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GUIDELINES FOR CLINICAL PRACTICE

Pancreatic islet transplantation

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Abstract

Type 1 diabetes mellitus is an autoimmune disease, which results in the permanent destruction of β-cells of the pancreatic islets of Langerhans. While exogenous insulin therapy has dramatically improved the quality of life, chronic diabetic complications develop in a substantial proportion of subjects and these complications generally progress and worsen over time. Although intensive insulin therapy has proven effective to delay and sometimes prevent the progression of complications such as nephropathy, neuropathy or retinopathy, it is difficult to achieve and maintain long term in most subjects. Reasons for this difficulty include compliance issues and the increased risk of severe hypoglycemic episodes, which are generally associated with intensification of exogenous insulin therapy. Clinical studies have shown that transplantation of pancreas or purified pancreatic islets can support glucose homeostasis in type 1 diabetic patients. Islet transplantation carries the special advantages of being less invasive and resulting in fewer complications compared with the traditional pancreas or pancreas-kidney transplantation. However, islet transplantation efforts have limitations including the short supply of donor pancreata, the paucity of experienced islet isolation teams, side effects of immunosuppressants and poor long-term results. The purpose of this article is to review recent progress in clinical islet transplantation for the treatment of diabetes.

INTRODUCTION

The primary treatment for type 1 diabetes is multiple injections of exogenous insulin together with regular monitoring of blood glucose levels. Intensive insulin therapy can help prevent long-term diabetic complications and the introduction of insulin pumps into clinical practice has raised the possibility of mimicking the basic, endogenous insulin secretion pattern, which directly relates to a better glycemic control[1-3]. Despite appropriate treatment, satisfactory and safe control of blood glucose levels still cannot be achieved in a small percentage of patients. Pancreatic islet transplantation has recently emerged as one of the most promising therapeutic approaches for improving glycometabolic control in type 1 diabetic patients. The first successful series of islet allografts was reported in 1990 in surgical diabetes[4], while the results in type 1 diabetes slowly improved during the 1990s until 1999. In 2000, the "Edmonton Protocol" introduced several modifications to the transplantation procedure, such as the use of a steroid-free immunosuppression regimen and transplantation of a mean islet mass of 11 000 islet equivalents per kilogram of patient's weight[5]. Since this exciting report, clinical islet transplantation activity has dramatically increased all over the world. A multi-center trial to evaluate the reproducibility of the Edmonton study organized by the Immune Tolerance Network reported variable rates of

success, indicating that the complexity of the procedure should not be underestimated by new centers entering the field[6]. In the centers with the most experience in the procedure, approximately 80% of patients treated with islet transplantation achieved insulin independence within the first year post-transplantation[6]. However, the Edmonton group showed in 2005 that only 7.5% of the 65 patients who received islet transplantation have reached insulin independence, although the majority of patients (82%) presented graft survival (C-peptide positivity)[7]. The data indicated the need of further advances in the preservation of the function of transplanted islets. Islet transplantation still faces major challenges, especially those related to cell loss during the process of islet isolation and the losses related to the graft site, apoptosis, allorejection, autoimmunity, and immunosuppression.

This review describes recent progress in clinical islet transplantation for the treatment of diabetes.

PANCREAS PROCUREMENT AND PRESERVATION

Pancreata are procured using a standardized technique in whole pancreas transplantation to minimize warm ischemia. University of Wisconsin (UW) solution is used for *in situ* perfusion of the donor. The pancreas is excised immediately after the liver and before the kidneys and is normally preserved in UW solution[8,9].

We recently reported that the ductal injection of 1 mL/g pancreas weight of a new preservation solution (modified Kyoto (MK) solution) before pancreas storage improves islet yields[10,11]. MK solution contains trehalose and ulinastatin as distinct components. Trehalose has a cytoprotective effect against stress, and ulinastatin inhibits trypsin. Ductal injection of the preservation solution increased the ATP level in pancreas tissue, reduced trypsin activity during the digestion step, and prevented islet apoptosis[10]. These data suggest that the ductal injection of preservation solution leads to improved outcomes for pancreatic islet transplantation.

Kuroda *et al*[12] were the first to report that the two-layer preservation method, in which the pancreas is stored at the interface of UW solution and oxygenated perfluorochemical (PFC), is effective for pancreas preservation. Since then, the two-layer method has been utilized for many clinical trials in islet transplantation[13-16]. However, UW solution has several disadvantages, including the inhibition of Liberase activity. We investigated the features of MK solution[17]. In porcine islet isolation, islet yield was significantly higher in the MK/PFC group compared with the UW/PFC group. Compared with UW solution, MK solution significantly inhibited trypsin activity in the digestion step; moreover, MK solution inhibited collagenase digestion less than UW solution. These data suggest that pancreas preservation with MK solution improves islet yield by trypsin inhibition and causing less collagenase inhibition.

ISLET DIGESTION

Human islet isolation is conducted using the standard Ricordi technique with modifications introduced in the Edmonton protocol. The introduction of the semi-automated method for controlled pancreatic digestion using a dissociation chamber (Ricordi Chamber) has dramatically increased islet yields from human pancreata[18] and the general principles of this method still form the basis of current islet isolation technology[19-22]. After perfusion through the pancreatic duct in a controlled fashion with a cold enzyme blend of collagenase with neutral protease, the distended pancreas is then cut into 7 to 9 pieces, placed in a Ricordi chamber, and shaken gently. While the pancreas is being digested by re-circulating the enzyme solution through the Ricordi chamber at 37℃, we monitor the extent of digestion with dithizone staining by taking small samples from the system. Once digestion is confirmed to be complete, dilution solution is introduced into the system. Then, the system is cooled to stop further digestive activity. The digested tissue is collected in conical tubes containing 25% HSA and washed with fresh medium to remove the enzyme.

