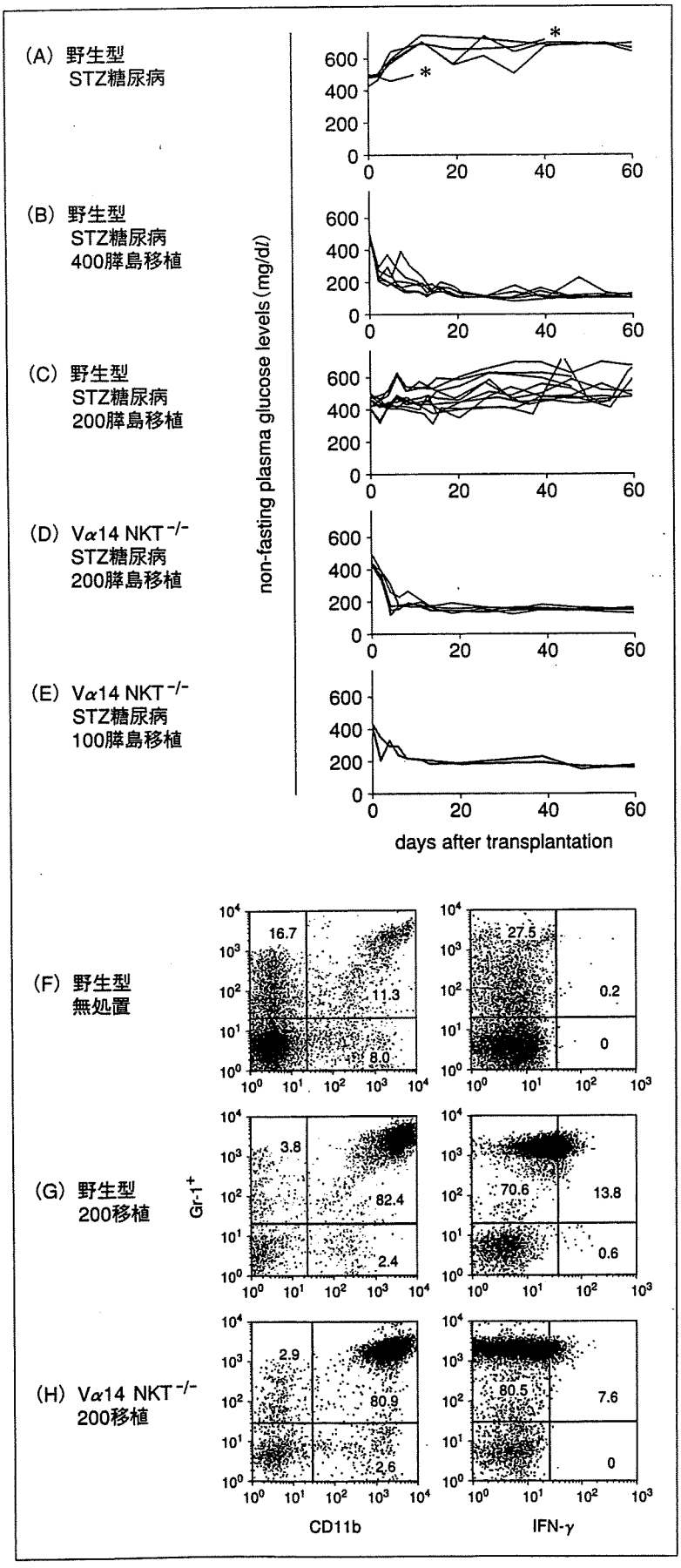

図 2　移植膵島障害におけるNKT細胞の役割

ストレプトゾトシン糖尿病マウス(C57BL/6)を作成し，膵島移植レシピエントに用いた(A)．同種同系単離膵島を野生型(B：400 個，C：200 個)，あるいは Vα14$^{-/-}$マウス(D：200 個，E：100 個)の経門脈的肝内移植した．膵島 200 個移植 6 時間後の野生型(G)または Vα14NKT$^{-/-}$(H)マウスより肝単核球を単離し，FACS で解析した．

ンが生理的に第1に肝臓に灌流する，局所麻酔で容易に移植できる，動物実験で効果が確認できているなどにより，肝臓が移植部位として選択され，ドナー膵島は経門脈的肝内に移植される．移植後に膵島は門脈末端に塞栓し，末梢側肝臓に虚血性変性が生じ，それに伴い炎症などの自然免疫反応が惹起され，移植膵島が破壊される．とくに肝臓は生体防御の第一線臓器として機能しており，強い自然免疫反応が起こりうる．

著者らは，その際に肝臓内に豊富に存在するNKT細胞が移植膵島障害に関与しているのではないかと想定した．その仮説を直接的に検証するために，Vα14 NKT細胞欠損マウス[4]を実験に使用した．ストレプトゾトシン糖尿病野生型マウス(C57BL/6)の経門脈的肝内に同種同系マウス1匹分の膵島200個を移植した場合，移植後レシピエントの血糖は正常化せず，高血糖で推移した(図2-C)．一方，2匹分400個の膵島移植後には正常血糖になった(図2-B)．このことはマウスにおいてもヒトと同様に，1匹の糖尿病レシピエントを膵島移植後にインスリン離脱するには2匹のドナーが必要であることを示している．驚いたことに，糖尿病Vα14 NKT細胞欠損マウスをレシピエントに用いた場合，200個(図2-D)のみならず100個(図2-E)の膵島移植で血糖は正常化した．さらに，細胞移入の実験においてもVα14 NKT細胞が移植早期膵島障害に必須の役割を担っていることが明らかになった．

FACS解析で，膵島移植後6時間の野生型マウス肝臓内にはIFN-γを産生するCD1dαGalCer-tetramer$^+$CD3$^+$(NKT)細胞が出現，さらにはIFN-γ$^+$Gr-1$^+$CD11b$^+$細胞が肝内に集積することが判明した(図2-G)．さらには200個の膵島移植レシピエントに抗Gr-1抗体，抗CD11b抗体，あるいは抗IFN-γ抗体を投与すると移植後正常血糖になった．Vα14 NKT細胞欠損マウスへの移植後では，Gr-1$^+$CD11b$^+$細胞が野生型と同様に肝内に集積するものの，IFN-γ産生はみられなかった(図2-H)．以上の知見は，NKT細胞に依存したGr-1$^+$CD11b$^+$細胞によるIFN-γ産生が移植早期膵島障害に必須の役割を果たしていることを示している．

つぎに，NKT細胞の合成リガンドであるα-galactosylceramide(α-GalCer)[5]を用い，NKT細胞を標的にした治療法を試みた．NKT細胞はα-GalCerの1回投与でIFN-γを大量に放出するが，繰り返し投与後にはIFN-γ産生は抑制されることが知られている．野生型糖尿病マウスに移植前α-GalCerを繰り返し投与(−15，−10，−7d)し，ストレプトゾトシンで糖尿病作成後に200個の膵島を移植すると血糖は正常化した．これらの知見は，NKT細胞を標的にした治療法で移植早期膵島障害が制御できることを示している．

移植免疫におけるNKT細胞の役割

NKT細胞にはエフェクター細胞，ならびに調節性細胞としての機能があることが報告されている[6]．著者らは移植免疫におけるNKT細胞の機能について解析し，マウス肝臓内CD4$^+$NKT細胞が調節性細胞としてラットからマウスへの異種膵島移植拒絶反応の制御にかかわっていることを明らかにした[7]．また，マウス同種膵島移植ではNKT細胞は拒絶反応の発現に関与しており，NKT細胞欠損マウスでは野生型と比較してグラフトの長期生存が得られることを報告した[8]．

今後の展望

臨床膵島移植の成績向上には，マウス実験系で得られた成果がヒトに応用できるかどうかを見極めることが急務と考え，現在ヒト肝リンパ球を用いた*in vitro*実験系で検討している．さらに，著者らが見出したNKT細胞を介した移植膵島障害の機序をもとに，臨床ですでに使用されている薬剤で制御できるものがないか，マウス実験系でスクリーニングしている．その結果，現在までにいくつかの有望な薬剤が判明しており，現在その臨床導入を検討している．また，著者らは膵島移植を通して，NKT細胞の動態・機能を解析してきたが，今後の研究によりいまだ明らかではないNKT細胞の内因性リガンドの発見につながる可能性があるのではないかと考え，研究を進めている．

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Improvement of pancreatic islet cell isolation for transplantation

