Clinical islet transplantation in type 1 diabetes mellitus: results of Australia's first trial

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ABSTRACT

Objective: To determine whether pancreatic islet transplantation can control diabetes and prevent severe life-threatening hypoglycaemia.

Design, setting and participants: A single-arm observation study of six patients undergoing islet transplantation. All patients had had type 1 diabetes mellitus for over 5 years and documented episodes of repeated severe hypoglycaemia. Islets were isolated from donor pancreases digested by Liberase. Separated islets were infused into the recipient's liver via the portal vein. Patients were immunosuppressed with daclizumab, sirolimus and tacrolimus. The transplants were performed at Westmead Hospital, NSW, between October 2002 and February 2005.

Main outcome measures: Normal blood glucose control without administration of exogenous insulin; demonstration of islet function and abolition of hypoglycaemia.

Results: Five of the patients received two islet infusions, and the sixth was withdrawn after one infusion following a portal vein thrombosis. Three patients became insulin-independent, with excellent glycaemic control. Two had islet function with circulating C-peptide, improved glycaemic control, reduced insulin requirement and abolition of severe hypoglycaemia. However, over a 2-year period, graft function deteriorated. Recipients who were initially insulin free remained C-peptide positive but required supplemental insulin. Complications included one postoperative bleed, two portal vein thromboses (which resolved completely), presumed recurrence of tuberculosis in one patient, and deterioration in renal function in one patient.

Conclusions: Islet transplantation is effective at improving glycaemic control and hypoglycaemia unawareness in the short to medium term. However, problems with long-term safety of immunosuppression, islet-induced thrombosis and early detection of loss of islet function remain to be addressed.

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Multiple injections of insulin are the mainstay of treatment for type 1 diabetes mellitus and, despite improvements in insulin administration and monitoring, long-term complications remain a problem for many patients. Between 30% and 60% of patients have evidence of end-organ complications within 15 years of their initial diagnosis.[1-3]

The concept that transplanting the islets of Langerhans would provide superior diabetic control was first proposed over 40 years ago. In 1966, Kelly et al[4] showed that normoglycaemia without exogenous insulin administration could be achieved by whole-organ vascularised pancreas transplantation. Further proof of the principle was demonstrated by Lacy and colleagues, who showed that transplantation of isolated islets could control streptozocin-induced diabetes in rodents.[5,6] However, reproducible, reliable and long-lasting diabetic control with islet transplantation has been difficult to achieve in humans.[7] More recently, a substantial improvement in the success rate of islet transplantation was achieved by Shapiro and colleagues.[8] Their success was based on principles that included selection of appropriate patients for transplantation, use of a non-toxic effective immunosuppressive regimen, isolation of appropriate numbers of viable islets, and transplantation of sufficient numbers of islets to control blood glucose levels. Their endeavours have stimulated renewed interest in islet transplantation as a therapy for a select group of patients in whom the risks of immunosuppression are considered less than the risks of continued dependency on insulin therapy. The patients most likely to fulfil these criteria are a small group of patients who, because of defective hormonal counter-regulation and/or autonomic neuropathy, develop life-threatening hypoglycaemia without the usual warning symptoms. These patients are at risk of undetected life-threatening coma unless a third party is present when coma occurs.

Here we report the findings of the first Australian clinical trial of islet transplantation to reverse diabetes. Our aims were to determine whether the results achieved by Shapiro and colleagues could be replicated, to confirm the efficacy of islet transplantation as a treatment for hypoglycaemia unawareness, and to obtain a preliminary evaluation of the safety and efficacy of islet transplantation in the short to medium term.

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METHODS

Patients

Patients eligible for selection for our study had had type 1 diabetes mellitus for more that 5 years and were aged between 18 and 65 years. Additionally, they had recurrent severe hypoglycaemia unawareness with coma that required constant monitoring or regular intervention by a third party. As a result, they had developed worsening diabetic control that could not be managed by intensive insulin therapy (which included trial of an insulin pump in two cases). The

risk of recurrent hypoglycaemia had to be judged by at least two of the investigators (P J O and D J H) to be greater than the overall risk of transplantation and immunosuppression. Patients had to be free of significant diabetic nephropathy (proteinuria < 300 mg/day) and renal impairment (glomerular filtration rate [GFR] > 60 mL/min/1.73m^2). All patients gave informed consent, and the protocol was approved by the Human Research Ethics Committee of the Western Sydney Area Health Service.

