Variables associated with islet yield in autologous islet cell transplantation for chronic pancreatitis

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The goal of total pancreatectomy followed by autologous islet cell transplantation is to manage pain and prevent surgical diabetes for patients with severe chronic pancreatitis. We performed this procedure in 17 patients from November 2006 to October 2009 at Baylor University Medical Center. All patients were included in this retrospective study and were divided into two groups based on islet yield in the final product based on patient body weight: a low-yield group (<5000 IE/kg) and a high-yield group (≥5000 IE/kg). There were significant differences between the two groups in the rate of pancreatic findings on computed tomography (low vs high group, 88% vs 22%: $P = 0.02$), Cambridge classification score for endoscopic retrograde cholangiopancreatography (3.8 ± 0.2 vs 2.1 ± 0.6: $P = 0.03$), number of positive endoscopic ultrasonography criteria (6.0 ± 0.8 vs 3.5 ± 0.4: $P = 0.04$), and distension score (1.9 ± 0.4 vs 3.7 ± 0.2: $P = 0.006$). A significant reduction in narcotics use after the operation was observed in both groups ($P = 0.03$ and $P = 0.009$ in the low and high groups, respectively, using a paired *t* test). Excellent graft function and glycemic control after the transplantation were also demonstrated in both groups. Patients in the high-yield group were in the early stage of chronic pancreatitis, which led to excellent pancreatic distention for islet isolation; however, the excellent clinical outcomes were observed in both low- and high-yield groups.

Chronic pancreatitis (CP) is a progressive inflammatory disease that destroys not only pancreatic acini but also islets in its late stage (1, 2). Episodes of severe abdominal pain are usually present in the natural course of CP, where both exocrine and endocrine function is also lost. Efforts such as decreasing smoking and alcohol use, taking oral pancreatic-enzyme supplements, and receiving endoscopic therapies such as sphincterotomy and stent placement are usually effective in managing pain and inhibiting disease progression; however, some patients have refractory or recurrent disease. Surgical options for CP treatment include drainage procedures such as the Puestow procedure and resections such as pancreaticoduodenectomy, distal pancreatectomy, or total pancreatectomy. These are effective in reducing severe abdominal pain but may not maintain endocrine function (3, 4).

Total or near total pancreatectomy (TP) followed by autologous islet cell transplantation (AIT) was developed for both pain management and maintenance of pancreatic endocrine function, especially glycemic control (5–7). A few institutes in the world have performed TP with AIT, since AIT requires special techniques for islet cell processing, and the effectiveness of this procedure has been reported (5).

We started allogeneic islet cell transplantation for patients with type 1 diabetes mellitus in 2005 (8, 9) and initiated AIT for CP in November 2006 at Baylor University Medical Center. Novel methods for pancreas procurement and preservation, which were originally developed for the processing of pancreatic islets from non–heart-beating donors in Japan (10, 11), were introduced in December 2007 to maximize the outcome of islet isolation. This retrospective study of our experience with TP with AIT aimed to investigate variables associated with increased islet yield.

METHODS

Patients

All 17 patients who received TP with AIT at Baylor University Medical Center at Dallas were included in this study: 2 patients had the procedure in 2006, 2 in 2007, 3 in 2008, and 10 in 2009. The patients were diagnosed by medical history, laboratory tests, and clinical image studies including endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic ultrasonography (EUS). This retrospective analysis was approved by the institutional review board, and written informed consent was obtained from all patients.

Pancreas preservation

In cases from 2006 to November 2007, the removed organs were placed in University of Wisconsin preservation solution following pancreatectomy. From December 2007, the ET-Kyoto solution (Otsuka Pharmaceutical Factory Inc., Naruto, Japan)

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was injected through a cannula inserted into the main pancreatic duct as previously described (8–12), and the pancreas was preserved using the oxygen-charged static two-layer method (ET-Kyoto solution/oxygenated perfluorocarbon) (13).