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Pancreatic islet transplantation is a promising treatment for diabetes but still faces several challenges. Poor islet isolation efficiency and poor long-term insulin independence are currently two major issues, although donor shortage and the need for immunosuppressants also need to be addressed. We established the Kyoto islet isolation method (KIIM), which has enabled us to isolate and transplant islets even from non–heart-beating donors. KIIM involves 1) cooling the donor pancreas in situ, 2) preserving the ducts with modified Kyoto solution, 3) using a modified two-layer pancreas preservation method, and 4) adjusting the density of the density gradient centrifugation and using an iodixanol-based solution for purification. KIIM has enabled us to transplant 17 islet preparations out of 21 isolations (an 81% success rate). All transplanted islets functioned, and all transplanted patients had improved glycemic control without hypoglycemic unawareness. Recently, we used KIIM for islet isolation from a brain-dead donor at Baylor, which resulted in a very high islet yield (789,984 IE) with high viability (100% by fluorescein diacetate/propidium iodide staining and a stimulation index of 4.7). This preliminary evidence suggests that KIIM may also be promising for islet isolation from brain-dead donors. In addition, to assess engrafted islet mass, we developed a secretory unit of islet transplant objects (SUITO) index: fasting C-peptide (ng/dL) / [fasting blood glucose (mg/dL) – 63] × 1500. This simple index has enabled us to monitor the engrafted islet mass. This index should be useful when deciding whether to perform additional islet transplantations to maintain insulin independence. Poor islet isolation efficacy and poor long-term results could be resolved with ongoing research.

Type 1 diabetes still represents a therapeutic challenge and remains a substantial burden for patients and their supporters. The Diabetes Control and Complications Trial showed that intensive insulin therapy improved glycated hemoglobin A_{1c} and protected against diabetic triopathy (1), but the penalty was a thrice-increased risk of serious hypoglycemic events, including recurrent seizures and coma (2). Whole-pancreas transplantation can make those patients insulin independent, but the morbidity of that procedure is too high to advocate it for most patients (3).

An attractive alternative is islet transplantation. The islet transplantation procedure doesn't involve major surgery, general anesthesia, or the complications related to exocrine enzymes. Since the first human islet allograft transplant was done in 1974 (4), this treatment has continuously improved, and a dramatic improvement was achieved with the Edmonton protocol in 2000 (5). Shapiro et al demonstrated that seven out of seven preuremic type 1 diabetic patients who received islet transplants became insulin independent, with a dramatic decrease in the frequency of hypoglycemic unawareness at 1 year posttransplantation.

Key elements of the Edmonton protocol are 1) avoidance of corticosteroids with combined sirolimus, tacrolimus, and anti-interleukin-2-receptor antibody therapy to protect against rejection and autoimmunity and 2) the use of two or more fresh islet preparations (within 3 to 4 hours after isolation) processed by the Edmonton islet isolation protocol. The Edmonton islet isolation protocol includes 1) procurement of the donor pancreas from a brain-dead donor and organ preservation in cold University of Wisconsin solution with a minimal storage period, 2) collagenase infusion via the main pancreatic duct using a pressure-controlled method, 3) pancreas digestion using the Ricordi system, 4) islet purification by continuous density gradient using Ficoll with a chilled COBE 2991 cell processor, and 5) removal of all xenoproteins from the islet isolation process (5). Isolated islets were transplanted immediately by simple gravity infusion.

There has been an exponential increase in clinical islet transplantation activity, with 471 patients transplanted at 43 international institutions (6). The University of Alberta, the University of Minnesota, and the University of Miami demonstrated that 82% of 118 recipients of completed transplants were insulin independent within the first year after transplantation (6). The University of Alberta further demonstrated progressive loss of insulin independence over time, leaving approximately 10%

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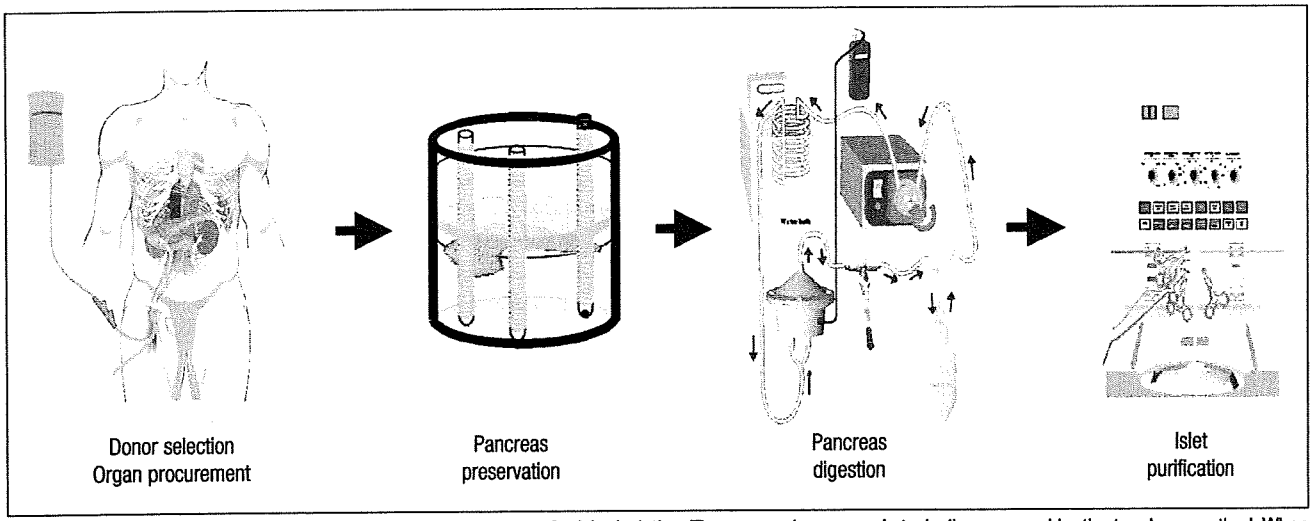

Figure. Donor selection and pancreas procurement is the first step for islet isolation. The procured pancreas is typically preserved by the two-layer method. When a pancreatic graft arrives at the islet isolation facility, the pancreas is distended with collagenase and digested in a Ricordi chamber. The final step is purification of isolated islets from exocrine tissues. A COBE 2991 cell processor is used for purification.

of patients still insulin independent at 5 years (7). However, more than 80% of patients continued to demonstrate persistent islet function at 5 years with effective prevention of recurrent hypoglycemia or severe lability combined with correction of hemoglobin A_{1c} (7). In addition, patients' quality of life was significantly improved by islet transplantation (8). Therefore, it should be reasonable to consider islet transplantation an option for the treatment of unstable type 1 diabetes.

The most difficult part of islet transplantation is islet isolation from a donated pancreas. Indeed, even in the leading centers, a transplantable yield of isolated islets is obtained in <50% of processed pancreata (9–11). In Japan, there is an extremely low number of brain-dead donors, and it has been almost impossible to utilize such donors for islet isolation. Therefore, alternative resources for islet isolation were sought, including islet isolations from non–heart-beating donors (NHBDs). The quality of the donor pancreas has a substantial impact on islet isolation. Therefore, using NHBDs for islet isolation is very challenging (12, 13).

In order to utilize pancreata from NHBDs for islet isolation, numerous protocols have been tested. Ultimately, an islet isolation method was established that enabled islet transplantation using pancreata from NHBDs (14–16). Very recently, this isolation method was applied to a regular brain-dead donor with promising preliminary data. In this article, we describe our endeavor to develop a new islet isolation method and our current effort to adapt this method to brain-dead donors.

THE PROCESS OF PANCREATIC ISLET ISOLATION

Pancreatic islet cell isolation starts at the point of donor selection and pancreas preservation *(Figure)*. When a pancreatic graft arrives at the islet isolation facility, the pancreas is distended with collagenase and digested in a Ricordi chamber. The final step is purification of isolated islets from exocrine tissues. After purification, islets are washed and put into a transplantation bag or culture medium before transplantation.

Donor selection

The quality of the donor pancreas is an important factor in successful islet isolation (17–20). However, pancreata are offered as whole organs first and only next for islet transplantation since whole-pancreas transplantation is considered an established treatment and islet transplantation is considered experimental.

