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Long-term outcome of islet transplantation on insulin-dependent diabetes mellitus: An observational cohort study

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ABSTRACT

Aims/Introduction: To investigate the long-term efficacy and safety of islet transplantation (ITx) compared with multiple daily injections (MDI) or continuous subcutaneous insulin infusion (CSII).

Materials and Methods: Among 619 patients diagnosed as insulin-dependent diabetes mellitus or type 1 diabetes at Kyoto University, Kyoto, Japan, seven patients were selected as the ITx group and 26 age-matched patients with no endogenous insulin secretion were selected as the MDI/CSII group. Hemoglobin A1c, aspartate aminotransferase/alanine aminotransferase (AST/ALT) and creatinine were assessed retrospectively at 1, 2, 5 and 10 years for both groups; serum C-peptide immunoreactivity was assessed for the ITx group. Major clinical events were also assessed.

Results: Hemoglobin A1c improvement in ITx was significant at 1 year (8.4% [7.8–9.9%] at baseline to 7.1% [6.3–7.4%] in ITx vs 8.2% [7.4–9.8%] at baseline to 8.1% [7.3–9.5%] in MDI/CSII, $P < 0.01$ between groups), and was maintained at 2 years (7.4% [6.3–8.2%] vs 8.4% [7.4–9.6%], $P = 0.11$). The increase of stimulated C-peptide immunoreactivity was significant at 1 year (0.57 ng/mL [0.26–0.99 ng/mL], $P < 0.05$ from baseline) and 2 years (0.43 ng/mL [0.19–0.67 ng/mL], $P < 0.05$), although it became insignificant thereafter. There was no significant difference in AST/ALT or creatinine at 10 years, although a transient AST/ALT elevation was observed in ITx. In regard to clinical events, the occurrence of severe hypoglycemia was 14% vs 31% (relative risk 0.46, $P = 0.64$), that of infectious disease was 43% vs 12% (relative risk 3.71, $P = 0.09$) and digestive symptoms was 43% vs 7.7% (relative risk 5.57, $P = 0.05$) in ITx vs MDI/CSII, respectively. No patient died in either group.

Conclusions: The present findings showed that ITx was considered to contribute to the reduction of hypoglycemia and better glycemic control with tolerable, but attention-requiring, risks over a period of 10 years compared with MDI/CSII.

INTRODUCTION

For decades after the discovery of insulin, the global standard therapy for patients with insulin-dependent diabetes mellitus (IDDM) was multiple daily injections of insulin (MDI) or continuous subcutaneous insulin infusion (CSII). However, due to the depletion of their endogenous insulin secretion and the limitation of mimicking endogenous glucose-stimulated insulin secretion by exogenous glucose-independent insulin complement, IDDM patients often suffer from "brittle" glycemic change and severe hypoglycemia, which requires other people's help, with inappropriately excess exogenous insulin infusion. Therefore, physicians have difficulty in attaining well-controlled glycemic change and avoiding the risk of hypoglycemia. In light of this background, transplantation therapies, such as pancreas transplantation and pancreatic islet transplantation (ITx), were launched. The goal of transplantation therapy is to restore

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endogenous and physiological insulin secretion by grafts, and thereby control unstable glycemic changes, and reduce severe hypoglycemia and the progression of diabetic complications.

The first ITx was carried out in 1974 as a simpler and less invasive procedure than pancreas transplantation[1]. In regard to the safety of the procedure, pancreas transplantation requiring abdominal surgery with vascular anastomosis and bladder or enteric drainage was more invasive, and resulted in a high rehospitalization rate due to transplantation complications[2], and a relatively high mortality rate mainly due to infections, bleeding and cardiovascular events[3–5] compared with ITx, which requires merely a small incision and has almost no procedure-related mortality[6]. However, regarding the graft outcomes so far, stronger results have been achieved by pancreas transplantation[2–4]. In regard to improvement of the graft outcomes of ITx, a clinically useful method of automated islet isolation was presented in 1988[7,8], and the immunosuppression protocol had also been improved[9]. In 1992, the Pittsburgh group showed that tacrolimus (calcineurin inhibitor) without a glucocorticoid regimen had better outcomes than that with glucocorticoid[10]. In 2000, an epoch-making glucocorticoid-free immunosuppressive protocol called the Edmonton Protocol by the University of Alberta using oral sirolimus (mammalian target of rapamycin inhibitor) and tacrolimus for loading and maintaining, and intermittent intravenous daclizumab (anti-CD25 antibody) succeeded in insulin independence of seven patients after a median of 11.9 months' follow-up (range 4.4–14.9 months)[11]. A following multicenter study also showed 82% of 118 patients among the Universities of Alberta, Minnesota and Miami became insulin independent at 1 year using this protocol[12].

Regarding middle-term outcomes of ITx using the Edmonton protocol, however, the Alberta group showed the rate of insulin independence declined to 8.5% at 5 years after transplantation, although 82% patients maintained a detectable C-peptide level that was thought to contribute to avoidance of severe hypoglycemic attack[13]. There were also some reports, such as a case series study about 5-year outcome[14], a comparative study about the 4-year outcome between ITx after kidney transplantation and ITx alone[15], and a case series study for 12 years[16]. The last study followed seven patients for 12 years, and it showed that no patient experienced severe hypoglycemia, opportunistic infection, or lymphoma, although only one of seven patients could maintain completely medication-free status[16]. As for comparative study of the outcome of IDDM patients between ITx and MDI/CSII treatment, there were a few reports for 12 months[17] or 7 years[18].