Islet isolation and assessment

Islets were isolated by the modified Ricordi method (10, 14). Liberase HI (Roche, Indianapolis, IN) or Collagenase NB with neutral proteases (SERVA Electrophoresis GMbH, Heidelberg, Germany) was infused into the main pancreatic duct (15). Pancreas distension was evaluated according to the following scores: excellent, 4; very good, 3; average, 2; and poor, 1. If pellet volume was larger than approximately 15 mL, islets were purified with the COBE 2991 cell processor (CaridianBCT, Inc., Lakewood, CO) with a continuous density gradient (9). The final preparation of islets was assessed by using dithizone staining (Sigma Chemical Co., St. Louis, MO) (2 mg/mL) for islet yield and purity. The islet yield was converted into a standard number of islet equivalents (IE, diameter standardizing to 150 μm) (16). Islet viability after purification was evaluated with fluorescein diacetate (10 μmol/L) and propidium iodide (15 μmol/L) staining (17). The average viability of 50 islets was calculated.

Transplantation

Isolated islets were infused into the portal vein via a mesenteric vein with heparin (70 U/kg body weight) over 30 to 60 minutes while the patient was under general anesthesia. During islet infusion, portal vein pressure (PVP) was monitored intermittently. If the PVP was >22 mm Hg, the infusion of islets was stopped and then restarted when the PVP decreased.

Imaging studies before transplantation

Pretransplant imaging studies, including transabdominal ultrasonography, abdominal computed tomography (CT), and ERCP and EUS were reviewed. ERCP images were classified according to the Cambridge classification, from normal (scored as 0) to marked (scored as 4) (18, 19). Previous reports showed that EUS is helpful in evaluating the diagnosis and severity of CP (20–23). EUS criteria included hyperechoic foci, hyperechoic strands, parenchymal lobularity, irregular main pancreatic duct margins, hyperechoic main pancreatic duct margins, visible side branch budding, main pancreatic duct dilatation, shadowing calcifications, and cysts; the presence of four or more criteria was used for diagnosis of CP (24, 25).

Assessment of transplanted islet function

After transplantation, transplanted islet function was assessed by C-peptide and the secretory unit of islet transplant objects (SUITO) index, which was shown to be a good clinical parameter for engrafted islet function in previous reports (26, 27). When the C-peptide value was below the detection level of the assay (0.1 ng/mL), the islet function was defined as "no function." When a patient achieved insulin independence, the islet function was defined as "full function." When the above two conditions were not entered, the islet function was defined as "partial function."

Pain assessment

Pain was scored according to the visual analogue scale from 0 (no pain) to 10 (severe pain). Information on opioid doses for pain control was also collected from the different prescriptions and converted to morphine-equivalent doses according to published data (28).

Statistical analysis

Data analyses were performed using SPSS 15.0 for Windows (SPSS, Chicago, IL). Patients were divided into two groups based on islet yield in the final product per patient body weight (IE/kg): a low-yield group (<5000) and a high-yield group (≥5000). The differences of means were tested by unpaired *t* test, and the Mann-Whitney U test was used for nonparametric data, including pain score, Cambridge classification score for ERCP, number of positive EUS criteria, and distension score. Categorical data were compared using Fisher's exact test.

Multiple linear regression analysis was conducted to identify the factors that independently predict islet yield in the final product per patient body weight (IE/kg), using a stepwise selection method with an entry level of 0.05 and an exit level of 0.10. The covariates considered included the following pretransplant factors: age at transplant, sex, body mass index (BMI), body surface area (BSA), duration of symptoms, history of alcohol abuse, history of smoking, fasting blood glucose level, cause of pancreatitis, history of pancreatic operations, pain score, equianalgesic dose of narcotics, pancreatic findings on transabdominal ultrasound or CT, the number of EUS criteria, the Cambridge classification score for ERCP, the length of cold ischemia time, use of ductal injection or the two-layer method, distension score, the type of collagenase used, and implementation of purification. For this analysis, categorical data were transformed to binary values. A P value <0.05 was considered statistically significant. Values are shown as mean ± standard error.