Pancreata from obese donors give higher islet yield than those from lean donors (11, 17). Recently, researchers at the University of Minnesota demonstrated that the average islet yield from a pancreas donor with a body mass index (BMI) >30 was 319,129 IE and from a donor with a BMI <30 was 215,753 ($P = 0.0002$) (11). In addition, the donor with a BMI >30 had a higher islet isolation success rate—defined as isolations yielding >300,000 islet equivalents per pancreas, with purities of >50% (37.3% vs 15.9%; $P = 0.009$). This study showed that successful islet isolation can be difficult with low-BMI donors.

Previously, older donors were considered more suitable for islet isolation than younger donors (17). However, investigators have recently confirmed the advantages of islets from young donors, both in vitro in terms of insulin secretory function (21) and in vivo after transplanting islets in diabetic mice (22). In addition, we discovered that high islet yields could be obtained from younger donors with a modified islet isolation and purification method (18). The main obstacle to gaining high postpurification islet yields from young donors lies in the higher percentage of mantled islets embedded in acinar tissue. To recover mantled islets, we individualized the density of the high-density purification solution for each islet preparation (18). We believe that if we can recover mantled islets from young donors, the islet preparations should be of high quality, both in function and in islet number.

The stability of the donor during brain-dead status is another important factor. Based on the Edmonton protocol, many islet centers, including ours, have several exclusion criteria related to stability. The first concerns circulation and blood pressure:

prolonged hypotensive episodes are exclusion criteria when they have caused significant biochemical abnormalities (e.g., elevation of serum creatinine levels by >50% of the initial value or elevation of transaminases to levels >2 times the normal values). Cardiac arrest is an exclusion criterion when constantly stable circulation cannot be achieved in the 2 days following the event or when significant biochemical abnormalities have occurred, as described above. A third exclusion criterion relates to vasopressors, specifically norepinephrine, if required for maintenance of stable circulation. However, we have recently developed an islet isolation method for marginal donors, especially NHBDs. With this method, we have successfully isolated islets from marginal donors, and we have changed the criteria from absolute contraindications to relative contraindications. This change will significantly increase the islet isolation number, and we estimate that about 50% of donor pancreata will be used for islet isolation (Matsumoto et al, manuscript in preparation).

We have found that histology-proven chronic pancreatitis has led to the worst islet isolations (18). The duration of pancreatic digestion was significantly longer, and the undigested tissue volume was significantly larger with chronic pancreatitis. In addition, the purity of isolated islets was significantly lower with chronic pancreatitis. This finding suggests that fibrotic pancreata are resistant to collagenase digestion, resulting in poor islet isolation.

Pancreas preservation

Traditionally, the pancreas is preserved in University of Wisconsin solution. However, even for a short duration, oxygenated perfluorocarbon (PFC) provides the best method for pancreas storage (23–25). With the oxygenated PFC, pancreas grafts are directly oxygenated and continuously generate ATP (26), and the viability of endothelial cells is maintained (27, 28). Because of these effects, oxygenated PFC seems to be the most suitable substance for preservation before pancreas transplantation and islet isolation.

When PFC is added to the University of Wisconsin solution or another solution—the "two-layer method"—it is necessary to oxygenate the PFC just before pancreas preservation, ensure adequate oxygenation of PFC just before storage, and ensure sufficient attachment of PFC to the pancreata. With inadequate oxygenation to the pancreas, ATP production was low, and the benefit of the two-layer method was lost (29).

Complete immersion of the pancreas into oxygenated PFC—the "one-layer method"—seems to be better than the two-layer method (30). Even for the one-layer method, the top layer is necessary to keep oxygen from escaping from the surface of the PFC.

Pancreatic ductal preservation seems important because collagenase is delivered through the pancreatic duct (31). Sawada et al demonstrated that a small amount of University of Wisconsin solution perfused into the pancreatic duct significantly improved the results of islet isolation in a rodent model (31), and a European group introduced the method in humans (32). We have demonstrated that ductal injection of a large amount of modified Kyoto solution into the main pancreatic duct significantly reduced apoptotic cell death of both exocrine tissue and islet cells (Noguchi et al, *Cell Transplantation*, in press). We avoided University of Wisconsin solution for ductal injection since it inhibits collagenase activity, which is essential for pancreas digestion and islet isolation (33). We preferred a large ductal injection because the solution protects not only pancreatic ducts but also exocrine tissues.

Collagenase selection and delivery

Selection of collagenase is important for successful islet isolation, and currently Liberase is used exclusively (34). Liberase is considered to be the best collagenase, but lot-to-lot variation has been a concern. Kin et al demonstrated that optimization of thermolysin dosage based on caseinase unit per gram of pancreas contributed to the islet isolation outcome, but the collagenase dosage provided by the manufacturer (Wünsch unit per gram of pancreas) was not a major determinant of islet isolation outcome (9). In addition, they pointed out that the lot-to-lot inconsistency of the enzyme's performance was explained not by the activity values provided by the manufacturer but rather by the proportion of class I collagenase and class II collagenase, as determined by an in-house assay (9). Specifically, the odds of successful isolation were 8.67 times higher when a vial with a class II:class I ratio of <0.204 was used than when a vial with a ratio of ≥0.204 was used.

Collagenase delivery with pressure monitoring is the current standard (35). It is widely believed that during the infusion of collagenase into the pancreas, the goal is excellent distension with minimum leakage. An important modification that we have made is the use of only one cannula, inserted from the duodenal orifice of the main pancreatic duct (one-cannula method) (25). For collagenase delivery, usually a pancreas is cut and cannulas are inserted into two or three pancreatic ducts; this has been done since if the pancreatic duct is not adequately preserved, one cannula cannot deliver collagenase through the pancreas. However, this technique inevitably causes collagenase leakage. The one-cannula method, which requires the pancreas to be preserved intact and not cut, has resulted in minimal collagenase leakage with excellent distension.

Pancreas digestion

The Ricordi method is a standard for pancreas digestion in clinical islet transplantation (36). The key component of this method is a special Ricordi chamber for pancreas digestion and the effective collection of digested pancreatic tissue (36). The Ricordi chamber is designed for effective pancreas digestion with meticulous temperature control and is useful for effective dilution and collection of digested pancreatic tissue with a large volume of solution. Besides the Ricordi method, other static digestion methods have been effective for pancreas digestion (37, 38); however, those methods may not be effective for dilution. Since islets are sensitive to overdigestion, effective dilution may be important. Therefore, dilution, temperature control, and neutralization of digestive enzymes are all important, and the Ricordi method is the best to provide these conditions.

Trypsin inhibitors may help to avoid overdigestion (38). Previously, we have shown that the use of the trypsin inhibitor

Pefabloc during islet isolation using the simple open-pan islet isolation method improved islet yield in nonhuman primate and human models (38). The University of Alberta also demonstrated that human islet isolation was improved with Pefabloc when pancreata were preserved for extended time periods (39). However, trypsin inhibition had no effect on improved islet isolation when pancreata were procured from brain-dead heart-beating donors using the Ricordi islet isolation method (40, 41). In addition, when collagenase activity is not strong enough, trypsin may actually help to digest a pancreas. Therefore, trypsin inhibition during islet isolation might not be important when an optimal pancreas is processed with the Ricordi islet isolation method.

Islet purification

Purification of islets from exocrine tissue is a critical step for maintaining high islet yields. The common method of islet purification is density gradient centrifugation. Ficoll is widely used for density gradients (42) with a COBE 2991 cell processor (43). However, an iodixanol-based solution has contributed to increased islet yield, especially for porcine islet isolation (44–47). Iodixanol has low viscosity and, therefore, it needs less force during centrifugation. Iodixanol-based purifications were clinically applied by others as well as by our own group with promising results (15, 16, 48, 49). We diluted iodixanol with ET-Kyoto solution. However, other solutions, such as culture media or other preservation solutions, could be examined.

THE KYOTO ISLET ISOLATION METHOD FOR NHBDS

In Japan, it is difficult to use brain-dead heart-beating donors for islet isolation and transplantation. Therefore, we pursued islet transplantation from NHBDs (14, 15) or living donors (50–52). To initiate islet transplantation using NHBDs, we established several criteria that resulted in the Kyoto islet isolation method (KIIM) (14–16).

