

Chapter 21

DCD for Islet Transplantation

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21.1 Introduction

Pancreatic islet transplantation has the potential to become the most physiologically advantageous and minimally invasive procedure for the treatment of type 1 diabetes mellitus. Since the first clinical islet transplantation was performed at the University of Minnesota in 1974 [1], the results have been far from ideal for more than two decades in spite of an improvement of islet isolation technique by Ricordi et al. [2–4]. The introduction of the Edmonton protocol, with a highly improved rate of insulin independency, encouraged us to promote clinical islet transplantation [5, 6]. In Japan, we organized the Working Group (The Japanese Islet Transplant Registry) in 1997 under the Japanese Society for Pancreas and Islet Transplantation for the purpose of starting clinical islet transplantation. The first issue of the Working Group was to construct a system of clinical islet transplantation in Japan including the registration of the recipients, procurement of the pancreas for islet isolation and transplantation of the isolated islets. In Japan, afterwards, various problems facing to a start of clinical islet transplantation have been discussed and we completed the guideline for clinical islet transplantation in Japan. The Japanese Organ Transplant Law was enforced in 1997 and organ transplantations using brain dead (DBD) donors were finally started. Since the islet transplantation was not included in the Japanese Organ Transplant Law because it was categorized as tissue transplantation, we were able to use the pancreas only from DCD donors for islet transplantation. The first islet isolation from the human pancreas was performed in 2003.9 and the

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first islet transplantation was performed in 2004.4 [7–9]. Sixty-five islet isolations and 34 islet transplantations were performed in our country from 2003.9.12 to 2007.3.11 [10].

In this chapter, we describe the current status of clinical islet transplantation using DCD donors in Japan.

21.2 Donor Criteria

Islet transplantation is categorized as tissue transplantation in Japan. Therefore, donor criteria for islet transplantation were based on the guideline issued by Japanese Society of Tissue Transplantation (JSTT) [11], which is shown in Table 21.1. According to the guideline, when a specific disease or state is observed in a donor, retrieval or transplant of tissue from the donor must not be permitted. It is necessary to conduct inspection and palpation of the donor, as detailed as possible. Interview of the donor's next of kin and studying the donor's medical records are also required. The results of a pathological autopsy, if performed, must be reviewed. For all tests performed, conduct the most current methodology. Criteria and methods of tests or examinations must be always updated at a tissue bank (Islet isolation center), in accordance with technical and scientific advancements and new findings in infectious diseases. (It is desirable to involve experts from related

Table 21.1 Exclusion criteria of tissue donors in general (Guideline on the safety, storage, and application of human tissue in medical practice. IV. Criteria for donor screening. Japanese Society of Tissue Transplantation (JSTT)) [11]

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- Death from unknown causes
 - Documented cases of sepsis or other systemic infectious diseases
 - CJD (vCJD) or its suspect of infection
 - Malignancy (as for primary brain tumors or solid tumors treated more than 5 years ago, and diagnosed to be cured, judgement may be given by the physicians to procure the tissue)
 - Hematologic malignancies, such as leukemia or malignant lymphomas
 - Severe metabolic or endocrine disorders. Autoimmune disorders such as hematological diseases or collagen disease
 - Syphilis positive
 - Hepatitis (HBV positive, HCV positive)
 - AIDS (HIV positive)
 - Adult T-cell leukemia (ATL), HTLV-1 positive
 - Parvovirus B19 positive
 - West Nile virus positive
 - SARS
 - Rabies
 - Other patient/donor who corresponds to exclusion criteria set for each tissue
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Patients or donors must be negative in interviews and tests (serodiagnosis or NAT) for all these infections and diseases listed above. Where necessary, a patient or donor is to be tested for negative infection of cytomegalovirus and EB virus

academic associations in the process of updating the criteria or collecting/evaluating information on infectious diseases, etc.) In accordance with the principle presented in the above notification of the Pharmaceutical and Medical Safety Bureau of MHLW, retrieval of tissue is prohibited when a patient or donor corresponds to any of the following items in Table 21.1.

In addition, the Japanese Islet Transplant Registry determined following four items for donor criteria of islet transplantation: (1) age ≤ 70 years, (2) warm ischemic time ≤ 30 min, (3) exclusion criteria of the JSTT guideline (Table 21.1), and (4) recommendation of UW solution or two-layer method (TLM) for preservation.

