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ORIGINAL ARTICLE

Impairment of renal function after islet transplant alone or islet-after-kidney transplantation using a sirolimus/tacrolimus-based immunosuppressive regimen

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Summary

The immunosuppressive (IS) regimen based on sirolimus/low-dose tacrolimus is considered a major determinant of success of the Edmonton protocol. This regimen is generally considered safe or even protective for the kidney. Herein, we analyzed the impact of the sirolimus/low-dose tacrolimus combination on kidney function. The medical charts of islet transplant recipients with at least 6 months follow up were reviewed. There were five islet-after-kidney and five islet transplantation alone patients. Serum creatinin, albuminuria, metabolic control markers and graft function were analyzed. Impairment of kidney function was observed in six of 10 patients. Neither metabolic markers nor IS drugs levels were significantly associated with the decrease of kidney function. Although a specific etiology was not identified, some subsets of patients presented a higher risk for decline of kidney function. Low creatinin clearance, albuminuria and long-established kidney graft were associated with poorer outcome.

Introduction

Islet transplantation is gaining recognition as a standard-of-care procedure for patients with type 1 diabetes. The implementation of the Edmonton Protocol has allowed an increase from 14% to 80% in the rate of insulin independence at 1 year [1–4]. One key element for the success of the Edmonton protocol resides in the steroid-free, high sirolimus/low tacrolimus-based immunosuppressive (IS) regimen designed to avoid the islet toxicity/diabetogenicity of conventional IS regimens [5]. This IS combination, originally applied in protocols of islet transplantation alone (ITA) in non-uremic patients with labile type 1 diabetes [3], is considered the standard regimen for islet transplantation and has yielded similar results in several other centers, including in islet-after-kidney (IAK) and simultaneous islet-kidney (SIK) transplantation [6–8].

Although the sirolimus/low dose tacrolimus regimen is generally considered safe, or even protective, for kidney function [9,10], a marked impairment of kidney function has been reported in a few islet transplant recipients [2,11]. We analyzed the impact of the sirolimus/tacrolimus combination on kidney function of the distinct nephropathy-prone population of patients with type 1 diabetes receiving an islet transplant.

Patients and methods

All patients transplanted with islets of Langerhans at Geneva University Hospitals between July 2002 and January 2004, in an ITA or IAK protocol, were included in the study. Islets were isolated using a local modification of the automated method [4,12,13] and transplanted intraportally with a transhepatic percutaneous approach [14]. Patients were transplanted with two sequential islet

infusions until a total of 10 000 islet equivalents (IEQ) per kilogram body weight was reached. In a few instances, patients received a third islet infusion with the aim to achieve insulin independence.

Ten patients fulfilled the inclusion criteria: five in the IAK group and five in the ITA group. Patients received their first islet infusion between July 2002 and April 2003, with a median (range) follow-up of 10 months (8–13) for the IAK group and of 15 months (11–17) for the ITA group. One patient was withdrawn from the study after 8-month follow-up, because of a deterioration of his kidney function leading to a return to dialysis and a total loss of islet graft function (no. IAK5). One patient elected to withdraw from the study after 1-year follow-up for personal reasons (no. ITA5). Patient characteristics are shown on Table 1.

All patients received a high-dose sirolimus/low-dose tacrolimus regimen. Recipients of IAK transplants were switched from their former IS regimen to the sirolimus/tacrolimus combination at the time of islet transplantation. Patients on steroid maintenance were progressively tapered to zero at the time of waitlisting. The target range for sirolimus (Rapamune; Wyeth, Zug, Switzerland) whole blood trough levels was 12–15 ng/ml for the first 3 months and was lowered to 10–12 ng/ml after 3 months. The target range for tacrolimus (Prograf; Fujisawa, Wallisellen, Switzerland) was 3–6 ng/ml during the whole study. Induction consisted in five doses of 1 mg/kg daclizumab (Zenapax; Roche, Basel, Switzerland) given at 14-day intervals from the day of transplant. In four instances of adverse events attributed to sirolimus, the regimen was modified by lowering/discontinuing sirolimus doses, increasing tacrolimus doses and/or introducing mycophenolate mofetil.

