Abstract

Objectives: Type 1 diabetes mellitus is an emerging epidemic worldwide and results from autoimmune destruction of insulin-producing β cells. Islet transplanting is a potential treatment for type 1 diabetes mellitus.

Materials and Methods: The Shiraz Organ Transplant Center is a leading center for organ transplants, especially pancreatic transplants, in Iran. For this reason, we want to establish an islet transplanting program. Here, we briefly describe our experience with islet isolation on 6 pancreata from deceased donors. We discussed the necessary equipment required for this procedure, as well as the professionals needed and a specially planned facility.

Results: Islet yield was ≤ 100 000 (islet equivalent), viability 40% to 45%, and the purity was 30% to 45%. We do not have a refrigerated COBE processor for purification; therefore, the yield was low. Our experience shows that we should improve things, so as to acquire more islets for developing clinical grade cell therapy.

Conclusions: Overall, isolation costs are high, and accessing a safer, more economic, and persistent source of material and reagents will improve this technique.

Key words: Hyperglycemia, Insulin, Pancreas, Langerhans, Endocrine

Introduction

Type 1 diabetes mellitus is an autoimmune disease with permanent destruction of the pancreatic islets of Langerhans. Destruction of pancreatic beta cells causes insulinopenia, hyperglycemia, and ketoacidosis. The lack of endogenous insulin is balanced by exogenous insulin injections, with regular monitoring of blood glucose levels. Intensive insulin therapy has been effective in delaying or preventing progression of complications including nephropathy, neuropathy, or retinopathy. However, it is difficult to maintain for the long term in most patients, either because of compliance issues or risk of severe hypoglycemic episodes. Whole organ pancreatic transplant is effective in restoring normoglycemia and long-term physiological glycemic control in more than 80% of patients. Simultaneous pancreas and kidney transplants are considered the standard therapy for patients with type 1 diabetes mellitus with end-stage renal failure. Transplanting of insulin-producing cells is another option for proper glucose regulation. Candidates for islet cell transplant are patients with unstable type 1 diabetes mellitus, who have a history of severe hypoglycemia unawareness, despite attempts to correct the condition by meticulous medical treatment.
in that initial attempt. \(^6\) Protocols improved slowly during the 1990s, until 1999, when Shapiro and associates reported insulin independence in 7 out of 7 consecutive type 1 diabetes mellitus patients treated with an islet transplant using a glucocorticoid-free immunosuppressive regimen.\(^7\) After that, clinical islet transplanting activity increased worldwide. The islets represent fewer than 2% of the organ, and this easily could be infused by interventional radiologic techniques, either intraportally or at other sites.\(^6\),\(^7\)

Although pancreatic transplant achieves insulin independence in many patients beyond the first year,\(^3\),\(^4\) it is associated with risks of major surgical procedures and long-term immunosuppressive drug therapy.\(^3\) In contrast, islet transplanting does not require any significant surgery or general anesthesia.\(^5\) In this article, we briefly describe our experience in islet cell isolation at the Shiraz University of Medical Sciences in Shiraz, Iran.

**Experimental pancreatic islet isolation**

The Shiraz Organ Transplant Center is a leading center for organ transplanting, and it was the first center for pancreatic transplants in Iran. Perfusion, digestion, and islet purification were performed on 6 pancreata between January 2011 and March 2012. All isolations were done at our islet isolation facility at the Transplant Research Center, affiliated with the Shiraz University of Medical Science. We also arranged visits to the Clinical Islet Laboratory at the Diabetes, Endocrinology & Metabolism of City of Hope, City of Hope, Duarte, CA, USA, where we obtained valuable information.\(^8\),\(^9\)

**Materials and Methods**

The pancreas was procured after obtaining written, informed consent. All protocols were approved by the ethics committee of the institution before the study began, and the protocols conformed with the ethical guidelines of the 1975 Helsinki Declaration. The donors were tested for human immunodeficiency virus, hepatitis B and C viruses, venereal disease reaction level, and cytomegalovirus. The organ was recovered, en bloc, together with duodenum and spleen.

University of Wisconsin solution was used for in situ flush and storage of organs under cold-storage conditions. The media and reagents were purchased from Invitrogen GmbH (Germany). Each step in the isolation procedure was written in the standard operating procedures.

Samples from tissue transport solution and final cell suspension were taken for microbiologic examinations (aerobic and fungi). Islets were isolated using the method described by Ricordi\(^10\) (Figures 1 and 2). A central incision into the pancreas was performed, and 2 cannulae were inserted into the main pancreatic ducts.