Thus, in regard to the long-term efficacy and safety of ITx over a period of 10 years compared with that of MDI/CSII, further supportive studies are required to confirm the evidence. The aim of the present study was to investigate the long-term outcome of ITx for 10 years compared with conventional MDI/CSII treatment.

METHODS

Study design

This was a single-center, observational cohort study carried out in Kyoto University Hospital, Kyoto, Japan. We collected data from medical records of our hospital.

First, we selected 619 patients diagnosed as type 1 diabetes mellitus and/or IDDM in our hospital before 2004. Among them, nine patients received first ITx from 2004 to 2005. Two patients moved and continued their follow up at another hospital. We could continuously follow up seven patients as the ITx group at our hospital. Then, 271 age-matched patients were selected from 610 patients aged within the 5–95 percentile range of the seven patients with ITx. Finally, among the 271 patients, 26 patients were eligible for the control MDI/CSII group, as their stimulated serum C-peptide immunoreactivity (CPR) by glucagon-stimulating test was under the detection level before 2004 and their baseline creatinine level was <1.5 mg/dL, and they were under continuous follow up at our hospital.

Islet transplantation

Six patients received 14 ITx in total from donors after cardiac death, and one patient received ITx once from a living donor. The procedures of preservation and transportation of donor pancreas, isolation and purification of islets, and transplantation to recipients were described in a previous report[14]. In regard to the immunosuppression regimen following the modified Edmonton Protocol for ITx alone patients, 20 mg basiliximab (anti-CD25 monoclonal antibody) was administered 2 h before and 4 days after transplantation. Sirolimus was administered orally once daily with a target trough level of 12–15 ng/mL during 3 months after transplantation, and 7–12 ng/mL thereafter. Tacrolimus was administered orally twice daily, with a target trough level of 3–6 ng/mL. If side-effects associated with sirolimus emerged, 1–1.5 g/day mycophenolate mofetil (MMF, antimetabolite) was used alternatively[19]. For ITx after kidney transplantation recipients who had already been administered an immunosuppressant, immunosuppression of kidney transplantation was continued with transient basiliximab use for the induction at ITx[19].

Clinical assessment

We evaluated the number of patients who had severe hypoglycemia or diabetic ketoacidosis (DKA) that required hospital care as clinical events for the effects of ITx. Severe hypoglycemia was defined as hypoglycemia requiring another person's help to recover from it. Infectious diseases and afebrile digestive symptoms (nausea, vomiting and diarrhea) that required hospital care, *de novo* cancer and mortality were also assessed as clinical events relating to possible adverse effects.

Regarding the blood test, we evaluated hemoglobin A1c (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine for both groups, and ad libitum (ad-lib) and glucagon-stimulated serum CPR for the ITx

group. If a glucagon stimulation test was not carried out at observation points due to high glucose or any other reason, ad-lib CPR was used as a substitute. The number of transplanted islets and the quantity of transplanted islets were also assessed in the ITx group. Observational points of the ITx group were pre-first transplantation, 75 days, 1 year, 2 years, 5 years and 10 years after first transplantation in accordance with the ongoing clinical trial ("Islet transplantation using brain-dead donors and donors after cardiac death for patients with insulin-dependent diabetes mellitus suffering from complicating hypoglycemia unawareness", UMIN000003977, launched on 1 November, 2010), and those of the MDI/CSII group were baseline, 1, 2, 5 and 10 years after baseline as a counterpart to the ITx group.

Ethical approval

This study was approved by the institutional review board, the Ethics Committee of the Graduate School and Faculty of Medicine, Kyoto University and Kyoto University Hospital (R1559).

Statistical analysis

All analyses were by intention-to-treat. Continuous variables were presented as the median and interquartile range (IQR) along with the ITx group, which could not be assumed to have normal distribution due to the sample size. Paired continuous variables were compared using the Wilcoxon signed-rank test. Independent continuous variables including non-normal distribution and heteroscedastic data were compared with the Brunner–Munzel test. Data of continuous variables were presented in figures as a box and whisker plot, in which the box shows the IQR with the inclusive median, the line in the box shows the median and the whiskers depict the range from minimum to maximum without outliers, if they exist. Data points below Q1 − 1.5 IQR or above Q3 + 1.5 IQR were regarded as outliers. Mean values of the data including outliers were presented as cross marks with a line chart as supplemental information. Categorical variables were compared using Fisher's exact test based on Cochran's rule by which the test is suitable when the smallest expectation is less than five[20], and are presented with the risk ratio (RR) and 95% confidence interval. RR was calculated as the ratio of the probability of events in the ITx group compared with those of the MDI/CSII group. Serum C-peptide levels under the detection limit were counted as 0.00 ng/mL for analysis. A P-value <0.05 was considered statistically significant and otherwise considered not significant (NS). Statistical analysis was carried out using Microsoft Excel (Microsoft Japan Co., Ltd., Tokyo, Japan) or BellCurve for Excel version 2.15 (SSRI Co., Ltd., Tokyo, Japan).