Creatinin clearance (CrCl) was calculated using the Cockcroft-Gault formula and was followed up at each patient visit. A persistent (>1 month) decrease of CrCl >20% of the pretransplant value was considered significant. A persistent (>1 month) increase of albuminuria in a second morning sample was only considered significant if the value reached 200 mg/l in case of pre-Tx microalbuminuria, and if the value reached 20 mg/l in case of normal pre-Tx albuminuria. The increase of albuminuria was considered significant only if it did not return to pretransplant levels.

Kidney graft biopsies were taken in IAK patients whenever deemed necessary, but at the latest 1 year post islet transplantation. No native kidney biopsies were taken from ITA recipients. Islet graft function was assessed by daily insulin requirements, basal C-peptide levels, glycosylated hemoglobin (HbA1c) and fructosamine. Data were analyzed by the Student's *t*-test. Values of $P \leq 0.05$ were considered significant.

Results

Patients were allocated to two groups corresponding to preserved (group 1) or impaired (group 2) kidney function. The overall CrCl statistically significantly decreased over time (mean CrCl 72 before islet Tx vs. 57 for the worst mean post-Tx CrCl, $P = 0.009$, paired bilateral *t*-test). Of the 10 study patients, four presented a significant

Table 1. Patient characteristics.

	Group 1			Group 2			
	IAK 1	IAK 2	IAK 3	IAK 4	IAK 5		
Gender	F	F	F	F	M		
Age (years)	30	52	35	54	45		
CrCl pre-Tx (ml/min)	72	40.6	45.9	47.1	40		
Albuminuria (mg/l)	+	0	0	0	++		
Nb islet infusions	2	2	2	2	2		
IEQ/kg	18134	12559	13212	11124	11235		
Interval kidney-islet Tx (years)	3.9	12	3.6	22.8	15.3		
	ITA 1			ITA 2	ITA 3	ITA 4	ITA 5
Gender	M			F	M	M	M
Age (years)	35			56	37	28	58
CrCl pre-Tx (ml/min)	119.6			91	107	83	66.5
Albuminuria (mg/l)	0			0	0	++	0
Nb islet infusions	3			1	3	3	3
IEQ/kg	16646			9098	17744	18925	20058

Albuminuria levels were separated in normal (0: <20 mg/l), low (+: 20–200 mg/l) and high (++: >200 mg/l).
IAK 1 to IAK 5 and ITA 1 to ITA 5 are Patient IDs.
IAK, islet-after-kidney; ITA, islet transplantation alone.

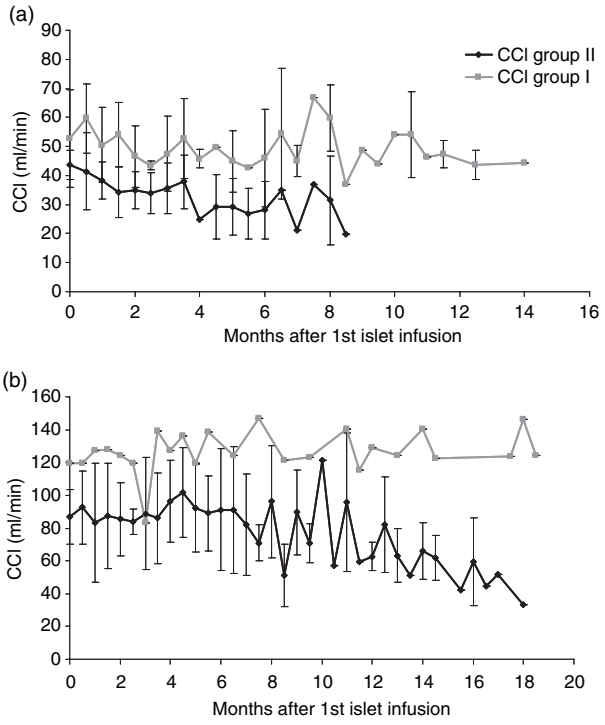

Figure 1 (a) Creatinin clearance (CrCl) in IAK patients. $n_{\text{CCI group I}} = 3$, $n_{\text{CCI group II}} = 2$. (b) CrCl in ITA patients. $n_{\text{CCI group I}} = 1$, $n_{\text{CCI group II}} = 4$. Values of CrCl, calculated according to the Cockroft-Gault formula, are shown over the duration of follow-up and expressed as mean ± SD.