ISLET PURIFICATION

Islet purification minimizes the risks associated with islet infusion through the portal vein by reducing the amount of transplanted tissue. Large-scale continuous purification using the COBE2991 cell processor, with Ficoll solutions, is the current gold standard method[20-24]. Recently, the Ficoll-based gradient has been progressively replaced by iodixanol-based gradients[16,22,25]. We recently showed the effectiveness of iodixanol-controlled density gradients on the islet purification step[25]. Islet yield after purification and rate of post-purification recovery were significantly higher using iodixanol-based solutions than with standard continuous gradient purification by Ficoll solutions. The data suggest that using an iodixanol-controlled density gradient improves the islet recovery rate in human islet isolation.

Recently, Ichii *et al*[26] have reported that an additional gradient purification method following regular purification with bottom loading could be of assistance in maximizing the number of islet preparations successfully used for transplantation by improving the efficiency of the purification of trapped islets, which often come from younger donor pancreata. This supplemental purification following regular purification could maximize the islet yield and improve clinical islet transplantation.

ISLET CULTURE/PRESERVATION

Culturing islets prior to transplantation provides flexibility for evaluation of isolated islets and pre-treatment of patients. However, it is well known that isolated islets deteriorate rapidly in culture. Optimum culture conditions

should provide sufficient oxygen and nutrients, in order to allow islet cells to recover from isolation-induced damage, maintain the three-dimensional structure of the clusters and reduce islet mass loss. Although the Edmonton protocol required freshly isolated islets to be transplanted without keeping them in culture[5], most transplant centers culture isolated human islet preparations before transplantation[7,16,21,23,34,27-29].

We evaluated optimal temperature for culture/preservation of isolated human islets before transplantation. Isolated islets were cultured or preserved for 48 h in the following culture/preservation conditions: preservation at 4℃ in UW solution, culture at 22℃ or 37℃ in culture medium. Islet morphology after 4℃ preservation was similar to that of fresh islets, whereas islet diameter after 37℃ or 22℃ culture was smaller than that of fresh islets. Islet yield significantly decreased at higher temperatures (24% loss in 37℃ culture and 19% loss in 22℃ culture, but less than 5% loss in 4℃ preservation). When cultured/preserved islets were transplanted into diabetic nude mice, the attainability of post-transplantation normoglycemia was significantly higher in the 4℃ preservation group than in 22℃ and 37℃ culture groups. These data suggest that preservation of isolated islets at 4℃ improves the outcome of islet transplantation more efficiently than preservation at either 22℃ or 37℃[30].

ISLET TRANSPLANTATION

Isolated islets are transplanted into the recipient liver through the portal vein with a percutaneous transhepatic cannulation under sonographic and fluoroscopic guidance[31]. The potential complications of the method include portal vein thrombosis, portal hypertension, and bleeding[32,33]. Heparin has been added to the process in order to reduce the clotting process, termed the instant blood mediated inflammatory reaction (IBMIR)[34,35]. Moreover, the use of heparin or anti-coagulative agents for several days following islet transplantation, together with intensive insulin treatment for the first weeks after transplantation have recently been reported as critically important variables which improve the efficiency of initial islet engraftment.

IMMUNOSUPPRESSION

A steroid-free immunosuppressive protocol with a combination of sirolimus and tacrolimus, which the Edmonton group reported, has been utilized in many clinical islet transplant trials. Although short-term results of islet transplantation using this protocol have been promising, with approximately 80% of patients maintaining insulin independence at 1 year posttransplant, the proportion of recipients maintaining insulin independence declines after the first year posttransplant[7,20]. The reason for this decline remains unclear, but suggested causes include alloimmune rejection, autoimmune recurrence, and/or toxicity of immunosuppressive medications[36,37]. Allo/autoimmunity and drug toxicity may be ameliorated by refined immunosuppressive protocols.

Bellin *et al*[38], recently showed improved success with a modified immunosuppressive protocol, usage of antithymocyte globulin (ATG) plus etanercept as induction therapy. Recipients received cyclosporine and everolimus for maintenance immunosuppression for the first year posttransplant, with mycophenolic acid or mycophenolate mofetil subsequently substituted for everolimus. Four of six recipients who received islet transplantation maintained insulin independence for more than 3 years. These results suggest that modifications of immunosuppressive protocol have been a key to improve long-term graft survival.

CONCLUSION

Islet transplantation is an alternative method to whole pancreas transplantation in patients with type 1 diabetes because of its low invasiveness and safety to the recipient[39,40]. Significant progress in clinical islet transplantation has occurred during recent years, with a progressive improvement of short-term and long term outcomes. The most recent results indicate that islet transplant recipients can maintain islet graft function without deterioration beyond 5 years, progressively closing the gap with the results of whole organ, pancreas transplantation. Although experiments in β-cell regeneration from stem cells are ongoing[41-48], there is still no reliable method to generate β-cells. Until a new method to generate β-cells is developed, improving the efficacy of islet transplantation seems the most realistic and prudent method to cure diabetes.

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