**Figure 1.** Distended Pancreas After Collagenase Infusion

**Figure 2.** Islet Cells Are Stained With Dithizone That Binds Zinc Ions Present in the Islet’s Beta Cells and Therefore, Stains the Islets Red

Perfusion was achieved with collagenase NB1 (Serva Electrophoresis, Heidelberg, Germany) using a peristaltic pump. The collagenase solution was slowly warmed to 37°C. The pancreata were initially distented at 80 mm Hg for 5 minutes, after which the pressure was increased to 180 mm Hg for 15 minutes. The distented organ was cut into 6 to 8 pieces. Then, the fragments were transferred to the Ricordi

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Negar Azarpira et al /Experimental and Clinical Transplantation (2014) 2: 139-142

Exp Clin Transplant
chamber and mechanically dissociated. Constant agitation aided digestion. During digestion, samples of the pancreatic tissue were taken and stained with dithizone to determine an optimum digestion endpoint. Dithizone was used to stain the islets a bright red color to distinguish them clearly from exocrine tissue. When the majority of the islets were free of exocrine tissue, the dissociation chamber was flushed with 8 liters of cooled Royal Park Memorial Institute medium solution containing bovine serum albumin to terminate the enzyme activity of the collagenase. Pancreatic digest contained both endocrine and exocrine tissue.

The digest is then added to cold University of Wisconsin solution. Because of the difference in densities between the endocrine and exocrine fractions, the lysate purified using discontinuous Ficoll gradients. After centrifugation, the interface containing the islets was removed and prepared for yield and purity. Viability was measured by combined fluorescent dye staining with propidium iodide and fluorescein diacetate. Then, the islets cultured in CMRL media and maintained in a humidified incubator at 30°C with 5% CO₂. Unfortunately, the glucose challenge test was not performed to determine the functional activity of isolated cells.

### Results

All donors were brain-dead males (mean age, 32.3 ± 17 y; age range, 16 to 50 y). Motor vehicle accidents, stroke (intracranial hemorrhage), and brain tumors were the major causes of death (Table 1). The cold ischemia time was 12 ± 4 hours. Islet yield was ≤ 100 000 IEQ (islet equivalent), viability was 40% to 45%, and the purity was 30% to 45%. The islets were shaped oval-round, with intact membranes. Overall, the majority of islets were free, but a few islets that were embedded in residual exocrine tissue also were identified. We did not have a refrigerated COBE processor (COBE 2991, Cobe, GAMBRO.BCT, Lakewood, CO, USA) for purification, therefore our yield was low.

### Discussion

Type 1 diabetes mellitus, a noncommunicable disease, is rapidly rising and has become a major challenge globally. It is also a major public health issue in Iran. Type 1 diabetes mellitus is a disproportionately expensive disease, requiring long-term medical attention, with a loss of productivity. Whole organ pancreatic transplant is effective in restoring normoglycemia, and pancreatic islet transplanting is another noninvasive treatment.

The human pancreas contains 0.3 to 1.5 × 10⁶ islets per pancreas, of which only 30% to 50% can be isolated using current islet isolation protocols. It also has been estimated that about 65% of human islets are viable after isolation. Major concerns of islet preparations are sterility, personnel qualification, and good equipment to prepare cells with high purity and viability. Despite a variety of published protocols for islet preparation, the Edmonton Protocol is widely used around the world. Donated pancreas (heart-beating brain-dead donors vs non–heart-beating donors), pancreas procurement, preservation, and density gradient purification (Ficoll-Hypaque vs Iodixanol) are the main differences between protocols.

One of the major goals at the Shiraz Transplant Center is to establish a pancreatic islet transplant facility for treating diabetic patients. We have good access to an organ donation system, which is an important point that can help us to establish a cell

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>Donor Blood Group</th>
<th>Cause of Death</th>
<th>Cold Ischemia Time (h)</th>
<th>IEQ</th>
<th>Islet Specification Purity (%)</th>
<th>Viability (%)</th>
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<tr>
<td>5</td>
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</table>

**Abbreviations:** IEQ, islet equivalent
transplant.

In our experience, islet viability and purity were 40% to 45% and 30% to 45%, with a low yield. Unfortunately, because of sanctions in Iran, direct purchasing of materials and equipment from overseas suppliers is difficult. The costs of materials are more expensive than they are for other countries.

Our experience must be improved to achieve more yields to develop clinical grade cell therapy. Stringent donor inclusion criteria, with optimal continual gradient purification by a COBE cell separator, will be key factors in attaining good islets in the future. An important advantage of a continual gradient is that it allows collecting serial fractions with different degrees of purity that can be assessed separately to select those with the highest purity for transplant.

References