decrease of CrCl (IAK: $n = 2$; ITA: $n = 2$). Two additional ITA patients presented an isolated increase of albuminuria, CrCl remaining otherwise normal. CrCl follow-up is shown in Fig. 1. Only two patients from the IAK group were on prednisone at time of waitlisting. These two patients were tapered, one from 10 mg/day to zero over 2 years, the other from 5 to 4 mg/day over 6 months, before the time of first transplantation. Neither of these patients increased their serum creatinin levels during steroid weaning.

Two IAK patients from group 2 had kidney graft biopsies because of the decline of CrCl. Patient IAK no. 4 increased her serum creatinin levels 5 months after her first islet infusion. Interestingly, this occurred 1 month after steroid discontinuation in a patient who had been transplanted with a kidney 23 years earlier. Biopsy showed signs of chronic humoral rejection with C4d glomerular deposits, and she was successfully treated with a 5-day course of intravenous immunoglobulins (Sandoglobulin, Novartis, Basel, Switzerland).

Patient IAK no. 5 showed a progressive decrease of CrCl (from 40 to 20 ml/min), reaching preterminal kidney failure over a 4-month period. A biopsy taken 4 months after islet infusion showed typical features of chronic allograft nephropathy, without signs of rejection. He finally developed sirolimus-related interstitial pneumonitis [15], which commanded discontinuation of all IS drugs and led to total loss of kidney and islet function.

As shown on Table 1, patients from group 1 had normal or low albuminuria, higher CrCl (52.8 vs. 43.6 ml/min in IAK, 119.6 vs. 86.9 ml/min in ITA), shorter time lag since kidney transplantation (6.5 vs. 19.1 years) and younger age (38.0 vs. 46.3 years) at the time of islet transplantation as compared with patients from group 2.

Immunosuppressive whole blood trough levels were similar in patients with or without impairment of renal function (Fig. 2). It should be noted that target trough levels were achieved in all patients and even slightly exceeded in some. For four patients in group 2 (ITA: $n = 2$; IAK: $n = 2$), sirolimus had to be lowered or discontinued at least temporarily because of poorly tolerated side-effects, namely mouth ulcers ($n = 2$), arthritis ($n = 1$) and interstitial pneumonitis ($n = 1$).

All four patients in group 1 achieved insulin independence for more than 3 months as compared with four of six in group 2. Both patients in group 2 who remained on insulin significantly decreased their insulin requirements. Metabolic control and graft function were similar in both groups when C-peptide, HbA1c and fructosamine levels were compared. Mean values over the course of follow-up were similar in groups 1 and 2 for C-peptide (pmol/l) (809.87 vs. 984.2 in IAK, 473.67 vs. 503.23 in ITA), HbA1c (%) (7.02 vs. 7.09 in IAK, 6.69 vs. 6.57 in ITA) and fructosamine (μmol/l) (286.6 vs. 267.03 in IAK, 273.69 vs. 284.1 in ITA).

Discussion

In this study, we show that impairment of renal function is a common occurrence after islet transplantation using a combination of high-dose sirolimus and low-dose tacrolimus. This is a disturbing finding, as this regimen was a major key to the success of the Edmonton protocol and is the only currently available association that consistently leads to insulin independence after islet of Langerhans transplantation [3,7].

Patients from both groups had similar levels of HbA1c and fructosamine, indicating similar medium-term and short-term glycemic control. Although patient numbers in this study are too low for valid statistical analysis, it is noteworthy that renal impairment was observed in those patients with lower CrCl, higher albuminuria, older age and longer interval between kidney and islet transplantation for IAK, all parameters pointing to long-lasting exposure to diabetes (median exposure time 25 years for group 1 vs. 38.5 years for group 2).

