Kidney Function after Islet Transplant Alone in Type 1 Diabetes: Impact of Immunosuppressive Therapy on Progression of Diabetic Nephropathy

Running title: Kidney Function after Islet Transplant Alone

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Abstract

Objective. Islet transplantation alone (ITA) is an alternative for the replacement of pancreatic endocrine function in patients with type 1 diabetes. The aim of our study was to assess the impact of the Edmonton immunosuppressive protocol (tacrolimus-sirolimus association) on kidney function.

Research design and methods. 19 patients with type 1 diabetes and metabolic instability received islet transplantation alone and immunosuppressive therapy according to the Edmonton protocol. Serum creatinine (sCr), creatinine clearance (ClCr) and 24 hour urinary protein excretion (24h UPE) was assessed at baseline and during a follow-up of 339 patient month.

Results After islet transplantation we observed: 1) sCr within the normal range in all but two patients where sCr increased immediately after islet transplantation, despite immunosuppression withdrawal patients progressed toward end-stage renal disease (ESRD); 2) ClCr remained within the normal range for those patients who had normal baseline and decreased progressing toward ESRD in two patients with a decreased baseline CrCl; and 3) 24hUPE worsened (>300 mg/24 hours) in four patients. In the two patients who progressed to ESRD the worsening of 24hUPE occurred immediately after islet transplantation. In one patient 24hUPE worsening occurred at 18 months: after withdrawal of immunosuppression it returned within the normal range. In another patient 24hUPE increased at 24 months stable while continuing immunosuppression

Conclusions. In type 1 diabetic patients receiving ITA, the association of tacrolimus-sirolimus should be used only in patients with normal kidney function. Alternative options for immunosuppressive treatment should be considered for patients with even a mild decrease of kidney function.
The Diabetes Control and Complications Trial (DCCT) has shown that, in patients with type 1 diabetes, intensive diabetes treatment reduces incidence and delays progression of long-term complications (1). The Epidemiology of Diabetes Intervention and Complications (EDIC), a follow-up of the original DCCT cohort, had shown a sustained effect of intensive diabetes treatment on the development and progression of nephropathy and macrovascular disease (2). Furthermore, EDIC has shown that patients with type 1 diabetes with some endogenous C-peptide reserve have a lower risk of progression of retinopathy and neuropathy (2). However, the benefits of intensive diabetes treatment come with the price of severe hypoglycemia and increased body weight (1).

Several studies have reported a high rate of insulin independence, and normalization of blood glucose and glycosylated hemoglobin levels after either pancreas or islet transplantation (3-7). In patients with type 1 diabetes, pancreas or islet transplantation have improved kidney graft survival (8-9), while the positive impact of pancreas transplantation on the native kidney has been counterbalanced by the nephrotoxicity of immunosuppressants, namely calcineurin inhibitors (10-11).

Since the Edmonton protocol islet transplantation alone, i.e., regardless of the need for kidney transplantation, has been proposed to patients with type 1 diabetes at increased risk of acute or chronic complications (3). However, few data have been reported on kidney function after islet transplantation alone (12-13), despite immunosuppression according to the Edmonton protocol uses the association of two potentially nephrotoxic drugs, namely tacrolimus and sirolimus (14-16).

The aim of our study was to assess the impact of the Edmonton immunosuppressive protocol on kidney function following islet transplantation alone in patients with type 1 diabetes.

Research Design and Methods

Patients

For the purpose of this study we analysed data on n=19 patients who received islet transplantation at the San Raffaele Scientific Institute between February 2001 and March 2005. Patients with type 1 diabetes were eligible for islet transplant alone if they met the following criteria: (a) diabetes duration >5 years; (b) decrease awareness of hypoglycaemia; (c) metabolic instability; or (d) progressive chronic complications despite intensive insulin regimen (i.e., ≥4 insulin injection/day or continuous subcutaneous insulin infusion). Patients with severe cardiovascular disease, evidence of progressive nephropathy (urinary protein excretion >500 mg/24 h or serum creatinine >135 µmol/L), history of chronic infectious disease (viral hepatitis, tuberculosis) or malignancy, were not eligible.

Patients were 10 males and 9 females, with mean age of 37.2 ± 9.0 years (range 24-61) and mean duration of diabetes of 23.3 ± 9.0 years (range 11-37). All patients had decreased hypoglycemia awareness; eleven patients had retinopathy; twelve patients had peripheral neuropathy; one patient had gastroparesis. Four patients had hypertension and were treated with ACE inhibitors. Two patients had mild nephropathy: one patient had macroproteinuria for two years prior islet transplantantion and the other had serum creatinine of 132.60 µmol/L, normal albumine excretion rate and hypertension. None of the patients had macroangiopathy.