RESULTS

Baseline characteristics

The ITx group consisted of five women and two men, whereas the MDI/CSII group consisted of 16 women and 10 men. The median age of the ITx group and the MDI/CSII group was 39.0 (37.0–44.0) vs 38.5 (34.3–46.3) years, respectively (NS, $P = 0.77$). HbA1c was 8.40% (7.75–9.90%) vs 8.15% (7.35–9.80%; NS, $P = 0.59$). AST was 19.0 IU/L (18.0–23.0 IU/L) vs 20.5 IU/L (16.3–23.8 IU/L; NS, $P = 0.83$) and ALT was 17.0 IU/L (16.5–22.5 IU/L) vs 17.5 IU/L (14.3–24.5 IU/L; NS, $P = 0.69$). Creatinine was 0.70 mg/dL (0.60–0.75 mg/dL) vs 0.70 mg/dL (0.60–0.70 mg/dL; NS, $P = 0.83$; Table 1).

Islet transplantation and graft function

Of the seven patients of the ITx group, two patients received ITx once, two received it twice and three received it three times during the 10-year observation period. The median islet quantity was 408,000 (365,000–477,000) islet equivalent/time (Table 1). Ad-lib serum CPR was 0.00 ng/mL (0.00–0.00 ng/mL), 0.29 ng/mL (0.21–0.60 ng/mL, $P = 0.03$ from baseline), 0.37 ng/mL (0.25–0.47 ng/mL, $P = 0.03$), 0.22 ng/mL (0.08–0.33 ng/mL, $P = 0.04$), 0.00 ng/mL (0.00–0.05 ng/mL, NS, $P = 0.18$) and 0.00 ng/mL (0.00–0.00 ng/mL, not applicable for comparison test) at baseline, 75 days, 1, 2, 5 and 10 years after transplantation, respectively. Stimulated serum CPR was 0.0 ng/mL (0.00–0.00 ng/mL), 0.88 ng/mL (0.66–1.34 ng/mL, $P = 0.04$ from baseline), 0.57 ng/mL (0.26–0.99 ng/mL, $P = 0.03$), 0.43 ng/mL (0.19–0.67 ng/mL, $P = 0.04$), 0.00 ng/mL (0.00–0.22 ng/mL, NS, $P = 0.11$ from baseline) and 0.00 (0.00–0.00, not applicable for comparison test), respectively (Figure 1).

Glycemic control

In the ITx group, HbA1c was significantly reduced from baseline at 75 days (6.40% [6.10–6.75%], $P = 0.02$), 1 year (7.10% [6.30–7.40%], $P = 0.02$) and 2 years (7.40% [6.27–8.15%], $P = 0.03$), and no significant difference was observed at 5 years (7.60% [7.05–9.05%], $P = 0.61$) and 10 years (7.30% [7.15–8.80%], $P = 0.08$; Figure 1). In the MDI/CSII group, HbA1c

Table 1 | Baseline characteristics and islet transplantation data

	ITx ($n = 7$)	MDI/CSII ($n = 26$)
Sex	5 F, 2 M	16 F, 10 M
Age (years)	39.0 (37.0–44.0)	38.5 (34.3–46.3)^{NS}
AST (U/L)	19.0 (18.0–23.0)	20.5 (16.3–23.8)^{NS}
ALT (U/L)	17.0 (16.5–22.5)	17.5 (14.3–24.5)^{NS}
Creatinine (mg/dL)	0.70 (0.60–0.75)	0.70 (0.60–0.70)^{NS}
HbA1c (mmol/mol)	68.3 (61.2–84.7)	65.6 (56.8–83.6)^{NS}
HbA1c (%)	8.40 (7.75–9.90)	8.15 (7.35–9.80)^{NS}
Times of transplantation		
Once	2	NA
Twice	2	NA
Three times	3	NA
Islet quantity (IEQ/time)	408,000 (365,000–477,000)	NA

Data are shown as median and interquartile range. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CSII, continuous subcutaneous insulin infusion; HbA1c, hemoglobin A1c; IEQ, islet equivalent; ITx, islet transplantation; MDI, multiple daily injection; NA, not applicable; NS, not significant.

