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ORIGINAL ARTICLE

One-year results of basiliximab induction and tacrolimus associated with sequential steroid and MMF treatment in pediatric kidney transplant recipient

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Summary

We report the 1-year results with a triple immunosuppressive regimen in pediatric recipients of a first kidney transplant, in order to evaluate its safety and efficacy in the prevention of acute rejection and in the reduction of steroid side effects. The immunosuppression is as follows: (i) basiliximab (20 mg if body weight >30 kg; 10 mg if <30 kg) is given pretransplant and at day 4; (ii) tacrolimus (Tac) is administered in order to obtain blood trough levels of 10–20 and 5–10 ng/ml during and after the first 2 months post-transplant, respectively; (iii) steroids are tapered during the first 6 months and then replaced by mycophenolate mofetil (depending on previous rejection episodes, infection status and the result of a routine biopsy) at a dosage of 4–600 mg/m^2 body surface area. Fifty-three children (median age 13 years, range 2–20) have entered this protocol. One-year patient and kidney survival are 100% and 94% respectively. During the first year a total of nine rejections in seven patients (13% of the cohort study) occurred, all but one responsive to steroids. Renal function was satisfactory throughout the first year (mean CrCl was 63.8 ± 18 and 60.9 ± 15.5 ml/min/1.73 m^2 at 6 and 12 months respectively). Subclinical signs of rejection were absent in more than 80% of biopsies (grade I Banff) at 6 months ($n = 47$); at the 12th month biopsy ($n = 42$) score I was stable in 20 patients (16 after stopping steroids) and had worsened in eight biopsies (six after stopping steroids). Major complications were insulin-dependent diabetes in three (5.6%) children with the need of insulin for a mean of 3 months; transient hyperglycemia (11 patients), treated with a dietary regimen, symptomatic viral infections (in 11 patients: two parvovirus B19, three cytomegalovirus and two Epstein–Barr virus systemic infections, three interstitial pneumonia, two BK nephritis). Tac doses more than 0.3–0.4 mg/kg/day are at significantly higher risk of viral infection. In conclusion, this immunosuppressive regimen is associated with a low percentage of clinical (13%) and subclinical rejections, but with a relatively high number of infections, prevented by a reduction in Tac doses (<0.3 mg/kg/day) during the first 2 months after transplantation. The assessment of steroid withdrawal needs a longer follow-up.

Introduction

Kidney transplantation represents the ultimate goal in the treatment of end-stage renal failure patients. When the recipients are children, it is important to bear in mind that their life expectancy is, hopefully, quite long. Consequently, graft and patient survival have to be assessed in the very long-term. Any factors that have a negative influence on long-term graft survival should be minimized, as well as adverse events and effects of drugs, in order to improve the clinical conditions and the quality of life of our young patients.

The incidence of acute rejection in the first 6 months after transplantation ranges from 10 to 35%, even with the new drugs available for immunosuppression [1,2]. Previous studies have indicated that early acute rejection is a significant risk factor for subsequent allograft nephropathy and long-term graft loss, which still is the leading cause of late loss of the transplanted kidney [3]. In a pediatric population the use of steroids produces well-known adverse metabolic effects and growth impairment [4–6]; furthermore, it has recently been reported that adult patients, in whom steroids have been withdrawn, experience a better quality of life [7, 8]. The side effect profile and morbidity associated with corticosteroids have stimulated attempts to withdraw these agents, but they are generally associated with an increase in acute or chronic rejection [9–11]. On the contrary, recent studies have shown that steroid withdrawal can be safe and improves growth in selected groups of children, when no early clinical acute rejection episodes have been documented, or when a careful clinical follow-up, including protocol biopsies, is undertaken [12–14].

With the availability of new monoclonal antibodies targeting the interleukin-2 receptor (the chimeric monoclonal antibody basiliximab) and the immunosuppressors tacrolimus (Tac) and mycophenolate mofetil (MMF), we designed an immunosuppressive protocol with the aim of (i) reducing the number of acute rejections; (ii) reducing the secondary side effects of steroids, stopping their usage 6 months post-transplantation and monitoring the graft by means of protocol biopsies. In this paper we describe the one-year results in 53 patients.