21.3 Procurement and Preservation of the Pancreas

In Japan, the pancreas from DCD donors are used for islet isolation since the pancreas from DBD donors are usually used for pancreas or pancreas/kidney transplantation according to the Japan Organ Transplant Law. The withdrawal of a respirator is rarely performed even though the donor is diagnosed to be brain dead (Maastricht category 4 [12]). Moreover, the donors usually are not examined to diagnose brain death, and thus, a cannulation into an abdominal aorta with a double-balloon catheter via a femoral artery and a systemic heparinization are not permitted before a cardiac arrest (modified Maastricht category 5 [13]). To shorten warm ischemic time and maintain a viability of the pancreas for islet isolation, we use in situ machine washout (ISMW) technique [14, 15], which was originally developed for the procurement of the kidneys from DCD donors. The efficacy of ISMW on clinical kidney transplantation from DCD donors is described in detail in another chapter. For the procurement of the pancreas from DCD donors, a double-balloon catheter position needs to be changed to perfuse celiac artery and superior mesenteric artery as well as bilateral renal arteries. We usually place the distal balloon in the aorta which is located above the celiac axis and only distal balloon is inflated. X-ray and ultrasound sonography are used for the confirmation of the collect location of the balloon. The University of Wisconsin (UW) solution is most frequently used as the perfusate of ISMW for the procurement of pancreas.

TLM, which was developed by Kuroda Y et al., was frequently used for the preservation of the pancreas before islet isolation. TLM was considered to be advantageous for the preservation of the pancreas for islet isolation, which resulted in higher yield and purity [16–22]. However, Caballero-Corbalán J et al. reported that a TLM had no beneficial effect as compared with the UW solution on human islet isolation and transplantation [23]. Currently, the use of TLM depends on the consideration of each islet isolation and transplantation center.

Procurement of the pancreas from DCD donors is different from that of the DBD donors in Japan. Since pancreatic islet transplantation is categorized as tissue transplantation, the pancreas must be procured after en bloc procurement of bilateral kidneys. Pancreas is procured with or without duodenum from the DCD donor. In back table, pancreas was isolated from the surrounding tissue including the

duodenum, spleen, blood vessels, and lymph nodes. When TLM is used for preservation of the pancreatic graft, the graft is moved into TLM container in which oxygenation was already done with 30 min oxygen bubbling. Some institutions perform the ductal injection with UW solution or other solutions into the main pancreatic duct [24–27].

21.4 Quality Control of Islet Transplantation

Quality control (QC) is important for the safe performance of islet transplantation. QC includes infection control and viability assay of the isolated islets. QC committee was placed in the Japanese Islet Transplant Registry, performing QC of all procedures in clinical islet transplantation in Japan [28]. As shown in Table 21.2, infection control is the major issue in QC. In the procurement of the pancreas from DCD donors, donor criteria must be strictly obeyed and past history of the infectious diseases should be carefully checked. Before islet isolation, the checking of the bacterial contamination of the preservation solution must be performed. Islet isolation is permitted to proceed in the authorized islet isolation centers which were acknowledged by the Japanese Society for Pancreas and Islet Transplantation. These centers have GMP-level-regulated cell processing rooms (center) and provide

Table 21.2 Quality control of pancreatic islets of islet transplantation (Committee of Quality Control, The Japanese Islet Transplant Registry, 2006.9) [28]

Quality control of pancreatic islets

1. Infection control
 1. Donor screening and assessment of the infection
 2. Checking of contamination during islet isolation and culture procedure
 3. Checking of endotoxin levels of the media before transplantation
2. Morphological and functional assay of the isolated islets (viability assay)
 1. Microscopic and electron microscopic examination
 2. Trypan blue staining
 3. Static incubation and/or perfusion studies

Algorithm of quality control

Procurement team

Procurement of the pancreas: checking of the active infection and past history of the infectious diseases

Islet isolation center

1. GMP level regulated cell processing room must be used (all procedures must be performed in sterile field)
2. Islet isolation: checking of the contamination (preservation solution, isolation media, culture media, etc.), static incubation
3. Cryopreservation of the islets: checking of the contamination (samples of cryopreserved islets), static incubation of samples of cryopreserved islets)

Islet transplantation center

1. Final checking of the contamination and endotoxin level of the islet suspended media
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the sterile field on all procedure of islet isolation and islet culture before transplantation. After islet isolation and culture, the media were sampled and checked for bacterial contaminations.

