the secondary complications of diabetes, since most existing insulin formulations cannot mimic the natural regulatory ability of the insulin-producing beta cells of the endocrine pancreas. Of note, more recent innovations in insulin delivery and therapy, such as insulin degludec (an ultra-long-acting basal insulin analog that can last up to 40 hours) has been shown to potentially mimic normal body glycemic control better than existing 24 hour-based regimens using long-acting insulin analog glargine [6].

Many insulin-dependent type I diabetes patients have some degree of hypoglycemia unawareness, the inability to sense low blood glucose, thus the benefits of stringent glycemic control may be outweighed by the risk of a potentially fatal insulin overdose [7]. Up to 10 percent of mortality in patients with type I diabetes is the result of hypoglycemia unawareness [8], which potentially causes loss of consciousness or inability to awaken from sleep ("dead-in-bed" syndrome) [9]. That autonomic neuropathy is both caused by hypoglycemia ("hypoglycemia-associated autonomic failure") and makes hypoglycemia more likely, forming a vicious cycle [7]. Despite advances in insulin therapy, the frequency of hypoglycemia unawareness has not declined in the past two decades [10], thus alternative therapies are required. In the following sections, we will discuss efforts to restore physiologic glucose homeostasis by beta cell replacement, which can be accomplished either via whole pancreas or isolated islet transplantation.

PANCREAS TRANSPLANTATION

The first successful pancreas transplantations were simultaneous kidney-pancreas (SPK) transplants performed in 1966 in two patients with end-stage diabetic nephropathy [11]. One of the two recipients from this original study achieved near-normal glycemia for approximately 2 months post-transplant [12]. Type I diabetes patients that have undergone a successful kidney transplant are candidates for pancreas-after-kidney (PAK) transplantation. Furthermore, type I diabetes patients with normal renal function and affected with severe hypoglycemic unawareness and glucose lability have been treated with pancreas transplant alone (PTA). The success rates of SPK and PAK transplants have continued to improve, while peri- and post-operative morbidity and mortality have steadily declined. SPK and PAK transplants improve quality of life and reduce hyperglycemia-related complications, including decreasing or reversing diabetic neuropathy [12].

However, these benefits come with the risks of chronic immunosuppression, surgical complications, and graft rejection [13]. Standard medical therapy with insulin and close glucose monitoring are safe and successful in most patients with type I diabetes, thus the American Diabetes Association believes that isolated pancreatic transplants carry unjustified risks [14,15]. Due to the significant improvements in the length and quality of life in patients with type I diabetes who adhere to intensive medical therapy alone, one study claims that PTA and PAK transplantation are associated with an in-

creased mortality rate at 4 years following transplant, by 57 percent and 42 percent respectively, compared to patients with type I diabetes who remained on the transplant waiting list [13]. However, this study was challenged by another report that found a significant benefit for patients undergoing pancreas transplants [16,17].

ISLET TRANSPLANTATION

The advantage of islet transplantation is avoidance of the major surgery needed for whole pancreas transplantation. However, like pancreas transplants, isolated islets are susceptible to immunologic rejection despite maintenance immunosuppression. The modern era of islet transplantation began in 1972 with reports from two laboratories demonstrating successful reversal of diabetes in rodents by isolated islet transplantation [18,19]. Since then, a number of critical milestones have been achieved, providing a platform for translation of this innovative therapy for treatment of type I diabetes patients. These include: 1) selection of the portal vein and liver as a site for inoculation of isolated islets [20]; 2) an automated method for isolation of human pancreatic islets [21]; and 3) improvements in collagenase enzyme blend for isolation and purification of islets.

According to the Collaborative Islet Transplant Registry (CITR) report, the periprocedural complications of islet transplantation have an estimated 20-fold lower morbidity risk than pancreatic transplants [22]. Another advantage of islet transplantation is that healthy islets can be isolated from pancreases that may not be used for whole pancreas transplantation, which is extremely valuable given the shortage of donor organs [23].