Patients and methods

Immunosuppressive regimen

Our treatment protocol is summarized in Fig. 1. Two basiliximab (Simulect; Novartis Pharmaceuticals, East Hanover, NJ, USA) injections were administered, one immediately pretransplant and the second on the fourth day postoperatively, at the usual dosage of 10 mg if body weight (bw) <30 kg and 20 mg if bw >30 kg. Tac (Prograf; Fujisawa GmbH, Munich, Germany) was given at an initial dose of 0.3 mg/kg/day in order to achieve trough blood levels of 10–20 ng/ml during the first 2 months after transplantation and thereafter 5–10 ng/ml. Steroids were given intravenously for the first 2–3 days (10–15 mg/kg/day) and then orally, tapering down from 1 mg/kg/day at day 3, to 0.1–0.2 mg/kg/day at 6 months and subsequently even further with the aim of complete cessation 2–3 months after graft biopsy. Before cessation of steroid administration, MMF (Cell Cept; Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany) is introduced at a dose of 4–600 mg/m^2 body surface area.

The criteria for steroid withdrawal were: one or no acute rejections in the previous 6 months, panel reactive antibody titer <40% and a graft biopsy at 6 months with a score of 1 on the Banff 97 classification for acute rejection [15].

The clinical definition of rejection was a stable increase in creatinine concentration of at least 20%, isolated or in

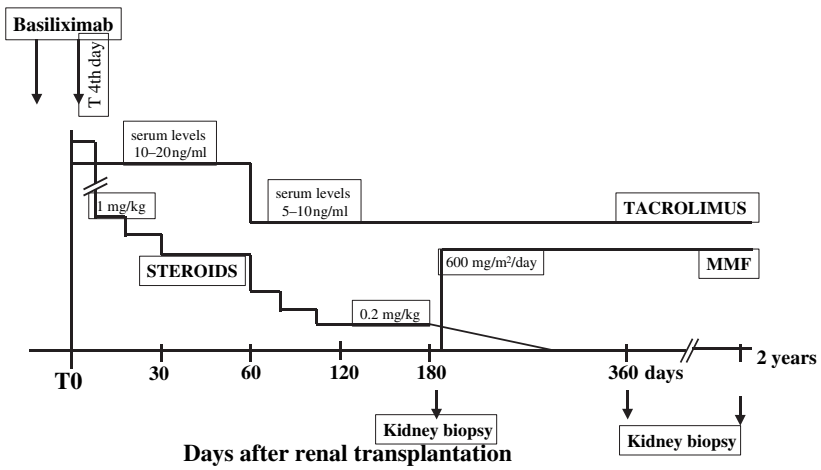

Figure 1 Treatment protocol.

combination with any of the following: reduced urine output, ultrasound signs of rejection, fever, histological evidence of rejection and the decision to initiate an anti-rejection treatment.

Anti-infection prophylaxis

All patients received i.v. antibiotic treatment (generally a second or third generation cephalosporin) for a mean of 7 days after surgery. Antiviral prophylaxis was given for 3–6 months after transplantation with acyclovir (40 mg/kg/day) in cytomegalovirus (CMV)+ recipients (R) from - donors (D) or gancyclovir (50 mg/kg/day) in CMV R−/D+, R+/D+ and R−/unknown status of the donor. Intravenous immunoglobulins (100 mg/kg/dose for three doses) were performed if a R− received a kidney from a D+. No antifungal prophylaxis was given.

Patients

Fifty-three consecutive first kidney transplant patients, transplanted between June 2000 and July 2002 in the participating centers, were enrolled in the study. Thirty-one were male and three had a transplant from a living relative, mean human leukocyte antigen A, B, DRB1 mismatch was 3.3 ± 0.8. The median age of the patients was 13 years, range 2–20; 10 patients were under 6 years of age. The primary diseases were the usual spectrum of diseases which produce end-stage renal failure in children [uropathy 24%, hypodysplasia 23%, hereditary disorders 21%, glomerulopathy (+FSGS) 19%; others or unknown 13%]. Patients or their parents gave informed consent to this immunosuppressive regimen.

We divided the cohort of the patients into two groups: (i) group A: 27 (18 males, median age 13.3 years, range 2–20) and (ii) group B: 26 patients (13 males, median age 12.2 years, range 2.5–20) transplanted before and after mid-July 2001 respectively. At this time a preliminary analysis had shown a relatively high incidence of viral infections (10 in the first 27 transplanted children). We therefore decided to keep the Tac trough blood levels in the lower part of the protocol-required ranges, especially during the first 2 months after transplantation, with a maximum dosage of 0.4 mg/kg, even when the blood levels were not completely satisfactory.

Donor and recipient CMV and EBV status

Pretransplant screening for anti-CMV and Epstein–Barr virus (EBV) antibodies was performed for almost all patients. Twenty-four and 31 children had a previous, but inactive CMV and EBV infection (IgG+) respectively; in two patients the EBV status was unknown. The remaining recipients were CMV or EBV negative. The CMV status was known in 45 donors (32 were IgG+): 19 kidneys from +donors went to −recipients. The donor EBV status (12 IgG+) was known only in 16 cases: five kidneys from +donors went to −recipients.