Viability assays of the isolated islets include the results of the isolation such as yield and purity of the islets, volume of the pellet of islets, morphological examination using microscope with dithizone staining, and electron microscope and functional assays.

The Japanese Islet Transplant Registry determined static incubation as the standard method of functional assay of the isolated islets. The technique of the static incubation is shown as follows. Briefly, five aliquots of ten islets were placed into 12-well Transwell microplates with 1 mL RPMI 1640 containing 3.3 mmol/L D-glucose and 0.1 % BSA as the basal medium. After 60 min, the culture Transwells were transferred into new 12-well microplates with RPMI 1640 containing 20 mmol/L D-glucose and 0.1 % BSA (glucose stimulation). After 60 min, the culture Transwells were transferred to new 12-well microplates to add basal medium again for an additional 60 min culture. Each medium was centrifuged and immediately frozen for a later assay of the insulin concentration by ELISA. The stimulation index was calculated by comparing the insulin content in the glucose stimulation medium with the second basal medium.

In addition to the static incubation, we perform the perfusion study to assess the ability of insulin release from the islets. Perfusion study was performed using following technique. Using our original perfusion system, the 50 islets were perfused in a low-glucose medium (Krebs Ringer solution with 3.3 mM D-glucose) for 80 min and then perfused in a high-glucose medium (Krebs Ringer solution with 16.7 mM D-glucose) for 30 min, followed by 60 min perfusion in a low-glucose medium. Stimulation indices were calculated from the ratio of insulin released into the low-glucose medium and the high-glucose medium.

Whether the isolated islets are alive or dead is the first viability assay. Chromogenic dyes such as neutral red or trypan blue are able to visualize live or dead cells. The islet, however, consists of a number of cells; therefore, chromogenic dyes cannot evaluate the islet itself, while fluorescein diacetate (FDA) is used to quantitate the proportion of cells that are intact or that are damaged, which can evaluate the islet more precisely [29]. Live islet is not necessarily able to respond appropriately to glucose stimulation; therefore, *in vitro* biochemical techniques have predominantly been used to assess islet viability. Insulin release from islets after stimulation with high glucose concentration has commonly been used in static incubation or perfusion study [30–33]. Glucose utilization [32], protein synthesis [34–36], and oxygen utilization [37] have also been used to assess islet function.

In addition to *in vitro* studies, syngeneic or autologous islet transplantation is preferable to assess islet viability, which simulates the outcome of islet transplantation. Nude (athymic) mice, which were induced with diabetes by intravenous injection with streptozotocin, are commonly used for *in vivo* assay of human islets [38, 39].

According to the outcome of islet isolation, the Japanese Islet Transplant Registry determined the criteria for fresh islet isolation. When the outcome of the isolation fulfill the criteria ((1) yield $\geq 5,000$ IEQ/kg (recipient body weight); (2) purity

Table 21.3 The rules for recipient selection from the recipient pool in Japan [28]

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1. Recipients must be registered to the islet isolation center
 2. ABO blood type
 3. Second or third transplantation
 4. Waiting days
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Table 21.4 Indications for the recipient of islet transplantation in Japan [28]

Indications

1. Insulin dependent diabetes mellitus (serum C-peptide level: <0.1 ng/mL)
2. Unstable blood glucose levels even under the treatment by a diabetologist
3. Age: <75 years old (preferable)
4. Sufficient informed consent obtained from the patient, family, and family doctor

Contraindications

1. Severe heart disease or severe liver disease
 2. Alcoholism
 3. Active infectious disease
 4. Malignancy
 5. Severe obesity
 6. Untreated retinopathy
 7. Others
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≥30 %; (3) final cell pellet ≤10 ml; (4) viability ≥70 %; and (5) endotoxin ≤5 EU/kg (recipient body weight)), the recipient is selected from the recipient pool by the Japanese Islet Transplant Registry according to the rules for recipient selection (Table 21.3) [28]. The patients were registered to each transplantation center according to the indications of the recipient for islet transplantation in Japan (Table 21.4) [28].