Immunosuppression

All patients were treated according to the Edmonton protocol (3). Briefly: (a) daclizumab, 1 mg/kg every 2 weeks for 10 weeks, repeated after each additional islet infusion; (b) sirolimus, loading dose of 0.2 mg/kg, followed by a maintenance dose of 0.1 mg/kg day, with target plasma levels of 12-15 ng/mL during the first 3 months and then 10-12 ng/mL thereafter; and (c) tacrolimus, twice daily, starting at the dose of 2 mg/day adjusted to achieve a target plasma level of 3-6 ng/mL. In six patients sirolimus was withdrawn due to side effects (mouth ulcers; joint pain; oedema) after 24 ± 14 weeks, and replaced by mycofenolate mofetil (MMF), 2 grams/day. After 12 months of immunosuppression with
tacrolimus and MMF, one patient was changed from tacrolimus to cyclosporine because of tremor.

Other medications
Short-term antibiotic prophylaxis was administered immediately before and after islet infusion (IV cephtazidima, 1 gram t.i.d. for one day). For three months after islet infusion patients were treated with trimetoprim (800 mg/day once a day), sulphametoxazole(160 mg/day once a day) and acyclovir (200 mg t.i.d.) to prevent pneumocystis carinii and cytomegalovirus infection. In six patients acyclovir was stopped because of gastric intolerance. Fifteen patients were treated with statins because of hypercholesterolemia and four patients with ACE inhibitors because of macroproteinuria (n=1) or hypertension (n=3). During the first three days after islet infusion, insulin was administered IV using an infusion pump, and then SC until withdrawal.

Islet transplantation
Islet were isolated from pancreas obtained from heart beating cadaveric multiorgan donors, using an automated method, modified as previously described (17). The purification was performed by the centrifugation on discontinuous Ficoll gradients (Sigma Chemical Co., St Louis, MO) and was assayed by computerized morphometric method (Leica Imaging System LDD, Cambridge, UK). Islets were cultured in M199 medium supplemented with 10% fetal calf serum, 1% L-glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin and incubated at 30° C in 5% CO2 and 95% humidified air for 2 to 48 hours. Islets were tested for sterility, endotoxin (Chromogenic LAL test; Bio-Whittaker, Walkersville, MD), and mycoplasma (Mycoplasma detection kit, Boehringer Mannheim, Indianapolis, IN). Islets were infused in the liver according to the protocol approved by our Institutional Review Board, as previously reported (18-19). In brief, an ultrasound imager was used for guidance during portal vein puncture with a 22 gauge needle under local anaesthesia. Portography was performed before and after islet infusion to confirm the correct positioning of the catheter and the patency of the portal vein. Two patients received one islet infusion, 11 patients received two islet infusions and 6 patients received three islet infusions (mean islet infusion 2.1±0.7). Mean islet equivalent was 11,477±3,970 number of islet/kg of body weight.

Follow-up
Nineteen patients contributed with 3 months of follow-up, 18 patients with 6 months, 17 patients with 12 months, 13 patients with 18 months and 8 patients with 24 months. Total follow-up was 339 patient months; median follow-up was 18 patient months (range 3-24 months). Two patients dropped out from the study at 8 and 12 months, respectively, when immunosuppression was withdrawn because of deterioration of kidney function. One patient elected to withdraw from the study at 4 months because of intolerance to immunosuppression; one patient withdrawn immunosuppression after 21 months because of graft failure.

The following variables were collected at baseline and every three months after the first islet infusion: glycosylated haemoglobin (HbA1c, %), fasting C-peptide (nmol/L), exogenous insulin requirement, episodes of severe hypoglycemia, serum creatinine (sCr, µmol/L), creatinine clearance (CrCl, mL/sec) estimated using Cockcroft-Gault equation (20) and 24 hour urinary protein excretion (24hUPE, gram/24 h).

Statistical analysis
Statistical analysis was performed using SPSS for Windows, version 10.1 (SPSS Inc., Chicago, IL).

Data are presented as mean±standard error (SD). A two sided paired Student’s t test was used to compare means at baseline versus follow-up. P< 0.05 (by two tailed testing) was considered statistically significant.

Results
Islet function
Pre-transplant HbA1c% was 8.6±0.03% and decreased significantly after islet transplantation: 6.6±0.2% at 3 months (p<0.001 vs pre-transplant), 6.2±0.2% at 6 months (p<0.001 vs pre-transplant), 6.8±0.2% at 12 months (p<0.001 vs pre-transplant), 6.9±0.3%
at 18 months (p<0.001 vs pre-transplant), 6.4±0.2 at 24 months (p<0.02 vs pre-transplant). Fasting C-peptide was 0.01±0.01 nmol/L at baseline. Fasting C-peptide >0.17 nmol/L was detected immediately after the first islet infusion in all patients. Mean fasting C-peptide values during follow-up were 0.33±0.03 nmol/L at 3 months, 0.40±0.03 nmol/L at 6 months, 0.46±0.07 nmol/L at 12 months, 0.53±0.07 nmol/L at 18 months and 0.50±0.03 nmol/L at 24 months (p<0.001, vs pre-transplant). The need for exogenous insulin therapy is reported in figure 1. No episodes of severe hypoglycemia were recorded after islet transplantation, even when patients were receiving exogenous insulin therapy.