First, we inserted a double-balloon catheter before cessation of heart beating to chill the pancreas immediately after cardiac arrest (53). This technique enabled us to minimize warm ischemic time. It was demonstrated that islet yield and function deteriorated after 30 minutes of warm ischemia in rat and dog models (54). As a matter of fact, without this technique, warm ischemic time is >30 minutes and results in unsuccessful islet isolation. Second, we introduced ductal injection immediately after procurement, as described in the pancreas preservation section. Third, we modified the two-layer (modified Kyoto solution and oxygenated PFC) method of pancreas preservation and recently switched to a one-layer method. Fourth, we used ulinastatin for trypsin inhibition during islet isolation. Ulinastatin is not only a trypsin inhibitor but also an antiinflammatory drug (55). Therefore, this drug might be useful for an ideal donor with the Ricordi method. Finally, we performed density measurements on exocrine tissue since acinar tissue density could decrease during warm ischemia. Adjusting the density of the gradient solution enabled us to recover embedded islets. In addition, we used iodixanol instead of Ficoll for islet purification because iodixanol has low endotoxin activity and low viscosity, which should be less harmful for islets.

We have isolated 21 human pancreata using KIIM from NHBDs. Double-balloon catheters inserted before cardiac arrest were combined with kidney retrieval in 18 cases (18). The average transplanted islet yield was 382,945 ± 44,146 IE (4,589 ± 504 IE/g), and the purity was 46.8 ± 3.3%. The viability of transplanted islets, as assessed by acridine orange/propidium iodide, was 96.2 ± 0.7%, and all of the samples were above 85%. The average insulin stimulation index was 4.2 ± 1.8. Islet preparations from 17 cases (16 cases with a double balloon and one case without a balloon) met transplantation criteria. These islet preparations were transplanted into eight type 1 diabetic patients. In all cases after islet transplantation, hemoglobin A_{1c} levels were improved and there was no hypoglycemic unawareness. Thus, 17 out of 21 islet preparations (81%) achieved success. Compared with results of leading institutes, KIIM provided a very high success rate of transplantation, even using NHBDs (9–11).

SECRETORY UNIT OF ISLET TRANSPLANT OBJECTS INDEX

To assess engrafted islet mass, we developed the secretory unit of islet transplant objects (SUITO) index (56, 57). The formula of the SUITO index is as follows: fasting C-peptide (ng/dL) / [fasting blood glucose (mg/dL) – 63] × 1500. A SUITO index of 100 reflects 100% pancreatic beta-cell function in a healthy person. If the fasting C-peptide level is 0.8 ng/dL and blood glucose is 103, the SUITO index will be 0.8 / (103–63) × 1500 = 30.

Previously, we have shown that a SUITO index of >25 is necessary for insulin-independent status (56). The SUITO index of islet-transplanted patients with cultured islets from NHBDs was significantly lower than that of patients with fresh islets from NHBDs (56). In addition, living-donor islet-transplanted patients showed the highest SUITO index with insulin-independent status (56).

Recently at Baylor, we performed islet transplantation from a brain-dead donor without culturing the islets. After a single islet infusion, the average SUITO index from day 3 to day 30 was 29.7 ± 10.4. The patient's glycemic control improved substantially without hypoglycemic unawareness. The insulin dosage has been substantially reduced, and the patient is expected to be insulin independent. After single islet infusion with NHBDs, the average SUITO index from day 3 to day 30 was approximately 12. Therefore, islets from brain-dead donors seem to be of higher quality than those from NHBDs.

APPLICATION OF KIIM FOR ISLET ISOLATION FROM A BRAIN-DEAD DONOR

Recently, we applied KIIM for islet isolation from a brain-dead donor (except for the double-balloon technique, which is necessary only for NHBDs). In this case, the islet team joined the Baylor University Medical Center donor team for the pancreas procurement. The donor was a 52-year-old woman with a BMI of 39.1 and a pancreas weight of 98 g. After the pancreas was retrieved, the attached spleen, duodenum, and fat tissue were immediately removed. A single cannula was inserted from the duodenal orifice of the main pancreatic duct. Approximately 100

mL of modified Kyoto solution was infused. An accessory pancreatic duct was identified and was ligated with a hemoclip. The pancreas graft was preserved by the two-layer method (modified Kyoto and oxygenated PFC) and transported to the islet isolation laboratory at the Baylor Institute for Immunology Research. The cold storage period was approximately 3 hours. Upon arrival at the islet isolation laboratory, the pancreas was immediately immersed in the decontamination solution since it had already been trimmed. Chilled collagenase solution (Serva collagenase with neutral protease) was infused using a single cannula with pressure control. The pancreas distended excellently with minimum collagenase leakage. The distended pancreas was cut into nine pieces and put into the Ricordi chamber for digestion. Islets were purified using a density-adjusted Kyoto-iodixanol continuous density gradient with a COBE 2991 cell processor.

Islet yield after digestion and before purification was 803,467 IE. After purification, the islet yield was 789,984 IE with approximately 40% purity. A viability assay with fluorescein diacetate/propidium iodide showed that 100% of the islets were viable after purification, and the stimulation index with static glucose challenge was 4.7. Thus, the first trial of KIIM for islet isolation from a brain-dead donor is promising.

CONCLUSIONS

At this time, islet transplantation is the most promising method to cure diabetes with minimum risks—although the success rate for islet isolation is still relatively poor. However, continuous improvements in islet isolation are occurring. Single-donor islet transplantation for insulin independence should be established with an advanced islet isolation technique. The SUITO index is a powerful tool to estimate engrafted islet mass (56). We should supply islets to maintain a SUITO index of >30; this practice should result in patients who remain insulin independent for the long term. Current major concerns of poor islet isolation efficacy and long-term results could be resolved with ongoing research.

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Estimation of Donor Usability for Islet Transplantation in the United States With the Kyoto Islet Isolation Method

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The quality of donor pancreata is important for successful islet isolation. However, in some countries like Japan, the number of donor pancreata is very low; therefore, marginal donors have been used with less restrictive donor criteria. In order to use marginal donor pancreata, we established the Kyoto islet isolation method (KIIM). According to United Network for Organ Sharing (UNOS) in 2005, more than 6,000 pancreata were not clinically used in the US. In this study, we applied the KIIM for brain-dead donors and reevaluated donor usability based on the Japanese islet donor criteria. Islets were isolated with the Ricordi method using pancreata stored in University of Wisconsin (UW) solution (UW group) or by the two-layer method (TLM group) or the TLM combined with ductal injection (DI group). We implemented the KIIM (KIIM group) to confirm the effect of the KIIM on brain-dead donors. Donor charts in Texas from 2005 to 2006 were reviewed. If pancreata were not used clinically, the reason was reviewed and donors were reevaluated based on Japanese criteria. There were no significant differences of islet yield, viability, and purity between the UW and TLM groups. The DI group significantly improved islet yields and isolations were further improved in the KIIM group [UW: 251,663 ± 60,217 islet equivalent (IE); TLM: 243,738 ± 54,170 IE; DI: 498,639 ± 28,853 IE; KIIM: 678,286 ± 55,853]. The KIIM provided high-quality islets in high numbers from islet isolations from brain-dead donors. A total of 236 donor charts were reviewed and 194 pancreata (82%) were not used. Of these, 185 cases identified the reasons that the pancreata were not used. When we applied the Japanese criteria, an additional 82 cases out of 185 (44%) seem to be suitable for islet isolations. With the KIIM, more than 2,500 additional donor pancreata can be used for islet isolation in the US every year when the Japanese criteria are applied.

Key words: Donor; Ductal injection; Islet transplantation; Kyoto islet isolation method; Kyoto solution; Two-layer method

INTRODUCTION

Islet transplantation is an option for the treatment of type 1 diabetic patients who maintain hypoglycemic unawareness despite maximal care (20,21). Successful islet isolation is the key to successful islet transplantation; the quality of the donor pancreas is important for successful islet isolation (1–3,5,7,15,22). Highly restricted criteria were made in order to assure high quality of donor pancreata for islet isolation (20). However, in some countries like Japan, the number of donor pancreata is very low; therefore, marginal-quality donor pancreata have been used. In order to use marginal donor pancreata, especially from non-heart-beating donors (NHBDs), we modified the Ricordi islet isolation method and developed the Kyoto Islet Isolation Method (KIIM) (11,14,16,17). Major implementations of this modification are pancreatic ductal preservation, modified two-layer pancreas preservation with Kyoto Solution and density-adjusted continuous density gradient purification (10). Using the KIIM, we successfully isolated islets and transplanted into type 1 diabetic patients in 17 out of 21 cases (81%) (8). This transplantation rate is remarkably high compared with previously published data (4,6,9). In fact, the transplantation rate of the majority of islet centers is less than 50%. Despite this low percentage, the majority of pancreata in the US, even from brain-dead heart-beating donors, are not clinically

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used. According to UNOS in 2005, only 1,466 pancreata out of 7,593 cases (19%) were clinically used, meaning that more than 6,000 pancreata were not used. However, we postulated that if we implemented the KIIM for islet isolation from brain-dead donors, we should be able to use marginal donor pancreata for islet isolation, meaning that more pancreata could be clinically used.