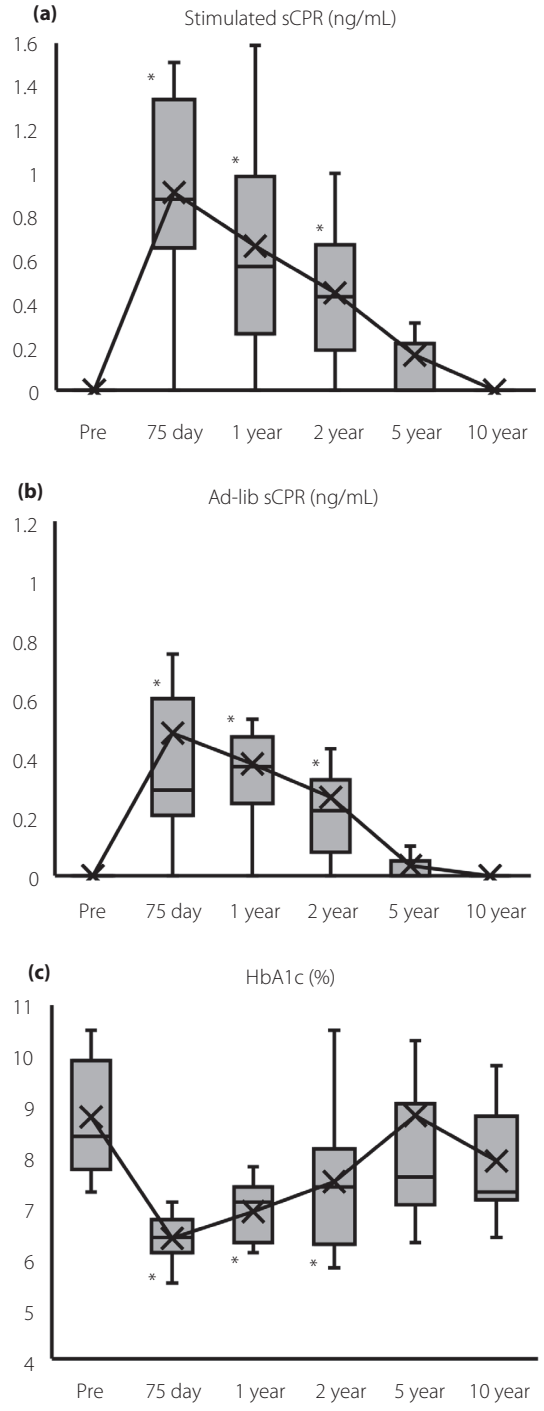

Figure 1 | Graft function and glycemic control after islet transplantation. Changes of (a) stimulated serum C-peptide immunoreactivity (sCPR), (b) ad libitum (Ad-lib) serum C-peptide reactivity and (c) hemoglobin A1c (HbA1c; %) in the islet transplantation group. Data are shown as box and whisker plots and a supplemental line graph of mean values that are presented as cross marks. *$P < 0.05$ from baseline. Pre, pre-first transplantation.

showed no reduction from baseline at 1 year (8.10% [7.30–9.50%], $P = 0.52$), 2 years (8.40% [7.40–9.60%], $P = 0.69$), 5 years (7.85% (7.43–8.58%), $P = 0.48$) and 10 years (8.45% [7.63–9.38%], $P = 0.56$).

In regard to comparisons between the two groups, better median HbA1c was observed in the ITx group at all comparable points, although a statistically significant difference was observed only at 1 year ($P < 0.001$); at other points, the difference was $P = 0.11$ at 2 years, $P = 0.60$ at 5 years and $P = 0.18$ at 10 years (Figure 2).

Differences of HbA1c between baseline and 10 years (ΔHbA1c) of the ITx group were significantly lower than those of the MDI/CSII group (−0.80% [−1.20 to −0.40%] vs 0.05% [−0.30 to 0.67%], $P = 0.04$; Figure 2). HbA1c improvement at 10 years from baseline was observed in six of seven patients (85%) of the ITx group, whereas it was 11 of 26 patients (42%) of the MDI/CSII group (NS, $P = 0.09$, not shown in figures).

Liver function

In the ITx group, AST and ALT elevation were observed at 75 days (33.0 IU/L [31.5–38.5 IU/L, $P = 0.03$ from baseline] and 33.0 IU/L [32.0–38.0 IU/L, $P = 0.02$ from baseline]), but it gradually recovered to baseline level (32.0 IU/L [28.0–34.5 IU/L, $P = 0.06$] and 25.0 IU/L [20.0–31.0 IU/L, $P = 0.24$] at 1 year, 28.0 IU/L [20.5–34.5 IU/L, $P = 0.17$] and 23.0 IU/L [15.5–31.5 IU/L, $P = 0.50$] IU/L at 2 years, 21.0 IU/L [20.0–24.0 IU/L, $P = 0.13$] and 13.0 IU/L [12.0–25.0 IU/L, $P = 0.74$] IU/L at 5 years, and 21.0 IU/L [19.0–26.5 IU/L, $P = 0.53$] and 13.0 IU/L [12.0–25.0 IU/L, $P = 0.45$] at 10 years; Figure 3). In the MDI/CSII group, no significant difference between baseline and any observational points was observed in AST and ALT (19.5 IU/L [16.8–25.0 IU/L, $P = 0.16$] and 17.0 IU/L [13.0–23.8 IU/L, $P = 0.38$] at 1 year, 21.0 IU/L [16.0–28.0 IU/L, $P = 0.25$] and 19.0 IU/L [14.0–25.0 IU/L, $P = 0.54$] at 2 years, 18.0 IU/L [16.0–22.5 IU/L, $P = 0.33$] and 16.5 IU/L [14.0–20.0 IU/L, $P = 0.39$] IU/L at 5 years, and 23.0 IU/L [17.3–24.8 IU/L, $P = 0.45$] and 18.0 IU/L [13.3–21.8 IU/L, $P = 0.78$] at 10 years; Figure 4).

Regarding comparisons between two groups, a significant difference was observed in AST at 1 year ($P < 0.001$), but no significant difference was observed thereafter ($P = 0.09$ at 2 years, $P = 0.06$ at 5 years and $P = 0.70$ at 10 years). No significant difference was observed in ALT ($P = 0.05$ at 1 year, $P = 0.57$ at 2 years, $P = 1.00$ at 5 years and $P = 0.68$ at 10 years). ΔAST and ΔALT were not significantly different between the ITx group and the MDI/CSII group (1.0 IU/L [−1.5 to 3.5 IU/L] vs 0.5 IU/L [−3.0 to 4.5 IU/L], $P = 0.85$ and −3.0 IU/L [−5.0 to 0.5 IU/L] vs 0.0 IU/L [−5.5 to 5.8 IU/L], $P = 0.51$, respectively; Figure 4).