RESULTS

Patient characteristics

Patient characteristics before transplantation are shown in *Table 1.* All patients had regular prescriptions of narcotics and prior endoscopic interventions including sphincterotomy and pancreatic duct stent placements. There were no significant differences in pain score and dose of narcotics between the two groups, but the low-yield group had a significantly higher rate of pancreatic findings at CT (P = 0.02), Cambridge classification score for ERCP (P = 0.03), and number of positive EUS criteria (P = 0.04). Marginally significant differences were found in sex, body weight, BSA, cause of pancreatitis, and previous history of pancreatic operations. Only one patient received insulin therapy for diabetes, and there was no significant difference in fasting blood glucose level between the two groups.

Table 1. Patient characteristics before total pancreatectomy with autologous islet cell transplantation

Variable	Low-yield group (n = 8)	High-yield group (n = 9)	*P* value
Female sex (n)	8	5	0.08
Mean age (years)	38.8 ± 4.9	41.2 ± 3.2	0.67
Body weight (kg)	64.7 ± 5.9	77.9 ± 4.5	0.09
Body mass index (m^2/kg)	24.7 ± 2.5	27.4 ± 1.1	0.36
Body surface area (m^2)	1.7 ± 0.1	1.9 ± 0.1	0.07
Cause of pancreatitis			0.06
Idiopathic (n)	2	7	
Other (n)	6	2	
Duration of symptoms (years)	7.4 ± 1.7	6.6 ± 1.0	0.71
Previous pancreatic operations (n)	3*	0	0.08
Pain score	7.8 ± 0.9	7.8 ± 0.6	0.88
Morphine equivalent requirements (mg/day)	323 ± 89	267 ± 67	0.62
Pain pattern			0.58
Constant (n)	6	8	
Intermittent (n)	2	1	
Current smoker (n)	1	4	0.29
History of alcohol abuse (n)	1	1	1.0
Fasting blood glucose (mmol/L)	5.6 ± 0.2	5.6 ± 0.7	0.99
History of diabetes treatment (n)	0	1	1.0
Image studies before islet transplantation			
Pancreatic findings of TA-US (n)	2	1	0.58
Pancreatic findings of CT (n)	7	2	0.02
Cambridge classification for ERCP	3.8 ± 0.2	2.1 ± 0.6	0.03
Number of positive EUS criteria	6.0 ± 0.8	3.5 ± 0.4	0.04

*Previous operations included Whipple resection, gastrostomy, and Puestow procedure.

TA-US indicates transabdominal ultrasonography; CT, computed tomography; ERCP, endoscopic retrograde cholangiopancreatography; EUS, endoscopic ultrasonography.

Table 2. Outcome of islet isolation

Variable	Low-yield group (n = 8)	High-yield group (n = 9)	*P* value
Pancreas weight (g)	80.7 ± 10.9	93.7 ± 5.9	0.30
Cold ischemia time (minutes)	41.1 ± 5.5	39.9 ± 5.2	0.87
Pancreas preservation			
Ductal injection with ET-Kyoto solution (n)	5	9	0.08
Two-layer method (n)	5	8	0.29
Distension score	1.9 ± 0.4	3.7 ± 0.2	0.006
Digestion time (minutes)	18.5 ± 2.4	13.6 ± 1.8	0.12
Collagenase			0.64
Liberase (n)	5	4	
Collagenase NB (n)	3	5	
Digested pancreas weight (%)	76.9 ± 5.9	85.1 ± 4.9	0.29
Dilution time (minutes)	44.6 ± 4.2	46.6 ± 3.9	0.73
Postdigestion			
Islet yield (10^3 IE)	168 ± 24	628 ± 36	<0.001
Islet yield/pancreas weight (IE/g)	2400 ± 466	6864 ± 522	<0.001
Islet yield/islet number	50.6 ± 7.2	55.6 ± 5.9	0.60
Tissue volume (mL)	6.4 ± 1.5	29.6 ± 4.7	0.001
Purification (n)	0	6	0.007
Final product			
Islet yield (10^3 IE)	168 ± 24	573 ± 37	<0.001
Islet yield/patient body weight (IE/kg)	2717 ± 406	7556 ± 683	<0.001
Islet yield/pancreas weight (IE/g)	2400 ± 466	6243 ± 503	<0.001
Islet particle number (10^3)	102 ± 13	297 ± 34	<0.001
Islet yield/islet number	1.6 ± 0.1	2.1 ± 0.2	0.12
Purity (%)	33.6 ± 13.4	28.0 ± 4.9	0.70
Viability (%)	96.8 ± 0.8	97.4 ± 0.5	0.52
Product volume (mL)	5.3 ± 1.2	15.1 ± 0.8	<0.001
Product volume (mL)/pancreas weight (g)	0.06 ± 0.01	0.16 ± 0.01	<0.001