21.5 Outcome of Clinical Islet Transplantation from DCD Donors in Japan

Sixty-five islet isolations were performed at five authorized centers of islet isolation and transplantation from December 2003 to March 2007. Only one isolation (the first isolation in Japan) was performed using the pancreas from DBD donor and the other 64 isolations were performed using DCD donors. Thirty-four successful isolations (53.1 % of 64 isolations from DCD donors) fulfilled the fresh islet transplantation criteria and were transplanted to the 18 patients. The analysis of the factors influencing the results of the islet isolation demonstrated that the donor age and a warm ischemic time did not affect the results. In contrast, duration of low blood pressure of the donor, cold ischemic time, and usage of Kyoto solution for preservation was significantly influenced factors [40]. Multivariate analysis selected usage of Kyoto solution as most important.

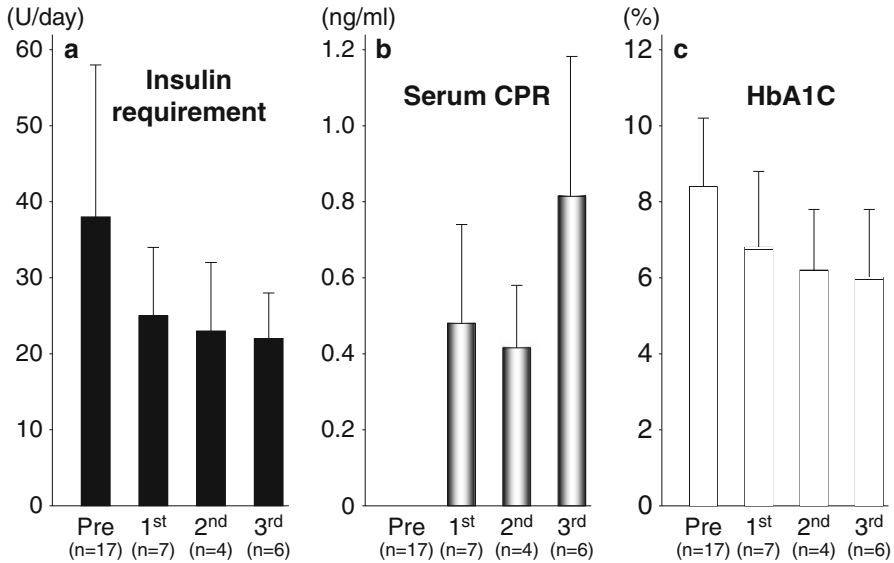


Fig. 21.1 The outcome of clinical islet transplantation in Japan: insulin requirement (a) decreased after the first (1st), the second (2nd), and the third (3rd) islet transplantation. Serum C-peptide levels (b) became positive after islet transplantation. Especially after the third islet transplantation, serum CPR showed 0.8 ± 0.4 ng/mL. Hemoglobin A1C (c) decreased after islet transplantation due to the stabilization of blood glucose levels

All 18 recipients were type 1 diabetic patients with the history of diabetes from 6 to 37 years. The age of the recipients ranged from 16 to 61 years (36.2 ± 10.9 years) and the gender was 5 males/13 females. Four patients underwent two sequential islet transplantations and six patients underwent three sequential islet transplantations. Out of four patients who underwent two islet transplantations, one patient showed insulin independency, and two out of six patients who underwent three islet transplantations achieved insulin independency. However, all of these patients returned to insulin dependency from 2 weeks to 6 months after achieving insulin independency. The islet transplantation technique was performed according to the Edmonton Protocol. The isolated islets were transplanted into the portal vein using an angiocatheter via an ultrasonography-guided puncture. A steroid-free immunosuppressive regimen was used with sirolimus, tacrolimus, and anti-CD25 antibody according to the Edmonton Protocol⁵. As is shown in Fig. 21.1, the serum C-peptide levels were 0.5 ± 0.4 ng/mL after the first, 0.4 ± 0.2 ng/mL after the second, and 0.8 ± 0.4 ng/mL after the third islet transplantation. The positive insulin secretion from the islets, as indicated by the serum C-peptide levels, resulted in the decreased insulin requirement: 39.7 ± 18.0 U/day before, 24.2 ± 11.0 U/day after the first, 21.4 ± 11.5 U/day after the second, and 21.0 ± 7.7 U/day after the third islet transplantation. In addition, the hemoglobin A1C levels (JDS) decreased from 8.8 ± 1.8 % before transplantation to 7.5 ± 1.4 % after the first, 6.5 ± 1.4 % after the second, and