Kidney function

In the ITx group, no significant difference from baseline in creatinine was observed at 75 days, 1, 2 and 5 years (0.70 mg/dL [0.60–0.80 mg/dL, $P = 0.27$], 0.70 mg/dL [0.55–0.75 mg/dL,

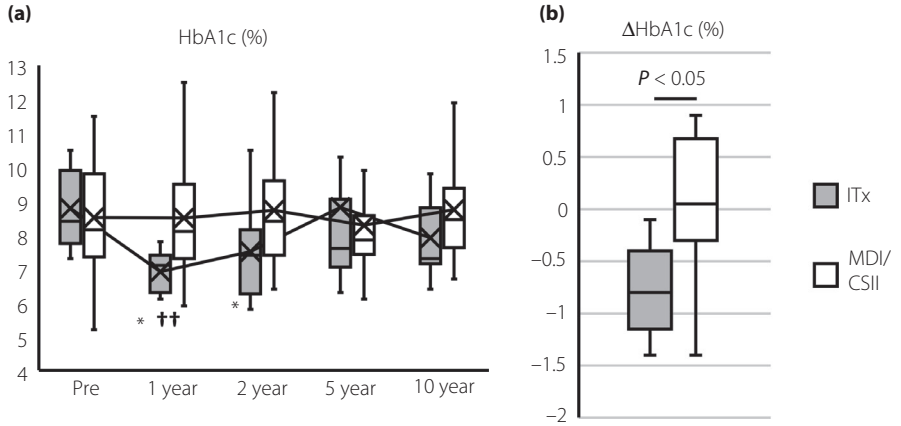

Figure 2 | Glycemic control of islet transplantation (ITx) group and multiple daily injections (MDI)/continuous subcutaneous insulin infusion (CSII) group. Comparison of (a) changes of hemoglobin A1c (HbA1c; %) and (b) box and whisker plots of ΔHbA1c (the difference between 10 years and baseline; %) in the ITx group and the MDI/CSII group. *$P < 0.05$ from baseline, $^{††}P < 0.01$ from MDI/CSII. Pre, pre-first transplantation.

$P = 0.86$], 0.80 mg/dL [0.65–1.00 mg/dL, $P = 0.22$], 0.80 mg/dL [0.70–0.80 mg/dL, $P = 0.14$], respectively), but significant elevation was observed only at 10 years (0.79 [0.74–0.87, $P = 0.03$]; Figure 3). In the MDI/CSII group, no significant difference from baseline was observed at any point (0.65 mg/dL [0.60–0.70 mg/dL, $P = 0.43$], 0.65 mg/dL [0.60–0.70 mg/dL, $P = 0.64$], 0.60 mg/dL [0.60–0.70 mg/dL, $P = 0.35$] and 0.69 mg/dL [0.63–0.79 mg/dL, $P = 0.07$] at 1, 2, 5 and 10 years, respectively).

However, there was no significant difference between two groups at any point ($P = 0.89$ at 1 year, $P = 0.13$ at 2 years, $P = 0.09$ at 5 years and $P = 0.14$ at 10 years), and no significant difference in Δcreatinine (0.10 mg/dL [0.04 to 0.22 mg/dL] vs 0.04 mg/dL [−0.02 to 0.11 mg/dL], $P = 0.08$; Figure 4). In the MDI/CSII group, one patient (3.8%) received hemodialysis at 10 years, whereas none of the patients in the ITx group received hemodialysis.

Clinical events

Severe hypoglycemia was observed in one of seven patients (14%) in the ITx group, whereas the number was eight of 26 patients (31%) in the MDI/CSII group. RR was 0.46 (95% confidential interval [CI] 0.07–3.12, $P = 0.64$). Severe hypoglycemia of one patient in the ITx group was observed at 5 years after the first transplantation. The graft function of this patient had declined; the stimulated serum CPR was reduced from 0.43 ng/mL at 2 years to an undetectable level at 5 years. DKA was zero of seven (0.0%) vs one of 26 patients (3.8%), respectively.

The occurrence of infectious disease was three of seven (43%) vs three of 26 patients (12%), respectively, with RR 3.71 (95% CI 0.95–14.55, $P = 0.09$). In the ITx group, there was one case of repeated pneumonia, one patient had *Clostridium difficile* colitis, and one patient had a neck abscess and urinary tract infection by *Klebsiella pneumoniae*. The occurrence of afebrile digestive symptoms was three of seven (43%) vs two of 26 patients (7.7%), with RR 5.57 (95% CI 1.14–27.12, $P = 0.05$). In the ITx group, one patient had severe stomatitis, and constipation and diarrhea like irritable bowel syndrome, and two patients had gastroenteritis after MMF had been started. Malignancy was three of seven (43%) vs one of 26 patients (3.8%), with RR 11.14 (95% CI 1.36–91.33, $P = 0.02$). In the ITx group, one patient had well-differentiated gastric adenocarcinoma and received endoscopic mucosal resection, one patient had papillary thyroid carcinoma and received surgery, and one patient had breast cancer and received surgery with adjuvant chemoradiation therapy. None of the patients of either group died during the observation period. Summarized data are shown in Table 2.