Outcome of islet isolation

There were no significant differences between the low-yield group and high-yield group in pancreas weight, cold ischemia time, or type of collagenase used. However, the low-yield group had significantly lower scores of pancreas distension than the high-yield group ($P = 0.006$, *Table 2).* Ductal injection with ET-Kyoto solution was used more frequently in the high yield group, even though the difference did not achieve statistical significance. After digestion, significant differences were found in islet yield ($P < 0.001$), islet yield per gram of pancreas ($P < 0.001$), and tissue volume ($P = 0.001$). No purification was performed in the low-yield group, and the rate of purification was significantly different between the two groups ($P = 0.007$). The final product also had significant differences in islet yield ($P < 0.001$), islet yield per gram of pancreas ($P < 0.001$), and product volume ($P < 0.001$). On multiple linear regression analysis, independent predictors of islet yield in the final product per patient body weight (IE/kg) were distension score

Table 3. Multiple regression analysis

Variable	B coefficient	B	*P* value
Distension score	1452	0.621	0.002
Cambridge classification	–891	–0.364	0.037
Constant	3705		0.067

The model has $r = 0.848$, adjusted $r^2 = 0.679$, $F = 5.302$, $P = 0.037$.

Table 4. Portal vein pressure during islet infusion

	Portal vein pressure (mm Hg)		
Period	Low-yield group (n = 8)	High-yield group (n = 9)	*P* value
Start	10.9 ± 1.1	8.1 ± 1.6	0.18
Maximum	13.9 ± 1.5	20.6 ± 1.4	0.005
End	12.7 ± 1.6	18.6 ± 1.1	0.007
Change	3.0 ± 1.2	12.4 ± 1.6	<0.001

Table 5. Clinical outcome

Variable	Low-yield group (n = 8)	High-yield group (n = 9)	*P* value
Follow-up period (months)	5.6 ± 2.5	8.9 ± 2.6	0.38
Endocrine function			
Islet graft function			1.0
Full function (n)	4	4	
Partial function (n)	4	5	
No function (n)	0	0	
SUITO index	39.2 ± 11.0	40.5 ± 12.4	0.94
Peak C-peptide (ng/mL)	1.1 ± 0.3	1.6 ± 0.4	0.27
Hemoglobin A_{1c} (%)	6.5 ± 0.7	6.8 ± 0.4	0.61
Pain management			
Regular use of narcotics at postoperative period (n)	6	5	0.62
Postoperative morphine equivalent dose (mg/day)	115 ± 39	41 ± 24	0.12

SUITO indicates secretory unit of islet transplant objects.

($P = 0.002$) and Cambridge classification score for ERCP ($P = 0.037$) *(Table 3).*

Portal vein pressure during islet infusion

All islet preparations were safely transplanted into the liver through the portal vein except for one case where 602,709 IE were infused into the liver and 117,067 IE into the intraperitoneal cavity. There was no significant difference in PVP at the start of islet infusion, but maximum PVP, PVP at the end of the procedure, and change of PVP were significantly higher in the high-yield group (*Table 4; P* = 0.005, 0.007, and <0.001, respectively). All patients received transabdominal ultrasonography and had no findings of portal thrombosis, except for one patient whose suspected thrombosis was found not to be present on angiography. Therefore, there was no portal thrombosis in this study.