6.2 ± 1.2 % after the third islet transplantation. All patients obtained stabilized blood glucose levels which resulted in the disappearance of hypoglycemic unawareness¹⁹. Overall graft survival defined as C-peptide level more than or equal to 0.3 ng/mL was 76.5 %, 47.1 %, and 33.6 % at 1, 2, and 3 years, respectively, whereas corresponding graft survival after multiple transplantations was 100 %, 80.0 %, and 57.1 %, respectively [40]. Almost all patients showed, however, negative C-peptide level at 5 years after the first islet transplantation.

21.6 Outcome of Clinical Islet Transplantation from DCD Donors in Chiba East National Hospital

From September 2003 to April 2007, 23 islet isolations were performed from the pancreas of non-heart-beating donors in our institution (GMP grade Bio-clean Cell Processing Center (CPC), Clinical Research Center, Chiba-East National Hospital). The age of the donors ranged from 10 to 69 years (37.5 ± 18.0 years), and the gender was 13 males/10 females. The causes of death were cerebrovascular disorder (ten cases), hypoxic encephalopathy (seven cases), trauma (five cases), and one brain tumor. After cardiac arrest, the pancreas were procured using our ISMW technique. In 9 of the 23 donors, permission was not given to insert a catheter into the aorta for systemic heparinization before a cardiac arrest, which resulted in a prolonged warm ischemic period. The pancreas were preserved using a TLM or a simple cold storage in UW solution and were transported to our CPC.

The islet isolation was performed according to the Edmonton Protocol with some modifications [41–48]. Briefly, the pancreas was distended with a cold Liberase solution (Liberase HI, Roche Diagnostics™, IN) by a ductal injection. Thereafter, the distended pancreas were cut into several pieces and put into a Ricordi chamber and digested using a closed automated system at 37 °C. The shaking of the Ricordi chamber was performed either by hand or by a shaking apparatus. The pancreatic digests were collected in a flask on ice and were purified on a Euro-Ficoll discontinuous solution using a COBE 2991 cell processor. When the results of the isolation fulfilled the criteria for fresh islet transplantation in Japan, the islets were immediately transplanted to the recipient.

Our technique of islet transplantation is the same procedure as in the Edmonton Protocol. The immunosuppression protocol was achieved by a triple therapy using sirolimus, tacrolimus, and anti-CD25 antibody. Four patients underwent six islet transplantations. All patients were type 1 diabetic patients (one male, three females) complaining of frequent hypoglycemic unawareness and showing an undetectable serum C-peptide levels (<0.03 ng/mL). The patient ages ranged from 16 to 33 years old. Two patients underwent two sequential islet transplantations.

The islet yield was 400–491,040 IEQ (mean 148,511) and the final purity was 1–70% (mean, 35.3). The stimulation index of static incubation was 1.38–11.69. Six isolations were used for transplantation because they fulfilled the Japanese criteria (success rate: 26.1 %). All patients, including two cases of single transplantation,