DISCUSSION

The present study examines the diabetic control, graft function, main organ function and major clinical events after ITx for a 10-year observation period in comparison with conventional MDI/CSII treatment. The results provide novel long-term evidence including reference to notable clinical events related to ITx.

Similar to the results of previous 12-month and 7-year cohort studies[17,18], the present ITx group showed a tendency toward risk reduction of severe hypoglycemia and also showed a reduction of glycated hemoglobin from baseline in the ITx group compared with the MDI/CSII group in a 10-year study. DKA was not observed in the ITx group at all; however, it was observed in only one of the 27 patients in the MDI/CSII group, so a contribution of ITx on DKA was not evident. The ITx group showed no mortality in 10 years, which supports the notion that ITx is a safe treatment.

As for engraftment, transplanted grafts might avoid acute graft rejection in many cases of ITx patients. Anti-CD25

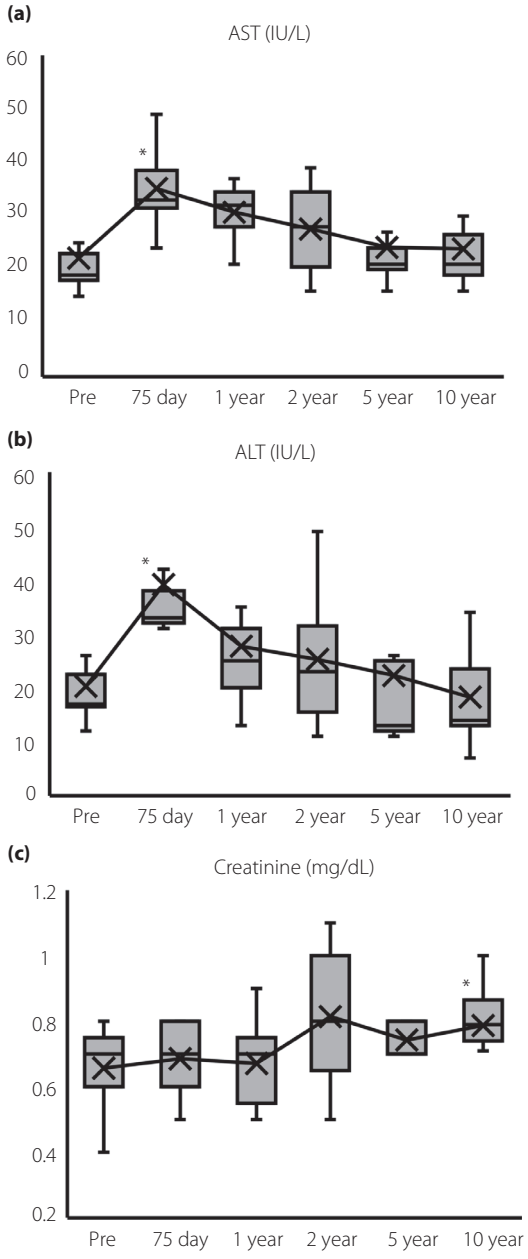

Figure 3 | Liver and kidney function after islet transplantation. Changes of (a) aspartate aminotransferase (AST), (b) alanine aminotransferase (ALT) and (c) creatinine in the islet transplantation group. *$P < 0.05$ from baseline. Pre, pre-first transplantation.

monoclonal antibody induction was thought to contribute to this outcome, playing an inhibitory role for binding of the interleukin-2 (IL-2) and IL-2 receptor, which leads to reduction of cytotoxic T lymphocytes. In terms of the difference between two types of anti-CD25 antibody, basiliximab and daclizumab, both of which contain the human-derived antibody domain, a previous prospective randomized study of deceased donor renal transplantation showed there was no significant difference in graft function and patient survival between them[21]. Regarding the graft function, ad-lib CPR and stimulated CPR were detected for several years after transplantation. According to the previous case study of 12 patients with ITx, the time from ITx to graft failure, which was defined as stimulated CPR being <0.3 ng/mL, was 2.8 ± 1.6 years[22]. In the present study, four of seven patients (57%) maintained stimulated CPR >0.3 ng/mL at 2 years, and two of seven patients (29%) at 5 years (data not shown). This acceptable rate of graft survival and capability of glucose-stimulated insulin secretion enabled the patients to maintain better glycemic control in the ITx group without hypoglycemia.

Another explanation for the contribution of ITx to hypoglycemia is counterregulatory hormones[23,24]; the glycemic thresholds to activate counterregulatory hormones and autonomic symptom responses to hypoglycemia appear normal in patients after ITx. A 1-year study also showed that ITx reduced hypoglycemia regardless of insulin independence[17]; therefore, this mechanism possibly had an additional role in the reduction of hypoglycemia, even after the graft failure occurred.

In regard to diabetic complications, differences in 10-year changes of proteinuria and retinopathy were not statistically significant between groups, although the results are presented merely for reference purposes, because only qualitative data were available (Tables S1,S2).