Islet preparation

Islets were separated by the closed loop method described by Ricordi et al.[9,10] Pancreases were removed from heart-beating deceased donors. The pancreas was disaggregated by infusing the ducts with cold Liberase enzyme (Liberase Human Islet, Roche Applied Science, Indianapolis, Ind, USA). Dissociated islet and acinar tissue was separated on a continuous Biocoll (Biochrom AG, Berlin) density gradient (polysucrose 400 and amidotrizoic acid) on a refrigerated apheresis system (Model 2991, COBE Laboratories, Lakewood, Colo, USA).

Purified islets were counted and islet number and mass were expressed in terms of islet equivalents (IEQ).[11] Islet preparations underwent a pre-transplant quality assurance process, which included a gram stain, purity and viability assessment, packed cell volume measurement and evaluation of islet morphology to exclude excessive fragmentation. Aliquots of transplanted islet preparations underwent microbiological culture, endotoxin assay and calculation of a glucose stimulation index (based on an in-vitro test of response to a glucose stimulus).[12]

Islet transplantation

To be suitable for islet transplantation, a pancreas had to yield more than 3000 IEQ/kg (recipient weight) in a packed-tissue volume of less than 10 mL. The islets were resuspended in 120 mL of medium 199 (ThermoTrace, Melbourne) containing 5000 U heparin and 20% human albumin. Patients received a general anaesthetic, and minilaparotomy was performed to access a mesenteric vein. An arterial angiographic catheter was inserted into the main portal vein with the assistance of image intensification. The islets were infused over 15–20 minutes. Portal venous pressure was monitored throughout. A Doppler ultrasound scan of the portal vein and liver was performed on Day 1 and Day 3 after transplantation to confirm that the portal circulation remained patent after the islet infusion.

Immunosuppression and care after transplantation

Immunosuppression was instituted at the time of transplantation. All patients received tacrolimus (Astellas), sirolimus (Wyeth) and daclizumab (Roche). Tacrolimus was commenced at a dose of 0.1 mg/kg per day in two equal divided doses, with the dose then adjusted to achieve a target blood concentration of 3–7 ng/mL. Sirolimus was commenced at 15 mg per day for the first day, followed by 7 mg per day, with the dose then adjusted to achieve a target blood concentration of 12–15 ng/mL for the first 12 weeks. Daclizumab was given as an infusion of 1 mg/kg every 14 days for a total of five doses commencing on the day of transplantation. In cases in which a second islet transplant was required more than 10 weeks after the first, a further five doses of daclizumab were given.

Cephazolin 1 g three times a day for 48 hours and a single dose of imipenem 500 mg were given perioperatively. Oral cotrimoxazole (sulfamethoxazole 400 mg and trimethoprim 80 mg) was given for the first 9 months after transplantation as prophylaxis for *Pneumocystis carinii*. Oral valaciclovir 1 g three times a day was given for 3 months to prevent cytomegalovirus and herpes virus infections. Vitamin E 800 IU/day, vitamin B6 100 mg/day and vitamin A 5000 IU on alternate days were given for the first 3 months as supplementary antioxidant therapy.

As prophylaxis for portal vein thrombosis, all patients received heparin 5000 U intravenously at the time of islet infusion. The original aim was for patients to receive full anticoagulation (ie, a heparin dose sufficient to double the activated partial thromboplastin time) for the first 48 hours. But this was changed to 15 000 U daily of subcutaneous prophylactic heparin after the first recipient suffered bleeding complications. Towards the latter half of the trial (after Patient 5 experienced portal vein thrombosis), full anticoagulation was again reinstituted.

Insulin therapy was ceased in the immediate postoperative period and was recommenced when blood sugar level rose above 10 mmol/L. Intensive insulin management was used to maintain normal glucose levels after transplantation (< 8 mmol/L postprandial), with the aim of reducing islet stress during engraftment. After 1 month, insulin use was gradually reduced and/or ceased, depending on glycaemic control (assessed by home blood glucose monitoring).