In this study, we first implemented the KIIM for islet isolation from brain-dead donors. Then we evaluated pancreas donors in our local area by reviewing well-documented donor charts and estimated how many pancreata in the US could be used for islet isolation when we apply the Japanese islet donor criteria in combination with an improved islet isolation technique, such as the KIIM.

MATERIALS AND METHODS

Pancreas Procurement and Islet Isolation

From January 2005 to August 2007, we performed 27 islet isolations using brain-dead donor pancreata. In all cases, pancreata were stored less than 8 h. All 27 islet preparations were manipulated according to Current Good Manufacturing Practice (cGMP) in the cell processing facility at Baylor Institute for Immunology Research.

The first protocol included the regular Ricordi islet isolation method (19,20) and pancreata were preserved in UW solution ($N = 6$, UW group) or by the oxygen static charged two-layer method ($N = 13$, TLM group) (13). For the next protocol, the islet team participated in pancreas procurement and pancreatic ductal injection was introduced at the donor site ($N = 3$, DI group) (10,11,16). Then pancreata were preserved by the oxygen static charged TLM and islets were isolated by the Ricordi method. For the final protocol, we implemented the KIIM ($N = 3$, KIIM group) (10,11,17). The key procedures of the KIIM are described as follows. We procured pancreata in conjunction with multiorgan procurement. We removed the duodenum and spleen from the pancreas at the procurement site. A cannula was immediately inserted into the procured pancreas through the main pancreatic duct from the direction of the pancreatic head and M-Kyoto solution was administered intraductally (ductal injection). M-Kyoto solution is 100,000 U/L of ulinastatin (Mochida Pharmaceutical Co. Ltd., Tokyo, Japan) in ET-Kyoto solution (Otsuka Pharmaceutical Co., Naruto, Japan).

For pancreas preservation during transportation, we use an oxygenated perfluorocarbon/M-Kyoto solution-based two-layer method. Before islet purification, we checked the density of the isolation aggregates with a test tube density adjustment method as follows (10). Six discontinuous density test tubes were prepared with 5 ml each of purification solution of different densities (1.085, 1.090, 1.095, 1.100, 1.105, and 1.110 g/cm^3). The different densities were achieved by adjusting the ratio of iodixanol (OptiPrep, Axis-Shield PoC AS, Norway) to M-Kyoto solution. Samples were taken from the isolation aggregates and added to the discontinuous density test tubes. The tubes were spun at 1,000 rpm for 5 min and the densities of aggregates were determined by whether they pelleted or floated in the different density solutions. Islets were purified with a continuous density gradient of iodixanol/M-Kyoto solution in an apheresis system (COBE 2991 cell processor, Gambro Laboratories, Denver, CO). The heavy density solution was chosen according to the test tube density adjustment method described above and the gradient was achieved by varying the ratio of iodixanol to M-Kyoto solution.

Islet Evaluation

Islet evaluation was independently judged by two investigators. Islet yield was determined with dithizone staining (2 mg/ml; Sigma Chemical Co., St. Louis, MO) under an optical graticule and converted into a standard number of islet equivalents (IE, diameter standardizing to 150 μm) (12,18). Purity was assessed by comparing the relative quantity of dithizone-stained tissue to unstained exocrine tissue. Islet viability was evaluated using fluorescein diacetate (FDA) and propidium iodide (PI) staining to visualize living and dead cells simultaneously (12,18). The recovery rate after purification was determined by dividing IE before purification by IE after purification. For qualification of transplantation, we used the original Edmonton protocol criteria (20). For a qualified transplantation, islet yield should be more than 5,000 IE/kg patient body weight (we used 60 kg as a default body weight, which means that the total islet yield should be more than 300,000 IE), viability above 70%, purity of more than 30%, and a tissue volume of less than 10 ml (20). In addition, the final product needs to have negative gram staining and have endotoxin levels below 5 EU/kg patient body weight (we used 60 kg as a default so that the total endotoxin level should be less than 300 EU).

Isolated islets from all three cases of the DI group were transplanted into two type 1 diabetic patients. One patient received two islet infusions and the other patients received one. We submitted the data obtained from the KIIM group to Food and Drug Administration (FDA) for the approval of clinical use of the KIIM.

Donor Chart Review

Two hundred and thirty-six donor charts from well-documented multiorgan procurements in Texas (Southwest Transplant Alliance; Dallas, TX and LifeGift Organ Donation Center; Fort Worth, TX, USA) from 2005 to 2006 were reviewed. Twenty-nine pancreata were

used for whole pancreas transplantations and 13 pancreata were used for islet isolation. Therefore, 194 (82%) pancreata were not used. In the unused 194 cases, the reasons that they were not used were also reviewed based on donor-specific inclusion and exclusion criteria at Baylor. Then unused donors were reevaluated based on Japanese islet specific donor inclusion and exclusion criteria.

Statistics

Values for the data collected represent means ± SE. Four groups were compared by means of ANOVA followed by Fisher's Protected Least Significant Difference post hoc test. The ratios between the two groups were compared using Fisher's exact test. Values of $p < 0.05$ were considered significant.

RESULTS

Donor Characteristics

In terms of donor characteristics among the four groups (UW group, TLM group, DI group, and KIIM group), there were no significant differences in donor age, body mass index (BMI), pancreas weight, or cold ischemic time (CIT) (Table 1).

Islet Isolation Outcomes

There was no significant difference in islet yields between the UW group and TLM group (251,663 ± 60,217 IE UW group vs. 243,738 ± 54,170 IE TLM group) (Fig. 1). Islet yields were significantly increased when we introduced ductal injection to the TLM method (498,639 ± 28,853 IE) (Fig. 1). The use of the KIIM further improved islet yields compared to the DI groups (678,286 ± 55,853 IE) (Fig. 1). There were no significant differences of viability or purity of isolated islets among the four groups (Table 2). The recovery rate after purification was significantly higher in the KIIM group compared with the UW and TLM groups (Table 2). Qualifying the transplantation criteria was 2/6 (33%) in the UW group and 4/13 (31%) in the TLM group. After the addition of ductal injection, both DI and KIIM groups had a 100% success rate for qualification of transplantation. The success rate of islet isolation was significantly improved with ductal injection (DI and KIIM groups) [6/19 (32%) without ductal injection (UW, TLM) vs. 6/6 (100%) with ductal injection (DI, KIIM), $p < 0.01$].

The clinical results in the DI group are shown in Figure 2. The daily insulin dose was gradually decreased after the first islet transplantation and the patient became insulin independent after the second islet transplantation (Fig. 2, top). Fasting glucose levels became well controlled after the first islet transplantation and further improved after the second islet transplantation (Fig. 2, middle). HbA_{1C} levels gradually decreased after the first islet transplantation and reached normal range (Fig. 2, bottom).

Donor Chart Review

Donor chart review revealed that out of 194 unused cases, the reasons that the pancreata were not used were identified in 185 cases, based on Baylor islet-specific donor inclusion and exclusion criteria (Table 3). Forty cases (21.6%) were aborted during procurement, 37 pancreata (20.0%) were not used due to donor age, 29 pancreata (15.7%) were not recovered due to high glucose, 17 pancreata (9.2%) could not be used due to diabetes, in 15 cases (8.1%) the family did not consent to the procurement, 14 cases (7.6%) had infectious disease, in nine cases (4.9%) no specific reasons were mentioned, eight cases (4.3%) were not attempted due to cardiac arrest events, seven cases (3.8%) were not performed for social reasons, five pancreata (2.7%) were not used due to fatty pancreata, and elevated creatinine levels prevented pancreas procurement in four cases (2.2%) (Fig. 3).