Kidney function: all patients
Serum creatinine, CrCl and 24hUPE values for individual patients are shown (figure 2). Serum creatinine levels at baseline were all in the normal range, except for one patient who had a sCr of 133 µmol/L. Serum creatinine remained within the normal range for the entire follow-up in all but two patients where sCr increased since immediately after islet transplantation. Despite immunosuppression withdrawal patients progressed toward end-stage renal disease (ESRD).

Similarly, all CrCl pre-transplant values were within the normal range, except for two patients who had a CrCl of 0.76 and 0.72 mL/sec, respectively. After islet transplantation CrCl remained within the normal range throughout the entire follow-up for those patients who had normal baseline CrCl and decreased progressing toward end-stage renal disease (ESRD) in the two patients with a decreased baseline CrCl.

After islet transplantation 24hUPE worsened (>300 mg/24 hours) in four patients. In the two patients who progressed to ESRD the worsening of 24hUPE occurred immediately after islet transplantation. In one patient 24hUPE worsening occurred at 18 months: after withdrawal of immunosuppression because of islet transplant failure 24hUPE returned within the normal range. In another patient 24hUPE increased at 24 months and remained stable, despite immunosuppression (data at 36 months not shown).

After an average of 4.5±1.3 months from the first islet infusion, sirolimus was withdrawn in six patients because of important side effects (mouth ulcers, joint pain, oedema) and patients were then started on MMF. Creatinine clearance and 24hUPE for these six patients are shown (figure 3). After shifting from sirolimus to MMF, creatinine clearance decreased in patient#1.6 ml/sec at baseline to 0.8 mL/sec at month 6, remaining stable thereafter (patient 11), while 24hUPE increased in another patient from 18 mg/24 h at baseline to 240mg/24 h at 24 months (patient #8).

Kidney function: patients with nephropathy prior to islet transplantation
Two patients had mild nephropathy prior to islet transplantation. Patient # 4 had microalbuminuria for two years prior to islet transplantation and was treated with ACE inhibitors. At baseline sCr was 88 µmol/L and 24hUPE was 195 mg/24 h. The patient received two infusions of islets and became insulin independent 4 weeks after the second infusion. HbA1c decreased from 11.6% to 6.2% in 3 months. After one month 24hUPE increased to 3,300 mg/24 h, without changes in sCr (85.75 µmol/L). At 6 months an increase of sCr (288 µmol/L) and a further increase of 24hUPE (4,600 mg/24 h) were observed. Immunosuppression was reduced and tacrolimus was stopped. Nevertheless, kidney function continues to deteriorate. Sirolimus was withdrawn at 9 months, however, with no improvement of kidney function. The patient started hemodialysis and was listed for a combined kidney-pancreas transplant. Patient # 6 developed hypertension one year prior to islet transplantation and was treated with ACE inhibitors. The patient received a single infusion of islets and became insulin independent after 3 weeks. HbA1c decreased from 7.5% to 6.1% at 6 months. Baseline sCr was 133 µmol/L and 24UPE was 133 mg/24 h. Serum creatinine increased to188 µmol/L at one month and to 235 µmol/L at 3 month. Proteinuria was detected for the first time at 3 months (3,330 mg/24 h). Because of the deterioration of kidney function, tacrolimus was withdrawn, but no improvement of kidney function was observed. At 7 months sCr
reached 277 µmol/L and also sirolimus was stopped, with no further increase in sCr. One year after completely withdrawing immunosuppression sCr was 327.08 µmol/L and 24UPE was 1,000 mg/24 h.

Conclusions

Our study shows that baseline kidney function among patients with type 1 diabetes receiving islet transplantation alone predicts deterioration of kidney function during immunosuppression according to the Edmonton protocol. In fact, during our follow-up of 339 patient months after islet transplantation, deterioration of kidney function occurred in two patients whose baseline kidney function was mildly decreased and in none of the patients whose baseline kidney function was normal.