Islet function was measured by assessing serum C-peptide levels. Patients also under-

1 Summary of patient characteristics and graft outcome

Patient number	Age (years)	Duration of diabetes (years)	Pre-tx GFR (mL/min/ 1.73 m^2)	Number of islet txs	Time from 1st to 2nd tx (months)	Graft survival (months)	Comment
1	37	28	143	2	5	> 38	Insulin free for 24 months; remains C-peptide positive
2	50	37	140	2	2.5	31	Insulin free for 24 months; failed graft
3	49	30	122	2	2.5	7	Insulin free for 2 months; ceased immunosuppression and withdrew from study at 7.5 months because of intolerance to medication
4	40	15	138	2	14	> 26	Awaiting third islet transplant
5	44	25	153	1	na	0	Withdrawn from study soon after first transplant because of right portal vein thrombosis; no graft function achieved
6	33	8	104	2	5.5	15	Antibodies to exogenous insulin detected; recurrence of tuberculosis; failed graft

GFR = glomerular filtration rate. na = not applicable. tx = transplant. ◆

went glucose tolerance testing at least annually. Glycosylated haemoglobin and lipid levels were measured, and renal function was assessed at least every 3 months.

RESULTS

Patient characteristics

Of 50 patients referred for evaluation by an endocrinologist, six were selected for our trial. Patient characteristics and graft outcomes are shown in Box 1. All had undetectable C-peptide levels before transplant and all had evidence of severe hypoglycaemia unawareness that had failed to respond to intense management of their insulin therapy. In addition to hypoglycaemia unawareness, one patient had antibodies to exogenous insulin and required immunosuppression with azathioprine to maintain adequate glycaemic control. Apart from hypoglycaemia unawareness, the patients were remarkably free of end-organ diabetic complications. Mean GFR was 132 mL/min/1.73m^2 (range, 104–153 mL/min/1.73m^2). Two had had laserphotocoagulation treatment for diabetic retinopathy, two had mild subclinical neuropathy, and two had hypertension (with blood pressure well controlled by an angiotensin-converting enzyme [ACE] inhibitor) and microalbuminuria (maximum protein excretion when not taking an ACE inhibitor was 257 mg/day but normal when taking medication).

The six selected patients underwent islet transplantation between October 2002 and February 2005. The median follow-up time was 18 months (range, 3–31 months). The mean number of islet equivalents transplanted was 17 958 IEQ/kg (range, 6995–26 480 IEQ/kg). The absolute number of islets transplanted did not correlate with success in achieving insulin independence (Box 2). Five of the six patients received two islet infusions. One patient developed a portal vein thrombosis after the first infusion, had no measurable C-peptide (indicating there was no graft function), and was withdrawn from the study. Of the five remaining patients, all had evidence of islet function after the first graft. C-peptide levels and total insulin dose before and after transplant are summarised in Box 3. Three recipients were able to cease insulin treatment completely for a period of time. However, one of them withdrew from the study after 7.5 months because of intolerance to the immunosuppressive medication, suffering nausea and mouth ulcers. The remaining two had evidence of substantial islet function, with evidence of C-peptide secretion and major reductions in insulin dose.

2 Isolation data for the 11 islet preparations used for transplantation

Patient number	Total IEQ	IEQ/kg body weight*	Total packed cell volume (mL)	Total islet number	Stimulation ratio†
1	717 037	11 117	6	262 400	nd
	570 766	8 849	5	207 333	9.7
2	494 690	6 776	9	202 500	nd
	537 345	7 361	10	163 000	1.0
3	712 960	16 580	7	184 000	7.8
	425 715	9 900	10	174 000	nd
4	1 108 216	14 976	10	170 125	5.0
	774 230	11 385	6	96 500	1.3
5‡	468 685	6 995	5	147 700	5.0
6	759 519	12 873	8	226 206	2.5
	468 080	7 933	8	100 800	5.8

IEQ = islet equivalents. *IEQ/kg = islet equivalents transplanted per kilogram of recipient's body weight. nd = not done. † Stimulation ratio reflects islet cell response to glucose in vitro. ‡ Patient withdrawn from study because of right portal vein thrombosis. ◆