Next, unused donors were reevaluated based on Japanese islet-specific donor inclusion and exclusion criteria (Table 3). Infection, lack of family consent, diabetic pancreata, and social reasons were considered as not

Table 1. Donor Characteristics

	UW	TLM	DI	KIIM
N	6	13	3	3
Age (years)	48.0 ± 3.5	43.6 ± 3.1	37.5 ± 14.5	34.3 ± 9.2
BMI (kg/m^2)	28.7 ± 2.0	29.5 ± 1.6	36.1 ± 1.2	35.7 ± 2.5
Pancreas weight (g)	115.0 ± 21.6	99.1 ± 7.2	121.4 ± 31.1	95.9 ± 4.4
CIT (h)	6.0 ± 0.6	4.8 ± 0.6	3.0 ± 0.6	3.3 ± 0.9
Gender (F/M)	2/4	3/10	2/1	1/2

There were no significant differences in all categories among all groups. UW, University of Wisconsin solution; TLM, Two-layer method; DI, TLM + ductal injection; KIIM, Kyoto islet isolation method; BMI, body mass index; CIT, cold ischemic time.

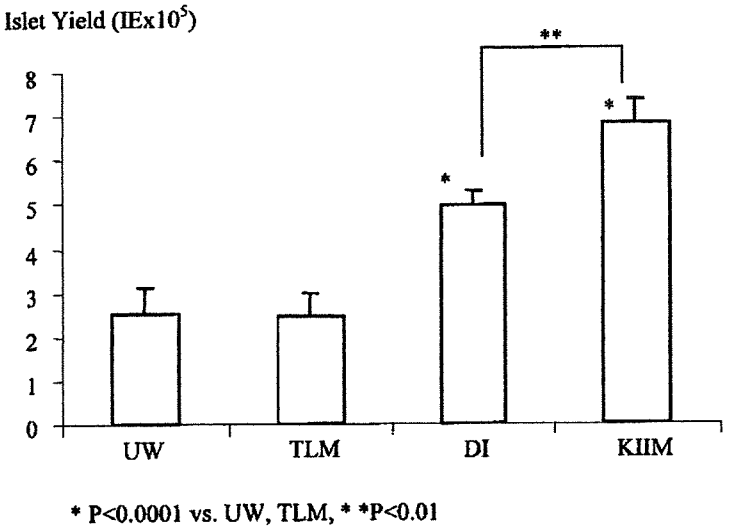

Figure 1. Islet yields of four different groups, including the Ricordi method using UW-stored pancreata (UW), the Ricordi method using TLM-stored pancreata (TLM), the Ricordi method using ductal preservation (DI), and the KIIM (KIIM). Islet yields were significantly higher in the DI group compared with the UW and TLM groups (*$p < 0.0001$ UW vs. DI and TLM vs. DI). Islet yields were significantly higher in the KIIM group compared with all other groups (*$p < 0.0001$ UW vs. KIIM and TLM vs. KIIM, **$p < 0.01$ DI vs. KIIM).

qualified cases (63 cases, 34%). Based on the Japanese criteria, high glucose without diabetes, fatty pancreas, or elevated creatinine levels are acceptable for islet donors; therefore, we counted those as qualified pancreata (47 cases, 25%). Ages less than 25 years old and up to 70 years old and cardiac arrest events with less than 30 min of warm ischemia are also acceptable. In some cases where the procedure was aborted, there were no contraindications. Out of 85 of those cases (age, cardiac arrest, aborted cases), 25 cases were qualified for islet donation. A total of 82 cases (44%) were qualified for islet donation.

When we extrapolated the 44% qualification rate that we identified here, but of the currently unused 6,000-plus pancreata, more than 2,500 pancreata could be used for islet isolation in 1 year in the US.

DISCUSSION

Currently, we have established the KIIM for islet isolation with marginal donor pancreata (8,10,11,14,17). With the KIIM, we successfully isolated transplantable islets from NHBDs in 17cases out of 21 isolations (81%) (8). All transplanted islets secreted insulin and all of the patients improved glycemic control without hypoglycemic unawareness. Three out of five multiple transplantation cases (60%) became insulin independent (8).

In this study, we first compared UW and TLM storage. We then tested ductal injection (DI group) and KIIM (KIIM group) to confirm the effect of DI and KIIM on islet isolation from brain-dead donors. We did not see any significant differences between the UW group and the TLM group. Previously, we demonstrated

Table 2. Islet Isolation Outcomes for the Different Groups

	UW	TLM	DI	KIIM
Viability (%)	87.3 ± 6.2	92.3 ± 0.8	95.7 ± 2.7	93.9 ± 0.9
Purity (%)	53.7 ± 7.9	67.8 ± 6.0	49.3 ± 3.8	43.0 ± 5.0
Recovery rate after purification (%)	53.9 ± 10.9	45.4 ± 6.1	61.2 ± 9.0	90.0 ± 6.9*
Qualified for transplantation	2/6 (33%)	4/13 (31%)	3/3 (100%)	3/3(100%)

*$p < 0.01$ versus TLM and $p < 0.05$ versus UW.

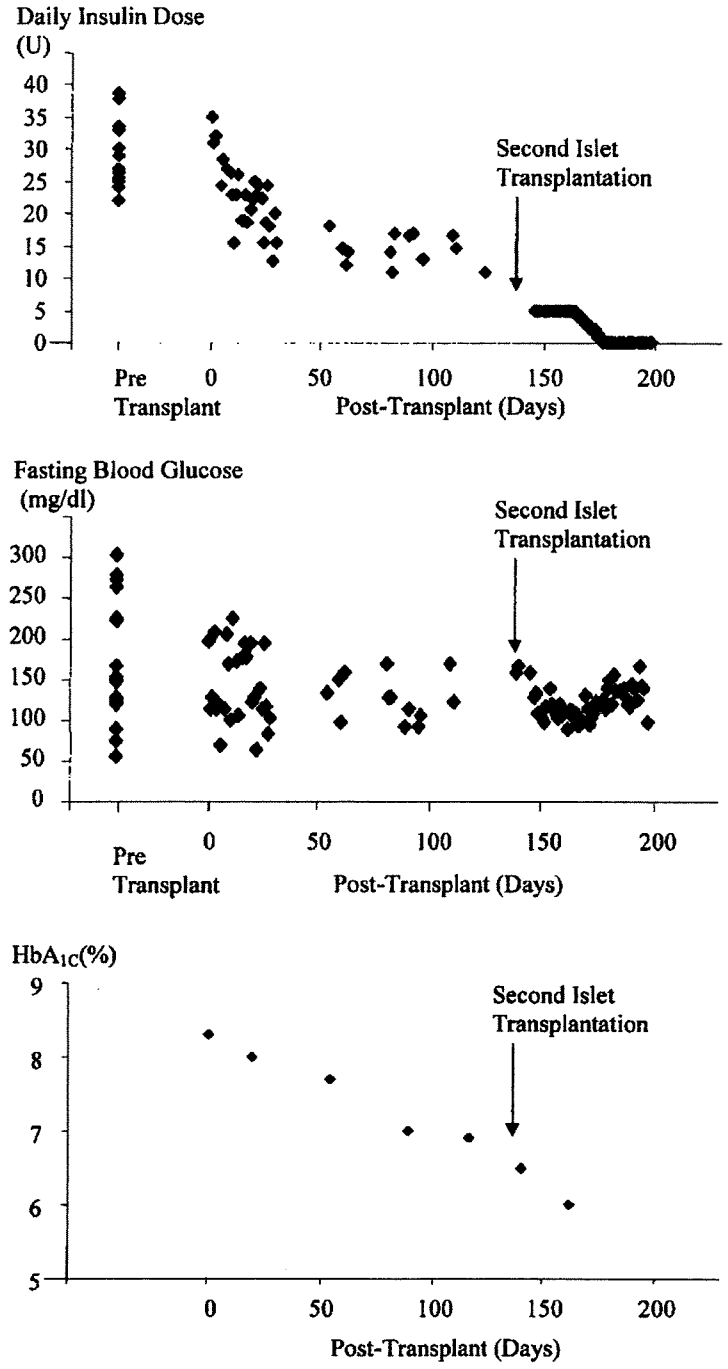

Figure 2. Daily insulin dose (top), fasting blood glucose (middle), and HbA_{1C} (bottom) before and after two islet transplantations with the islets from the DI group. Daily insulin dose decreased after the first islet transplantation and the patient became insulin independent after the second islet transplantation (top). Fasting blood glucose became more stable after the first islet transplantation and became more stable after the second transplantation (middle). HbA_{1C} continuously decreased after the first islet transplantation and achieved normal range after the second transplantation (bottom).