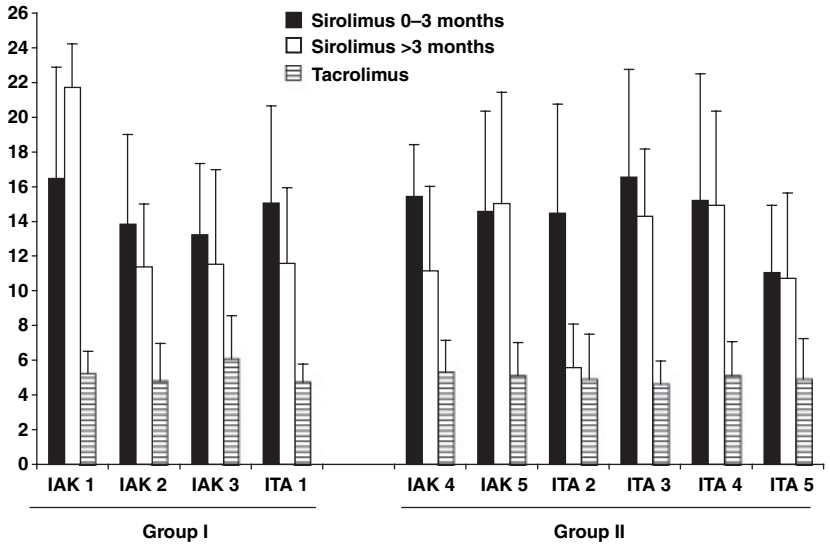

Figure 2 Immunosuppressive (IS) drugs whole blood trough levels. Mean trough levels of IS drugs are shown over duration of follow-up for each patient, and are expressed in nanogram per milliliter as mean ± SD. Black histograms represent mean sirolimus trough levels during the first 3 months, white histograms represent sirolimus levels beyond third month, dashed histograms represents tacrolimus levels.

The low-tacrolimus regimen, originally designed to minimize diabetogenicity, should also have protected study subjects from calcineurin-inhibitor nephrotoxicity. In fact, we observed a regression of the arteriolar toxicity of ciclosporine in two IAK patients who were switched from a high-ciclosporin to the low-tacrolimus regimen. Tacrolimus trough levels were successfully kept within the low target range in both groups, with near identical actual levels. Therefore, tacrolimus toxicity is unlikely to have been an isolated factor for the impairment of kidney function, but rather worked as a synergistic factor with other nephrotoxic agents or situations such as sirolimus therapy or diabetic nephropathy.

Although usually considered a harmless drug for the kidney, sirolimus has been associated with renal toxicity and delayed graft function in animal models and clinical trials [16–19]. Notably, tubular toxicity was demonstrated in a rat model [20,21]. Importantly, in the clinical setting, sirolimus seems mostly to potentiate the nephrotoxicity of calcineurin inhibitors, rather than to display an intrinsic nephrotoxicity of its own [22–25]. Although it could be argued that sirolimus levels were slightly higher than the target trough levels, they were similar in groups I and II and do not seem to explain why some patients developed kidney function impairment and others not. However, we cannot rule out that these high levels played a role in the alterations of kidney function observed.

The subset of IAK patients is confronted by the additional issue of rejection of the established kidney graft secondary to the change in the IS regimen, and indeed humoral rejection was demonstrated in the biopsy of one of our patients. Because pretransplant kidney biopsies were not performed, it is not possible to ascertain that these changes were pre-existing to islet transplantation. Additionally, all our patients were screened for anti-HLA lymphocytotoxic antibodies, and anti-GAD65 and anti-IA-2 autoantibodies prior to islet transplantation and during follow-up, and these studies have been negative in all our patients so far. The fact that our two patients with the longest time lag between kidney and islet transplantation, and thus the longest time on steroids before the weaning process, developed the most severe deterioration of renal function, indicates that special considerations must be had for IAK patients with a long-established kidney graft, and that complete steroid weaning might not be desirable in these cases.

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