3 Graft function and glucose control after islet transplantation

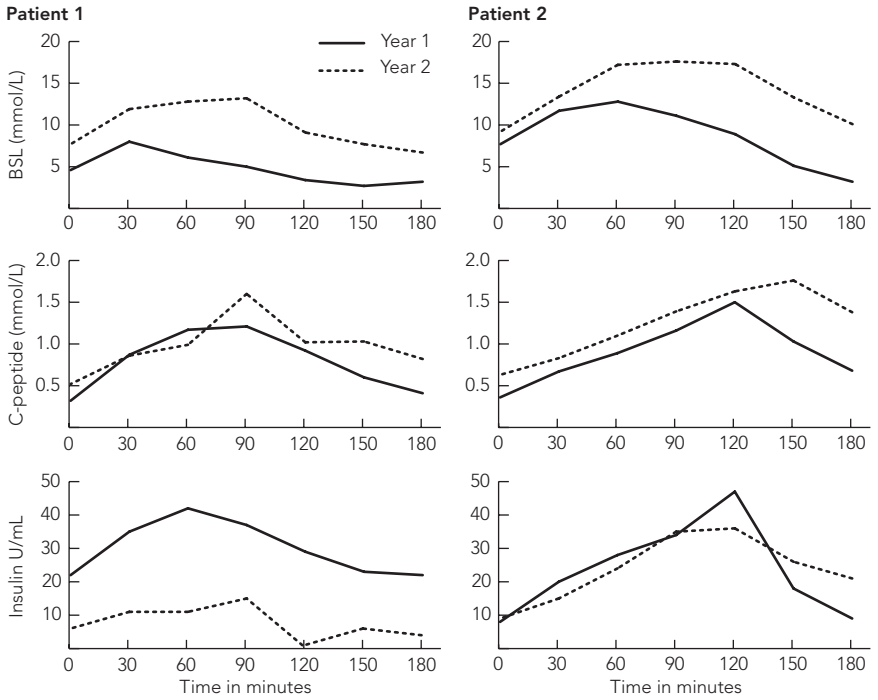
Patient number	Number of islet txs	Unstim_ ulated C-peptide level	Pre-tx daily insulin requirement	Post-tx daily insulin requirement	Pre-tx HbA_{1c} level*	HbA_{1c} level 3 months after tx	HbA_{1c} level 12 months after tx
1	2	1.05 nmol/L	28 U	0	8.0%	5.3%	5.5%
2	2	0.50 nmol/L	30 U	0	7.8%	5.1%	6.3%
3†	2	0.65 nmol/L	28 U	0	9.7%	6.5%	9.2%
4	2	0.3 nmol/L	48 U	7 U	8.0%	6.2%	7.4% (5.9%)‡
5§	1	< 0.1 nmol/L	35 U	35 U	8.4%	8.4%	NA
6¶	2	0.4 nmol/L	40 U	6 U	8.7%	6.7%	7.4%

NA = not applicable. tx = transplant. *Normal range < 6%. † Patient withdrew from trial at 7.5 months because of intolerance to immunosuppressive medication. ‡ Second islet transplant was 14 months after first; HbA_{1c} level 3 months after 2nd tx was 5.9%. § Patient developed right portal vein thrombosis after 1st tx. ¶ Presumed recurrence of tuberculosis at 12 months. ◆

Glycaemic control

All recipients with evidence of islet function showed marked improvements in blood glucose control and haemoglobin A_{1c} (HbA_{1c}) level (Box 3). In all patients, there was a reduction in insulin requirement and a fall in HbA_{1c} level after the first islet infusion. There was a further fall in insulin requirement after the second infusion, even in recipients who did not completely stop taking insulin. Severe hypoglycaemic episodes were abolished. Three patients had episodes of mild hypoglycaemia (blood sugar level, 2.5–3.5 mmol/L), but none required third-party intervention. These episodes became less frequent or ceased with time and were rarely seen 6 months after the second transplant. In the two patients (Patients 1 and 2) who received a second islet transplant and were insulin free for 2 years, there was evidence of loss of islet function with impairment of glucose tolerance towards the end of the second year (Box 4). Both patients have now returned to insulin therapy. Patient 1 has good control with 6–10 U insulin a day, and remains C-peptide positive with HbA_{1c} level < 7%. (Outcomes were the same for Patient 2 until the graft ultimately failed.) Episodes of mild hypoglycaemia did occur in Patient 1 following the onset of graft dysfunction, but none of these required intervention by a third party. In Patient 3, who ceased immunosuppressive treatment after graft loss, and Patient 5, who

4 Stimulated blood glucose, C-peptide and insulin levels after a standard oral glucose tolerance test in the two patients who were insulin free for 2 years*



* Solid line indicates results at 1 year after islet transplantation; broken line indicates results at 2 years after transplantation. ◆

never achieved any graft function, episodes of severe hypoglycaemia continued to occur.