Table 3. Comparison of Baylor and Japan's Donor-Specific Inclusion and Exclusion Criteria

	Baylor	Japan
Donor-specific inclusion criteria	Multiorgan donor	Multiorgan donor
	Adequate in situ hypothermic perfusion	In situ hypothermic perfusion: no limit
	Cold ischemia time: maximum 18 h	Cold ischemic time: no limit
	Age: 25 to 70 years	Age: less than 70 years
	Hospitalization stay: <96 h	Hospitalization stay: no limit
Donor-specific exclusion criteria	Warm ischemia exceeding 10 min	Warm ischemia exceeding 30 min
	Preexisting diseases: Diabetes mellitus type 1 or 2; Malignancies other than primary brain tumor; Septicemia	Preexisting diseases: Diabetes mellitus type 1; Malignancies; Septicemia
	Circulation/blood pressure/cardiac arrest: S-Cre >150% of initial value or ALT, AST >twofold of normal	Circulation/blood pressure/cardiac arrest: S-Cre, ALT, AST no limit
	Vasopressors: Norepinephrine	Vasopressors: no limit

that TLM improved islet yields and the effect was more apparent when pancreata were stored for longer periods (12). In this study, we restricted CIT to less than 8 h. Therefore, the effect of the TLM became less apparent. In addition, in the previous study, members of the islet team procured pancreata for the TLM. In this study, a multiorgan procurement team procured pancreata and stored them by TLM. The University of Alberta group also demonstrated that the TLM had no significant impact on islet transplantation (6). In their study, the multiple organ procurement team but not islet team procured pancreata. These facts suggest that the type of procure-

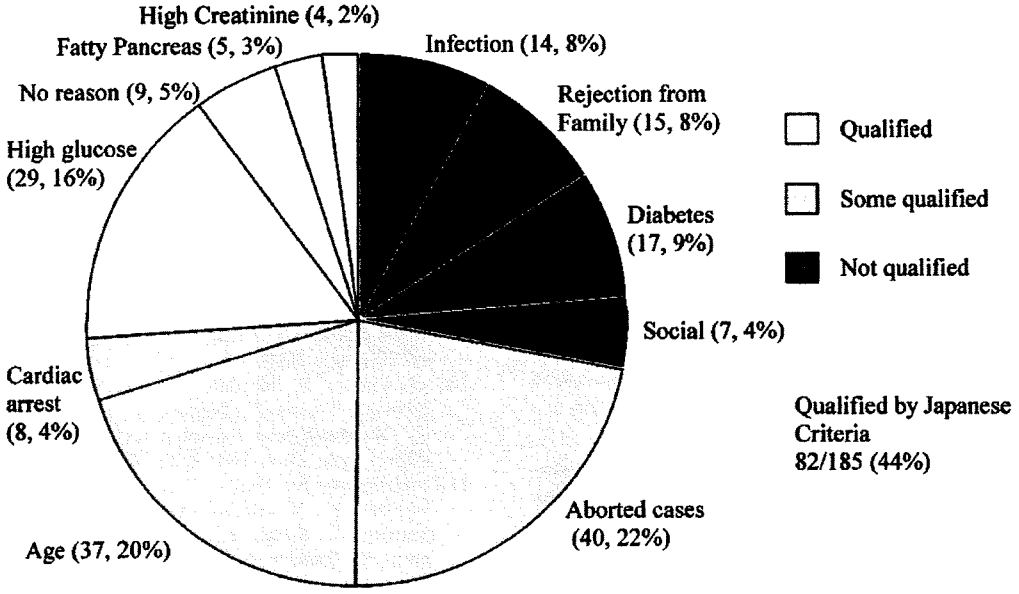

Figure 3. Reasons and number of unused pancreata from brain-dead donors in Texas. Out of 194 unused cases, the reasons that the pancreata were not used were identified in 185 cases, based on Baylor islet-specific donor criteria. The reasons were reevaluated by the Japanese criteria. Eighty-two cases out of 185 (44%) unused pancreata were qualified for islet donation based on the Japanese criteria. Values are number, percentage of total.

ment team has a significant impact on islet isolation. This is because the TLM requires expertise to perform properly (e.g., immersing at least two thirds of pancreas into PFC, removing fat surrounding pancreas before storage).

Even though the number is small, introduction of ductal injection and procurement by the islet team significantly improved islet yield; and introduction of the KIIM further improved islet yields. One of the major advantages of the KIIM is the density adjusted continuous density gradient with Kyoto solution plus iodixanol (10). This method enabled us to maximize the recovery rate after purification; and this study showed the significant improvement in recovery rate after purification. Therefore, the KIIM seems beneficial for islet isolation from brain-dead donors. The fact that transplanted islets from the DI group resulted in excellent glycemic control and insulin independence by the recipient further supported this concept. In order to confirm the benefit of the KIIM for isolating islets from brain-dead donors, further research is necessary to increase the case number. This is our current ongoing research.

In this study, we reevaluated pancreas donors in the Texas area with the Japanese criteria. Previously, we demonstrated that islet isolation by KIIM using NHBDs that had elevated blood creatinine levels and/or transaminase levels, or who had experienced cardiac arrest events, which are current contraindications in the US for donor eligibility, did not have a significant impact on the isolation results (8). Therefore, those factors could be eliminated from the list of contraindications and, in fact, they are not contraindications under the Japanese criteria. Under our current criteria in the US, ages of less than 25 years old are contraindication for islet donors. However, it was recently shown that younger donors could provide high-quality islets even though isolation of islets from young donors is difficult (5). In fact, we isolated islets with the KIIM from a 14-year-old donor pancreas. From this isolation, we obtained approximately 500,000 IE islets that resulted in successful islet transplantation. Therefore, we propose that donor ages of less than 25 years should not be a contraindication for islet donors in the US. The Japanese criteria do not impose such a limitation.

We showed that more than 40% of the unused pancreata were actually suitable for islet isolation under the Japanese criteria with the KIIM. We conclude that with the KIIM and the Japanese criteria, more than 2,500 additional donor pancreata might be used for islet isolations annually in the US.

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SUITO Index for Evaluation of Efficacy of Single Donor Islet Transplantation

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Evaluation of engrafted islets mass is important for clinical care of patients after islet transplantation. Recently, we developed the secretory unit of islet transplant objects (SUITO) index, which reflected engrafted islet mass. In this study, we evaluated the SUITO index for the prediction of clinical outcome after single islet transplantation. Single islet transplantations were performed into six type 1 diabetic patients. Isolated islets were quantitatively assessed at the time of transplantation. The SUITO index was calculated as follows: fasting C-peptide (ng/dl)/[fasting blood glucose (mg/dl) – 63] × 1500. Islet yield/recipient's body weight and SUITO index were evaluated, along with HbA_{1C}, relative insulin dose (insulin dose posttransplant/pretransplant), and M-values. HbA_{1C} improved in all cases, irrespective of the SUITO index score or islet yield/body weight. The average SUITO index from postoperative days 3 to 30 ($R^2 = 0.728$, $p < 0.04$), but not islet yield/body weight ($R^2 = 0.259$, $p = 0.303$), correlated with relative insulin dose. The daily SUITO index strongly correlated with the daily relative insulin dose ($R^2 = 0.558$, $p < 0.0001$) and weakly correlated with the daily M-values ($R^2 = 0.207$, $p < 0.02$). A SUITO index score of less than 10 was associated with increasing insulin dose even after islet transplantation. The SUITO index seems to be a better predictor of success of islet transplantations than islet yield/body weight. SUITO index is recommended to assess clinical outcome of islet transplantation.

Key words: SUITO index; Islet transplantation; Single donor; M-value

INTRODUCTION

Pancreatic islet transplantation is a promising treatment for type 1 diabetes (5,15). However, poor long-term insulin independence is currently one of the issues for islet transplantation. After 5 years of islet transplantation less than 10% of patients could maintain insulin independence but more than 70% patients maintained islet function (14,16). The patients with functioning islets could maintain excellent glycemia and, importantly, those patients could have substantial improvement of hypoglycemic episodes, even though insulin injection is necessary. Therefore, the current goal of islet transplantation has shifted from insulin independence to maintaining excellent glycemic control without hypoglycemic unawareness (16).

Recently, we demonstrated that single donor islet transplantation significantly improved glycemic control and reduced the basal insulin requirement (12). Those patients also had substantial improvement in their hypoglycemic episodes (3). In some countries, like Japan, organ donors for islets are extremely low and donor shortage is a serious issue. Therefore, we consider single donor islet transplantation to be an option for the treatment of type 1 diabetes with hypoglycemic unawareness.