Many studies demonstrated the effect of restoring endocrine pancreatic function, i.e., pancreas or islet transplantation, on the development and progression of diabetic nephropathy. Kidney biopsy studies by Fioretto et al. demonstrated that pancreas transplantation can reverse the glomerular changes of diabetic nephropathy, and that the reversal was evident after 10 years since pancreas transplant, but not after 5 years when only functional and morphological signs of cyclosporin nephrotoxicity were evident (10-11). However, immunosuppression consisted of only one potentially nephrotoxic drug (i.e., cyclosporin) and insulin independence was prolonged for a decade. In patients who underwent simultaneous pancreas-kidney or islet-kidney transplantation improved cumulative survival, kidney graft size and function were reported in the group with functioning pancreas or islet (21). Furthermore, insulin independence was not required for a positive effect on kidney function (8), supporting the EDIC finding that patients with type 1 diabetes with residual C-peptide function have a lower risk of diabetic nephropathy (2). The question of how to balance risks and benefits of islet transplantation in regards to kidney function is still unanswered.

In the Edmonton protocol immunosuppression after islet transplantation alone in patients with type 1 diabetes is based on the association sirolimus-tacrolimus (3). Tacrolimus nephrotoxicity is well described, while the effects of sirolimus on kidney function are just emerging (14). In kidney transplant recipients, sirolimus was not nephrotoxic, unless combined with calcineurin inhibitors (15). However, the association sirolimus-tacrolimus increased delayed graft function rate by three folds in kidney recipients (15) and caused acute graft failure in living donor kidney recipients (22). Furthermore, sirolimus can be nephrotoxic to the native kidney as reported in patients with chronic glomerulopathies (23). Sirolimus nephrotoxicity is due to direct tubular damage and, to a lesser degree, glomerular damage. In fact, sirolimus inhibits growth factor-induced proliferation of cultured proximal tubular cells and induces apoptosis (24). This effect is mediated by the inhibition of a 70 kDa S6 protein-kinase needed for cell-cycle progression (25). The early and progressive deterioration of kidney function that occurred in two of our patients who progressed to ESRD after islet transplantation may be explained by an increased sirolimus-tacrolimus nephrotoxicity in individuals with some degree of glomerular damage due to diabetic nephropathy and tubular damage due to the use of these immunosuppressive drugs. Furthermore, the extent of glomerular damage in patients with type 1 diabetes may somehow be masked by the widespread use of ACE-inhibitors, as indeed may have been the case of one of our patients (26).

Similarly, the tacrolimus-sirolimus nephrotoxicity, rather than progression of diabetic nephropathy, may have caused the progressive increase of urinary protein excretion that we observed in two patients who did not have any sign of diabetic nephropathy before islet transplantation. In fact, withdrawal of immunosuppression in one of them resulted in the decrease of proteinuria, while in the other patient proteinuria remained stable up to 36 months (data not shown), despite immunosuppression. Impairment of renal function was reported in few cases of islet after kidney transplantation that was switched from their former immunosuppressive regimen to
sirolimus and low dose tacrolimus combination (12). Recently Senior et al. (13) reported three cases of proteinuria in islet transplant recipient treated with the association sirolimus-tacrolimus. Proteinuria resolved after replacing sirolimus with MMF, and starting treatment with ACE inhibitors and angiotensin-2 receptor blockers. In these cases the reduction of proteinuria was associated with a reduction of creatinine clearance and both findings may be related to a progression of diabetic nephropathy, observed within 6 months.

Our observations on progression of diabetic nephropathy in patients who underwent islet transplant alone have to be considered in the risk benefit rate evaluation before the procedure. In patients with many years of diabetic disease and initial signs of microangiopathy, as microalbuminuria, or treated with ACE inhibitors, the association tacrolimus-sirolimus should be avoided since it can trigger the irreversible progression of diabetic nephropathy, in no case counterbalanced by the improvement of metabolic control.

In summary, in type 1 diabetic patients receiving islet transplant alone, the association of tacrolimus-sirolimus should be used only in patients with normal kidney function. Alternative options for immunosuppressive treatment should be considered for patients with even a mild decrease of kidney function.

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References
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Figure Legends

Figure 1. Insulin therapy in 19 patients with type 1 diabetes who received islet transplantation alone and the Edmonton protocol (3). Vertical Line across bars indicates additional islet transplantations.
Figure 2. Serum creatinine (mg/dL) (upper panel), creatinine clearance (mL/sec) (middle panel) and 24 hour urinary protein excretion (mg/24 h) (lower panel) in 19 patients with type 1 diabetes who received islet transplantation alone and were immunosuppressed according to the Edmonton protocol (3). Patients who withdrawn sirolimus and were changed to MMF are reported until sirolimus withdrawal.
Figure 3. Twenty-four hour urinary protein excretion (mg/24 h) (upper panel) and serum creatinine (µmol/L) (lower panel) in the six patients who withdrew sirolimus and were changed to mycofenolate mofetyl. The arrows indicate the time of drug change.