Side effects

Procedure-related side effects included a postoperative bleed in Patient 1 on Day 1 requiring transfusion and laparotomy for evacuation of haematoma. The patient had a rapid and full recovery. Patient 5 experienced right portal vein thrombosis, with eventual recovery, and Patient 6 had a partial left portal vein thrombosis that resolved completely within 9 days. In addition, all patients had a mild rise (a less than two-fold increase) in gamma-glutamyl transferase and alanine aminotransferase levels, and two patients had ultrasound evidence of fatty liver.

Side effects of immunosuppressive medication included mouth ulcers, raised cholesterol level, ankle swelling and, in one patient, a presumed recurrence of tuberculosis (symptoms were fevers, weight loss and ketonuria, which resolved with antituberculous therapy, although no organism was grown). Surprisingly, hypertension was not a major side effect, and no new patients commenced antihypertensive therapy. However, Patient 2 showed a significant fall in isotopic GFR at 18 months (from 143 mL/min/1.73m^2 pretransplant to 63 mL/min/1.73m^2) and required cessation of tacrolimus and substitution with mycophenolate mofetil. Patient 3 was diagnosed with a skin squamous cell carcinoma within 3 months of the transplant, but it is likely that it was present before commencement of immunosuppression.

DISCUSSION

Our trial confirmed both the potential advantages and current limitations of islet transplantation. Five of six transplant recipients had evidence of significant C-peptide secretion, improved glycaemic control and elimination of severe hypoglycaemic episodes. In three patients, insulin injections were ceased completely for a period of time. The procedure was well tolerated, with the majority of patients spending less than a week in hospital. Three of four patients who were not working before the transplant were able to return to work, confirming improved quality of life.

However, beyond 12 months, there was evidence of a progressive loss of β-cell function. Both patients with long-term insulin independence had to return to taking a small dose of exogenous insulin, even though there was evidence of ongoing islet function and prevention of hypoglycaemia. This was consistent with previously published reports showing chronic graft loss and a reduction in β-cell secretory responses at 12 months.[13,14]

The major drawback of the transplantation procedure remains the complications associated with long-term immunosuppression. This supports the current stringent selection criteria. Several patients had minor complications, such as transient mouth ulcers or oedema, that were generally treated successfully with local measures. However, one patient withdrew from the study because the side effects were considered intolerable. Another patient had a presumed recurrence of tuberculosis, and another had to stop taking tacrolimus after experiencing significantly reduced renal function. As the transplantation procedure improves, patients will remain on immunosuppression longer and hence remain at increased risk of serious side effects from immunosuppressive drugs. It is essential that only patients who have failed conventional insulin therapies be selected for transplantation.

The other major safety issue is the development of portal vein thrombosis, which occurred after two of the 11 islet infusions in our study. No characteristics of the islet preparations were considered predictive of this complication. However, in one of the patients, thrombosis was associated with an episode of line sepsis on the second day after transplant, which may have been a predisposing factor.

Our findings are consistent with similar North American and European studies.[8,14-17] In our study, 80% of patients achieved graft function, with 33% achieving insulin independence for 2 years. The Edmonton group's most recent published results of 65 recipients showed that 44 patients (68%) achieved insulin independence initially, but only 38% of those remained insulin-independent at 2 years.[18]

Currently, islet transplantation remains experimental and should not be undertaken except in the context of formal clinical evaluation. Although the procedure can achieve normal glycaemic control and relieve patients of debilitating hypoglycaemia, it is difficult to perform and requires a large multidisciplinary medical and laboratory team and intense monitoring to achieve a successful outcome. Ongoing clinical development is required to ensure more

robust long-term function and avoid early islet cell loss. The critical question of whether islet transplantation can effectively reduce secondary complications can not be evaluated until better long-term graft survival is achieved. This goal remains the primary focus of our research.

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COMPETING INTERESTS

Wyeth Australia provided the sirolimus for 1 year for each patient without charge. Wyeth had no role in the design, data collection, analysis, interpretation, writing or publication of this article.

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