From the viewpoint of adverse effects on major organs, no significant deterioration of liver and kidney function was observed after 10 years compared with the MDI/CSII group, although transient elevation of transaminase was observed after ITx in the present study. Two of the seven patients (29%) in the ITx group showed transient transaminase elevation twice from baseline, and six of the seven patients (86%) showed elevation of 1.5-fold from baseline (data not shown); however, the elevated transaminase lowered to normal levels compared with baseline levels, and none of the patients of the ITx group suffered from liver failure. The present result was the same as the report of the Collaborative Islet Transplant Registry that also showed the transient elevation of AST and ALT levels after ITx and the subsequent normalization[6]. Significant creatinine elevation was observed only in the ITx group at 10 years from baseline, but the difference from the MDI/CSII group at 10 years was not significant. According to the Diabetes Control and Complications Trial, and following the Epidemiology of Diabetes Interventions and Complications study, creatinine elevation of type 1 diabetes patients with MDI/CSII was from 0.68 to 0.73 mg/dL during a mean follow-up period of 6.5 years in the Diabetes Control and Complications Trial, and from 0.73 to 0.85 mg/dL during 16 years in the Epidemiology of Diabetes Interventions and Complications study[25]. Compared with the result of these studies, the creatinine elevation of the ITx group in the present study (from 0.70 to 0.79 mg/dL in 10 years) was within a reasonable range.

However, the tendency of an increase of infectious disease and digestive symptoms warned us to pay attention to

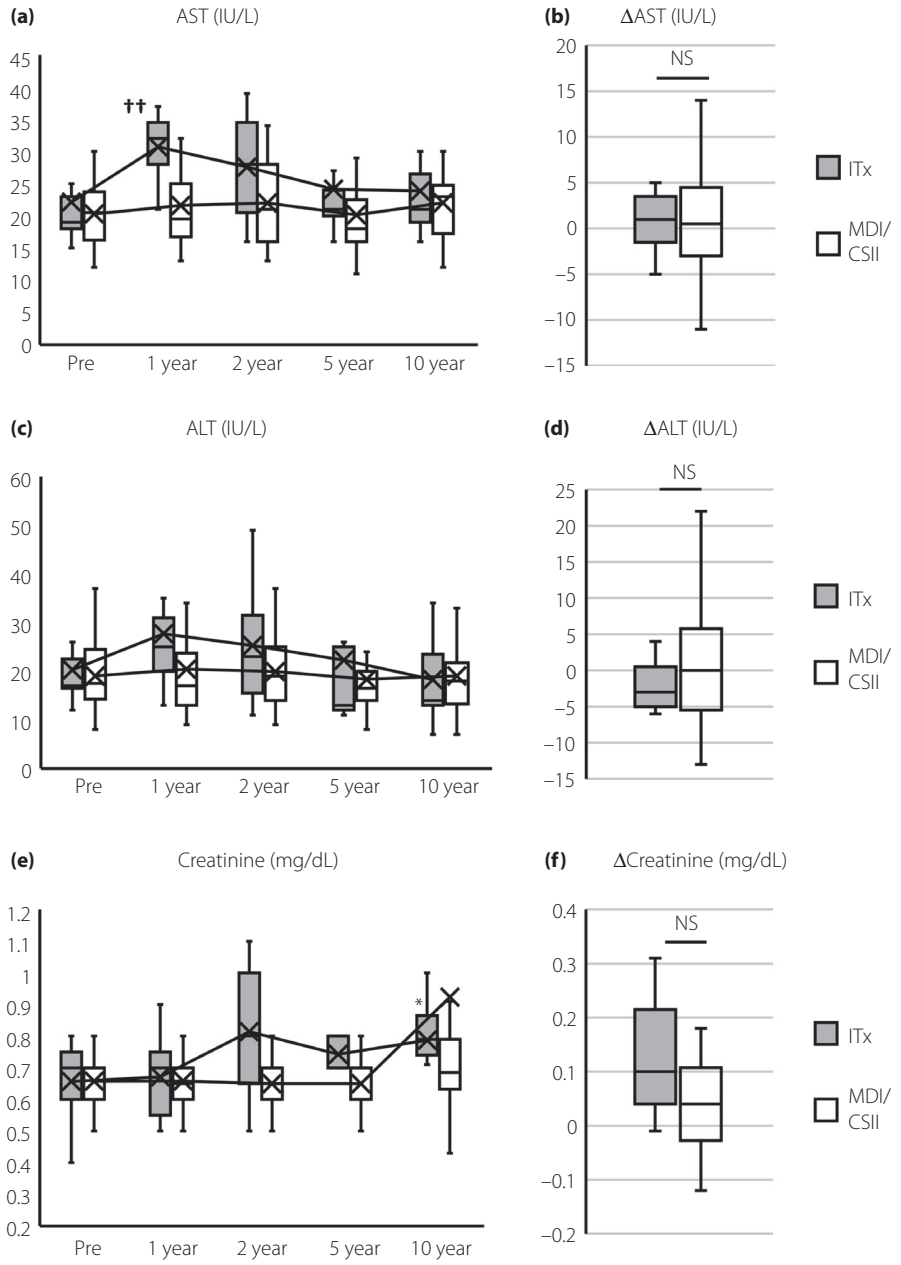

Figure 4 | Liver and kidney function of islet transplantation (ITx) group and multiple daily injections (MDI)/continuous subcutaneous insulin infusion (CSII) group. Comparison of changes of (a) aspartate aminotransferase (AST), (c) alanine aminotransferase (ALT) and (e) creatinine, and box and whisker plots of (b) ΔAST (the difference between 10 years and baseline), (d) ΔALT and (f) Δcreatinine in the ITx group and the MDI/CSII group.$^{††}P < 0.01$ from MDI/CSII, * $P < 0.05$ from baseline, NS, not significant; Pre, pre-first transplantation.