ISLET TRANSPLANTATION: EDMONTON PROTOCOL

The landmark trial of islet transplantation in 2000 by the Edmonton group [24] demonstrated successful reversal of diabetes in seven consecutive type I diabetes patients. The trial utilized induction immunosuppression with daclizumab (a monoclonal antibody to the IL-2 receptor) and maintenance with a glucocorticoid-free immunosuppressive regimen consisting of the calcineurin inhibitor tacrolimus and the mTOR inhibitor sirolimus. The islet transplant recipients had suffered from recurrent severe hypoglycemia. After transplantation, insulin treatment was stopped; if serum glucose reached >200mg/dL, another transplantation was performed. All seven patients required second islet transplantations, while one required a third. The investigators did not observe any acute rejection and recorded only mild side effects due to the immunosuppression, such as sirolimus-induced superficial buccal ulcerations. At 1 year of follow-up, all seven patients were insulin-independent, their serum glucose levels showed less fluctuation, and none suffered episodes of severe hypoglycemia. However, longer follow-up revealed progressive loss of beta cell function and recurrence of diabetes. In a trial of 65 patients conducted by the Edmonton group in 2004, only 10 percent of the patients remained insulin-independent at 5 years, although partial graft function persisted in 80 percent of the patients [25].

In 2006, the reproducibility of the Edmonton protocol was tested in 36 patients at nine international academic centers [26]. The trial achieved more sobering results and saw large differences in islet graft function among the various centers. After 1 year, only 16 of the 36 patients were still insulin-independent; 10 patients maintained partial graft function, and 10 had complete graft loss. After 3 years, only one patient remained insulin-independent. But even though nearly all patients resumed insulin therapy, patients with partial islet function nonetheless demonstrated improvement in glycemic control and protection from hypoglycemia compared to their pre-transplant states.

Some investigators saw the progressive graft loss and lack of reproducibility as fatal flaws of the Edmonton protocol [27]. Investigators from Bergamo, Italy, criticized the method and mission of islet transplantation and announced abandonment of their own clinical islet cell transplantation program

[28]. Because two to four whole pancreases were used for a single islet cell transplant, they believed islet transplants to be "a waste of valuable donated organs," especially with a persistent shortage of human donors. Additionally, they raised the concern that sirolimus and tacrolimus may inhibit beta cell regeneration and cause nephrotoxicity in the long term. Also, the potential development of alloantibodies after failed islet transplantation may increase the recipients' risk of later rejecting whole pancreas and kidney transplants, which may be required following islet cell transplant failure [29].

In response to the criticisms, the Edmonton group dismissed the Italian abandonment of islet transplantation as "entirely personal," and that "clinicians and researchers involved in the majority of programs do not share the pessimistic view expressed by the Italian group" [30]. They indicated that many patients did achieve complete insulin independence, and despite graft loss after a few years, recipients were nonetheless protected from severe hypoglycemia. Falling short of complete insulin independence, they wrote, should not call for abandonment of islet transplantation; even partial islet graft survival may suffice if the most important goal is to prevent hypoglycemia unawareness and tragic "death-in-bed" events.

ISLET TRANSPLANTATION: BUILDING ON EDMONTON

In 2004, the Clinical Islet Transplantation (CIT) Consortium was established in eight academic centers under the guidance of the National Institutes of Health (NIH) and U.S. Food and Drug Administration (FDA) to conduct islet transplantation in two cohorts: islet-alone transplantation in type I diabetes subjects with severe hypoglycemia unawareness (CIT-07) and islet-after-kidney transplantation in subjects with prior successful kidney transplantation (CIT- 06). A number of important modifications were implemented: 1) uniform and standardized manufacturing of isolated islets utilizing improved collagenase enzyme blend; 2) induction immunotherapy with polyclonal antithymocyte globulin ATG and TNFα antagonist [31]; 3) a steroid-free maintenance immunosuppression regimen composed of low-dose calcineurin inhibitor (tacrolimus) and mTOR inhibitor (rapamycin); and 4) *in vitro* culture of islets prior to transplantation.

ATG is a polyclonal antibody with the capacity to delete and inhibit anti-islet allo- and auto-immune T-cells and contains several species of antibodies targeting B-cells, adhesion molecules, and integrins needed for diapedesis of lymphoid cells [32]. TNFα antagonist interferes with early post-transplant immune response, blocks direct cytotoxic effect of TNFα on murine islet beta cells [33], and limits cytokine release syndrome associated with ATG administration.