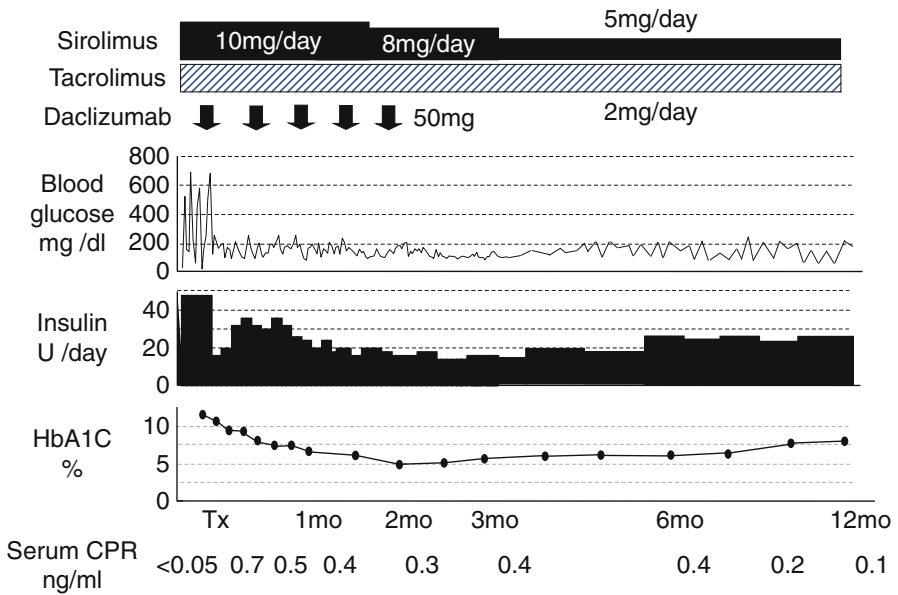


Fig. 21.2 Post-transplant course of the patient #1 (Department of Surgery, Chiba-East National Hospital): stabilization of the blood glucose levels and the decrease of the insulin requirement were obtained due to the positive serum CPR. However, serum CPR decreased gradually after islet transplantation

showed a positive serum C-peptide level (0.4–0.8 ng/mL) immediately after transplantation. Although insulin independency was not achieved, all patients achieved stabilization of blood glucose levels, reduced requirement of insulin, and the disappearance of hypoglycemic unawareness. The hemoglobin A1C levels (JDS) were significantly decreased from $9.4 \pm 3.1\%$ to $6.4 \pm 0.6\%$ at 4 months after transplantation. Although stomatitis and diarrhea, which were considered as the side effects of sirolimus, were observed in two patients, severe complications did not occur. In patient #1, the serum C-peptide levels decreased gradually after transplantation (Fig. 21.2). Blood glucose levels were, however, again stabilized after the second islet transplantation (Fig. 21.3). However, all four patients showed negative C-peptide levels at 5 years after the first islet transplantation.

21.7 Conclusions

Islet transplantation employing DCD can ameliorate severe hypoglycemic episodes, significantly improve HbA1c levels, sustain significant levels of C-peptide, and achieve insulin independence after multiple transplantations. Thus, DCD can be an important resource for islet transplantation if used under strict releasing criteria and

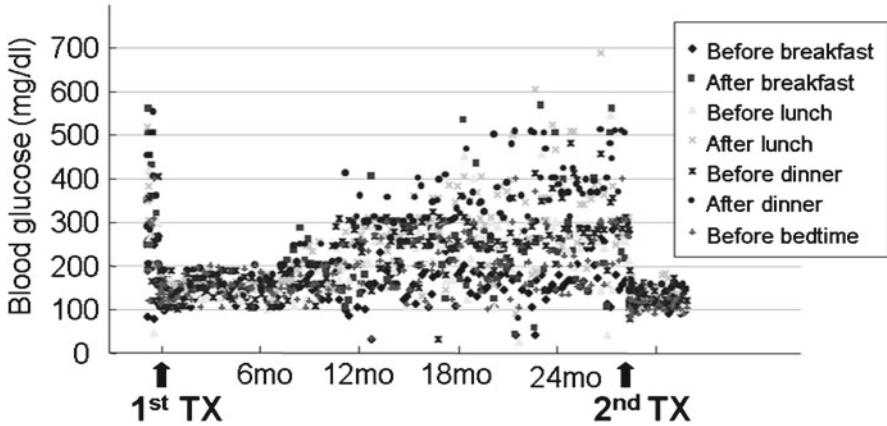


Fig. 21.3 Changes in blood glucose levels after islet transplantation in the patient #1: control of blood glucose levels became poor 12 months after transplantation. However, blood glucose levels were again stabilized by second islet transplantation

in multiple transplantations, particularly in countries where heart-beating donors are not readily available.

However, the outcome of clinical islet transplantation using Edmonton protocol is not satisfactory results. In particular, long-term islet survival has not been achieved, resulting in poor achievement of insulin independency. Based on these results, the Japanese Islet Transplant Registry currently introduced a newly designed clinical islet transplantation protocol including an immunosuppressive protocol using anti-thymus globulin and anti TNF α , and the use of DBD donors.

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