immunosuppressant adverse effects as described in a previous report[26]. Notably, most of the digestive symptoms involved diarrhea, and patients complained of diarrhea after MMF administration. MMF-induced diarrhea is known as a frequently observed side-effect of MMF[27,28]. An *in vitro* study showed that acyl glucuronide, which is a metabolite of mycophenolic acid, the active metabolite of MMF[29], promoted the tumor necrosis factor (TNF)-alpha and IL-6 release from mononuclear cells[30]. TNF-alpha and IL-6 are widely-known cytokines that are upregulated in inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease[31]. Furthermore, there was a case of MMF-induced refractory diarrhea with ulceration throughout the colon after kidney transplantation that was ameliorated by only a single dose of infliximab, a TNF-alpha inhibitor[32]. Interestingly, a 1-year follow-up study of ITx using a recent immunosuppressive regimen with anti-

Table 2 | Incidence of clinical events in islet transplantation group and multiple daily injection/continuous subcutaneous insulin infusion group

	ITx (n = 7)	MDI/CSII (n = 26)	RR [95% CI]	P-value
Severe hypoglycemia	1 (14%)	8 (31%)	0.46 [0.07–3.12]	0.64
DKA	0 (0%)	1 (3.8%)	NA	NA
Infectious disease	3 (43%)	3 (12%)	3.71 [0.95–14.55]	0.09
Digestive symptoms	3 (43%)	2 (7.7%)	5.57 [1.14–27.12]	0.05
Malignancy	3 (43%)	1 (3.8%)	11.14 [1.36–91.33]	<0.05
Mortality	0 (0%)	0 (0%)	NA	NA

CI, confidence interval; CSII, continuous subcutaneous insulin infusion; DKA, diabetic ketoacidosis; ITx, islet transplantation; MDI, multiple daily injection; NA, not applicable; RR, risk ratio.

thymocyte globulin, daclizumab and etanercept (TNF-alpha inhibitor) for induction, and MMF, sirolimus and no or low-dose tacrolimus for maintenance did not report apparent gastrointestinal symptoms[33]. These findings suggest a potential preventive role of TNF-alpha inhibitor for MMF-induced diarrhea, although further investigation is required.

Malignancy was more frequently observed in the ITx group than in the MDI/CSII group. Generally, post-transplant lymphoproliferative disorder is a well-known side-effect of solid organ transplantation and hematopoietic stem cell transplantation, together with the suppressed T-cell function[34,35]. To our knowledge, only a few cases of post-transplant lymphoproliferative disorder after ITx have been reported[36], and there were no patients of post-transplant lymphoproliferative disorder in the present study, as compared with another long-term case series study[16]. Malignancies observed in the ITx group of the present study were in stomach, breast and thyroid. According to the high-volume study of solid organ recipients that referred to the incidence ratio of various cancers, stomach and thyroid cancer were more frequently observed than expected, but breast cancer was less frequently observed[35]. Furthermore, screening frequency bias might have affected this finding, as described later. Thus, the present results regarding malignancy are controversial, and it seems too early for conclusions regarding the relationship between malignancy and ITx.

Taken together, the tendency of risk reduction of severe hypoglycemia and the better glycemic control without life-threatening adverse effects supports the evidence of the long-term safety and efficacy of ITx. Thus, ITx has a beneficial influence not only on a patient's physical condition, but also on their comprehensive quality of life, as documented previously[37–39].

There were some limitations to the present study that should be addressed. This was a single-center, non-randomized observational study with limited cases, and thus potential bias was not totally excluded. For example, the ITx group tended to more often have visited a hospital and received patient education or examination, including a blood test, computed tomography or magnetic resonance imaging, than the MDI/CSII group; the frequency of patient education or screening tests were not able to be controlled. There might be underestimation of hospitalization events, because the information includes only the medical records of our hospital. Another potential limitation is that the results might not be applicable to other ethnic groups. These biases should be taken into consideration when interpreting the results.

In regard to further prospects, the results of the ongoing clinical trial (UMIN000003977) using the immunosuppressive regimen with anti-thymocyte globulin are highly anticipated, as the previous 2-year study using a similar regimen showed encouraging results[40].

In conclusion, the present study confirms the safety of ITx, and the possibility of ITx to improve glycemic control and to reduce severe hypoglycemia for 10 years by comparison with control IDDM patients with MDI/CSII. No fatal adverse effects were observed in patients with ITx, although transient elevation of transaminase and mild creatinine elevation were observed. In addition, the present results also describe significant clinical events in the life-long management after transplantation. We showed the necessity of paying careful attention to infectious diseases, digestive symptoms and malignancy after ITx, although further evidence is required.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Baseline characteristics of proteinuria and retinopathy in the islet transplantation group and the multiple daily injection/continuous subcutaneous insulin infusion group.

Table S2 | Ten-year changes of proteinuria and retinopathy in the islet transplantation group and the multiple daily injection/continuous subcutaneous insulin infusion group.