Evaluation of the efficacy of islet transplantation is important to follow up the clinical course. Recently, we and others demonstrated that the ratio between fasting C-peptide levels and glucose levels correlated with insulin requirement after islet transplantation (1,4,10,17). We developed a secretory unit of islet transplant objects (SUITO) index, which reflects engrafted islet mass compared to β-cell function in a normal healthy person (4,10). The formula of the SUITO index is as follows: fasting C-peptide (ng/dl)/[fasting blood glucose (mg/dl) –

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63] × 1500. A SUITO index of 100 reflects 100% pancreatic β-cell function in a healthy person. For example, if the fasting C-peptide level is 0.8 ng/dl and blood glucose is 103, the SUITO index will be 0.8/(103 – 63) × 1500 = 30. The average SUITO index after islet transplantation from postoperative days 3 to 30 was shown to be correlated with insulin reduction (10).

In this study, we examined whether the SUITO index reflected the clinical outcome after single islet transplantation.

MATERIALS AND METHODS

Pancreas Procurement, Islet Isolation, and Transplantation

Single islet transplantations were performed between March, 2005 and March, 2007 at Baylor University Medical Center (Dallas, TX, USA). The procurement and allocation process of donated pancreata is governed by the United Network for Organ Sharing (UNOS) guidelines and managed locally by the Southwest Transplant Alliance or LifeGift, local organ procurement organizations. In four cases, pancreata were shipped to a remote center and islets were isolated at the remote center (2). In two cases, islets were isolated at our center (Baylor Institute for Immunology Research). In all cases, pancreases were preserved oxygen charged static two-layer method (9) and islets were isolated according to the Edmonton protocol (8,15). Islet yield was determined with dithizone staining ((2 mg/ml; Sigma Chemical Co., St. Louis, MO, USA) under optical graticule and converted into a standard number of islet equivalents (IE, diameter standardizing to 150 μm) (8,11). At least 4,000 IE/kg body weight islets were transplanted into type 1 diabetic patients. Patients were sedated and a percutaneous transhepatic approach was used to gain access to the portal vein for all patients. Once access was confirmed, the Seldinger technique was used to place the Kumpe catheter within the main portal vein. Islets were infused by gravity using the bag technique.

Immunosuppression consisted of maintenance with tacrolimus (Prograf®, Fujisawa, Japan), at a target trough level of 4–6 ng/ml and sirolimus (Rapamune®, Wyeth Pharmaceuticals, Inc., Madison, NJ, USA), at a target trough level of 12–15 ng/ml (15).

Assessment of Islet Transplantation Efficacy

In this study, we assessed islet transplantation efficacy using the SUITO index or islet yields per body weight after a single infusion of islets from brain-dead donors into six type 1 diabetic patients. Transplantation efficacy was evaluated by HbA_{1C}, relative insulin dose, and M-values. We avoided using the SUITO index from postoperative days (POD) 0 to 2, because broken islets release high levels of C-peptide during the first 24 h after transplantation (4,10).

The values of HbA_{1C} pretransplantation and 3 months after islet transplantation were compared.

Relative insulin dose was calculated as follows: daily insulin dose/insulin dose immediately prior to islet transplantation. When insulin independence is achieved, the relative insulin dose is 0% and when the insulin dose is the same as just before transplantation, the relative insulin dose is 100%.

M-values were calculated using blood glucose levels from six time points (before and after breakfast, before and after lunch, before and after dinner). M-values were calculated as follows: M-value = average of six measurements of absolute value of $\log_{10}$[blood glucose (mg/dl)/100]^3 (13).

We analyzed the relationships of HbA_{1C} with the average SUITO index (from POD 3 to 30) and the islet equivalent per body weight. We also analyzed the relationships of the relative insulin dose with the average SUITO index (from POD 3 to 30) and islet equivalent per body weight. The relationships of the average (from POD 3 to 30) M-value with the average SUITO index (from POD 3 to 30) and islet equivalent per body weight were also analyzed.

Then we analyzed the daily SUITO index, the daily relative insulin dose, and daily M value to examine whether the daily SUITO index is useful to predict clinical outcome. For this purpose, we plotted all daily SUITO index measurements against relative insulin dose and M-values of the six recipients.

Statistical Analysis

Values were expressed as mean ± SE. Correlations between two factors were analyzed by simple regression tests. Statistical analyses were performed with Stat View 4.0. A value of $p < 0.05$ was considered significant.

RESULTS

Recipient and Clinical Characteristics

Recipient characteristics are shown in Table 1. Islets were isolated at a remote center for the initial four cases and switched to a local center for the last two cases. Islets from case #5 were transplanted without culture and for the other cases islets were transplanted after culture. All isolated islets were qualified for transplantation based on the Edmonton protocol (15).

Islet yield per body weight, average SUITO index, and clinical characteristics are shown in Table 2. Ranges of islet yield per body weight were from 4,063 to 12,241 IE/kg. The averages (POD 3 to 30) of the SUITO index were from 6.1 to 24.6. The ranges of relative insulin dose were from 22.2% to 92.2%; therefore, even the

Table 1. Recipient Characteristics

	Gender	Age (Years)	Body Weight (kg)	BMI (kg/m^2)	Islet Yield (IE)	Isolation Center
1	F	28	63	25.6	293,796	remote
2	M	46	91	28.7	372,561	remote
3	F	28	64	23.6	482,507	remote
4	M	48	75	21.2	473,610	remote
5	F	58	57	24.2	697,763	local
6	F	55	70	25.6	342,216	local

BMI, body mass index.

most effective case still required 22.2% of pretransplant amount of insulin.

HbA_{1C} data showed that all cases improved glycemic control irrespective of SUITO index or islet yield/body weight (Table 2).

Comparison of Islet Yield/Body Weight and Average SUITO Index for Prediction of Clinical Outcome

The relationship between islet yield/body weight and relative insulin dose is shown in Fig. 1, left panel. There was no significant correlation between islet yield per body weight and relative insulin dose. The relationship between the average (POD 3 to 30) SUITO index and relative insulin dose is shown in Figure 1, right panel. There was significant correlation ($p = 0.031$) between average SUITO index and relative insulin dose.

The relationship between islet yield/body weight and the average M-values is shown in Figure 2, left panel. There was no significant correlation between islet yield per body weight and the average M-values. The relationship between the average (POD 3 to 30) SUITO index and the M-values is shown in Figure 2, right panel. There was no significant correlation between the average SUITO index and the average M-values.

Daily SUITO Index Correlated With Daily Insulin Dose and M-Value

Then we examined the relationship between the individual SUITO index versus daily relative insulin dose and daily M-values. The daily SUITO index strongly correlated with the daily relative insulin doses (Fig. 3, left) and weakly but significantly correlated with daily M-values.

When the SUITO index was less than 10, the average relative insulin dose was 117.1 ± 5.9% and when the SUITO index was equal or more than 10 the average relative insulin dose was 55.4 ± 8.4% (Table 3). The relative insulin dose was substantially lower when the SUITO index was equal to or more than 10 ($p < 0.0000001$).

When the SUITO index was less than 10, the average M-value was 18.3 ± 3.8 and when the SUITO index was equal to or more than 10 the average relative insulin dose was 11.5 ± 3.0 (Table 3). There was no significant difference in the average M-values between the group with a SUITO index less than 10 and the group whose index was equal to or more than 10 ($p = 0.21$).

DISCUSSION

Monitoring of transplanted islet mass and function is important to evaluate clinical outcome. Previously, we have shown that single donor islet transplantation from non-heart-beating donors could improve glycemic control without hypoglycemic unawareness (12). The concept of single donor islet transplantation is important for a country that has a limited number of donor pancreata, like Japan (6,7). We evaluated islet transplantation with non-heart-beating donors and living donors using the

Table 2. Characteristics of Transplant

	IE/kg Body Weight	Average SUITO Index	Relative Insulin Dose (%)	Pre-Tx HbA_{1c}	Post-Tx HbA_{1c}
1	4,663	11.1 ± 2.0	49.1	9.7	5.2
2	4,094	6.1 ± 0.9	92.2	9.9	5.8
3	7,539	7.7 ± 1.6	89.5	8.6	6.4
4	6,314	17.2 ± 3.1	37.5	7.2	6.0
5	12,241	24.6 ± 8.9	22.2	8.3	7.0
6	4,889	8.9 ± 0.6	48.3	7.4	5.0

IE, islet yield; SUITO, secretory unit of islet transplant objects; HbA_{1c}, glycosylated hemoglobin.