In vitro culture of islets for 48 to 72 hours yields a "cleaner" islet suspension with less immunogenic and thrombogenic collagen and debris, a higher yield of viable islet cells, and permits quality control metabolic testing and sterility prior to islet transplantation [34].

The consortium investigators seek to demonstrate effectiveness of islet transplantation in type I diabetes patients with severe hypoglycemic unawareness (at least one episode of severe hypoglycemia in the 12 months before enrollment). The primary endpoint of the trial is the proportion of subjects rendered insulin-independent and free of severe hypoglycemic events a year following the first islet cell transplant, as well as having a hemoglobin A1c (HbA1c) of less than 7 percent. Since HbA1c is a marker of red blood glycation as a result of exposure to plasma glucose, achieving a near-normal HbA1c is a good marker of glycemic control over a period of 2 to 3 months, which is the average lifespan of a red blood cell [35]. Estimated completion dates for analysis of the data are January 2013 for CIT-06 and September 2012 for CIT-07.

OTHER ADVANCES IN ISLET TRANSPLANTATION

Despite the paramount advances made in islet transplantation in the last few

decades, much remains to be done. For example, islet cell harvest from whole pancreas remains a limiting step, as the efficiency of harvest and cell viability post-harvest is relatively poor. While many laboratories are developing methods to improve these processes, given the severe shortage of donor pancreases, other investigators are exploring alternative sources of beta cells.

One possibility is to derive pancreatic islet cells *de novo* from human embryonic stem cells (hESCs). Recently, it has been shown that the small molecule (-)-indolactam V induces differentiation of hESCs into pancreatic progenitor cells *in vitro* [36]. The more plentiful pancreatic ductal cells isolated from human donor pancreases can be trans-differentiated into the more scarce beta-cells [37]. Similarly, mouse experiments have shown that bile duct epithelial cells [38], acinar cells [39], and hepatic cells [40] can also be trans-differentiated into beta-cells. The differentiation of human fibroblast-derived induced pluripotent stem cells (iPSCs) into beta-cells provides another alternative that is particularly enticing due to its potential avoidance of allogeneic rejection [41]. Additionally, since porcine insulin is closely homologous to human insulin, islet cell xenografts using porcine cells are also being investigated [42]. While these methods of *de novo* islet cell generation technologies hold great promise, much optimization and patient-safety testing remains before they will be feasible alternative sources of islet cells.

Further investigations are focused on creating an artificial immune-privileged micro-environment to prevent rejection of transplanted islets. Current attempts are under way to develop polymer encapsulations of islet cells that are permeable to oxygen, glucose, nutrients, and insulin, but not antibodies or cytokines. However, a major technical hurdle is inadequate oxygen perfusion of cells within the encapsulated islets, which leads to ischemic necrosis in their centers [43]. Maintaining graft survival and function remains the primary difficulty for developing safe and effective islet transplantation.

Advances in our understanding of immunobiology of organ rejection in general, specifically the induction of donor-specific tolerance, also provide a pathway for the widespread utilization of islet transplantation for treatment of type I diabetes [44].

CONCLUSION

Islet cell transplantation is a promising potential therapy for patients with type I diabetes with hypoglycemia unawareness or glycemic lability. Unlike pancreas transplantation, it does not require invasive surgery, and it can utilize islets isolated from suboptimal deceased donor pancreases. However, a number of technical challenges remain in optimizing islet transplantation, including the need to find less toxic immunosuppressive therapies to improve graft survival. The Clinical Islet Transplantation (CIT) Consortium has implemented logical steps to advance the field, and trial results are eagerly awaited. In addition to the challenges of immunosuppression, innovative therapies to achieve glucose homeostasis must work within or overcome the relative scarcity of organ donors. The ability to transdifferentiate non-islet cells into islet cells may create a more plentiful supply of islets for transplantation. In the future, islet transplantation could transform management of type I diabetes not just for patients with refractory difficulties in glycemic control, but for all type I diabetes patients.

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