Clinical outcomes

There were no significant differences in graft function and glycemic control between the two groups *(Table 5).* Pain management also had no significant differences, and a significant reduction of narcotics dose was observed in both groups: $P = 0.03$ in the low-yield group and $P = 0.009$ in the high-yield group using paired t test. No patient required a higher dose of narcotics postoperatively compared with preoperatively.

DISCUSSION

The objectives of TP with AIT are to improve pain management and to prevent surgical, brittle diabetes due to TP (5–7). All of our patients successfully reduced their narcotics doses after the procedure in this study, and overall 35% of patients stopped regular use of narcotics after the operation. Further reductions of narcotics are expected, since it often takes a long time to reduce narcotics requirements in patients who use the drugs regularly (29). On the other hand, all patients showed islet graft function and good glycemic control after transplantation (Table 5). Sutherland et al reported that islet function correlated with islet yield, and only 27% of the transplanted patients achieved insulin independence 1 year after the procedure when islet yield was <5000 IE/kg patient weight (30). Half of the patients who received <5000 IE/kg of isolated islets in our institute achieved insulin independence, although the follow-up period was short. At the same time, half of the high-yield group did not achieve insulin independence in our cohort. However, both the low-yield and high-yield group had high SUITO indices. In allogenic islet transplantation, a SUITO index >26.0 is an excellent predictor of insulin independence (27). Therefore, it is reasonable to predict that most patients who have a high SUITO index after AIT will become insulin independent. On the other hand, islet mass inevitably decreases after this procedure; therefore, blood glucose levels also inevitably increase until transplanted islets compensate their function. Until transplanted islets have full function, we prefer to use insulin injection to protect islets from glucotoxicity. We expect that the majority of patients will return to insulin independence when transplanted islets have full function.

This study revealed that islet yield per patient body weight was associated with the Cambridge classification score for ERCP, which suggests that progression of inflammation would worsen the outcome of islet isolation. Similar findings were observed in the rate of pancreatic findings by CT and the number of positive EUS criteria, where patients in the low-yield group had significantly higher values than those in the high-yield group (Table 1). In addition, islet yield per patient body weight was associated with distension score. We postulated that progression of inflammation makes the pancreas fibrotic, and that should be the reason for poor distension. The University of Minnesota

group also reported that fibrosis and acinar atrophy inversely correlated with islet yield in pediatric patients with CP (31). The timing for TP with AIT for patients with CP is quite important for better outcomes in islet isolation after transplantation.

Findings similar to those previously reported with allogeneic islet cell transplantation (32–36) were observed in our experience of AIT. The averages of body weight, BMI, and BSA in the high-yield group were higher than those in the low-yield group, although the difference was not statistically significant (Table 4). The islet isolations for allogeneic islet cell transplantation had a higher islet yield when cadaveric human donors had a higher body weight, BMI, or BSA (32–36).

This study also showed that the high-yield group had a significantly higher PVP at maximum, end of procedure, and change during islet infusion than the low-yield group, although no patient had a severe adverse event (Table 3). This phenomenon was already reported in the setting of allogeneic islet cell transplantation as well as AIT (37). Sufficient time to transplant islets with careful monitoring of PVP and use of heparin might be effective to prevent complications of islet infusion.

We have recently implemented into AIT pancreas preservation and islet isolation methods that were originally developed for pancreata from non–heart-beating donors in Japan (8, 10–12). This study has shown the effectiveness of ductal injection with ET-Kyoto solution, as the rate of using this pancreas preservation method was higher in the high-yield group with a marginally significant level, as well as in the setting of pancreata from cadaveric donors (8–12, 38, 39) (Table 2). We postulated that ductal injection using cold ET-Kyoto solution could immediately chill down the resected pancreas, which could minimize warm ischemic injury. In addition, trehalose, which is a major ingredient of ET-Kyoto solution, might have a cytoprotective effect. The benefit of the two-layer method was not apparent. This might be due to the short preservation period.

In summary, excellent clinical outcomes after TP with AIT were observed in our cohort. Clinical imaging findings, which include CT, EUS, and ERCP, were associated with outcomes of islet isolation. The patients will continue to be evaluated, since long-term clinical benefits are expected.

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