Follow-up

Patients were followed-up on a routine outpatient basis, monitoring renal function, Tac blood concentrations, blood pressure, fasting blood glucose and other usual biochemical parameters. Infection monitoring was focused on CMV (antigenemia levels pp65), EBV and parvovirus B19 (PB19) using PCR. BK virus infection was monitored by means of PCR evaluation of urine and blood, the remaining parameters of viral status (i.e. herpes zoster virus, herpes simplex virus, hepatitis virus) were monitored by means of the determination of the serological status.

A general clinical and laboratory evaluation and a renal biopsy were performed at 6 and 12 months after transplantation. In the graft specimen renal histology and immunohistochemical markers of renal damage were evaluated. The Banff 97 working classification was utilized to evaluate the lesions occurring for acute rejection semi-quantitatively. No patients were lost to follow-up.

Efficacy and safety parameters

The following efficacy parameters were evaluated: incidence of acute rejection, the Banff 97 grading obtained from the routine biopsies, patients who did not withdraw steroids or needed re-introduction of steroid treatment, renal function measured by creatinine levels or creatinine clearance (Schwartz formula) [16], graft loss and recipient death. The safety parameters were the incidence of opportunistic infections (in particular viral infections), adverse events and in particular hyperglycemia, arterial hypertension and death.

Statistical analysis

Data are expressed as mean ± SD or median and range as appropriate. Comparisons between different groups were made using Student's *t*-test or Mann–Whitney test for normal distributions and distribution-free variables respectively. The Pearson's chi-squared test was utilized to evaluate the difference in the prevalence of infections. $P < 0.05$ was considered to be statistically significant.

Results

All the 53 patients have reached the 1 year follow-up. The main clinical and biochemical data are summarized in Table 1, which documents satisfactory kidney function.

Table 1. Main clinical and biochemical data.

	First month	Sixth month	Twelfth month
Kidney survival	53/53	50/53	50/53
SCr (μmol/l)	92.5 ± 52.8	98.6 ± 41.1	97.8 ± 34.0
CrCl (ml/min/1.73 m^2)	69.2 ± 21.7	63.8 ± 18.4	60.9 ± 15.5
Proteinuria (g/day)	0.12 ± 0.14	0.1 ± 0.13	0.13 ± 0.17
Tac dose (mg/kg/day)	0.32 ± 0.15	0.2 ± 0.15	0.16 ± 0.08
Tac trough levels (ng/ml)	13.5 ± 4.5	8.5 ± 2.6	8.1 ± 2.1
Steroid dose (mg/kg/day)	0.5 ± 0.13	0.18 ± 0.1	0.10 ± 0.1
Blood glucose (mmol/l)	4.9 ± 0.8	4.9 ± 0.9	4.8 ± 0.6
Rejections	3 (1SR)	4	2
Steroid withdrawal			32
MMF (mg/day)			42 (697 ± 270)

Patient survival was 100% at 6 and 12 months; kidney survival was 94% at 6 and 12 months. Three kidneys were lost, one because of recurrent FSGS, one because of microangiopathy secondary to PB19 associated with a ureteric stenosis, and one because of nephropathy secondary to BK virus. One patient was switched from Tac to cyclosporin A (CyA) because of biopsy-proven nephrotoxicity.

There were three episodes of rejection in the first month after transplantation, four episodes of rejection from 1 to 6 months, and two from 6 to 12 months. There were a total of nine rejections in seven patients. At 12 months, 13% of our patients experienced at least one acute episode of rejection.

Steroids were withdrawn from 32 patients [main reason for not stopping steroids in 21 patients were: graft lost ($n = 3$), clinical rejections and/or routine biopsies with Banff (97) score >1 ($n = 10$), recurrent FSGS ($n = 1$), switch to CyA because of Tac toxicity ($n = 1$), EBV chronic infections ($n = 2$), nonadherence to the protocol ($n = 2$) and adverse effects secondary to the introduction of MMF ($n = 2$)]; MMF was started in 42 patients at a mean dose of 591 ± 128 mg/day.

Routine biopsies

The results according to the Banff 97 classification for acute rejection as applied to 6 and 12 months are shown in Table 2; 37/43 (86%) adequate specimens at 6 months did not show signs of acute rejection.

Here we describe the histological course (between the sixth month and the 12th month specimen) of the 42 biopsies obtained at 12th month: grade I was stable in 20 graft biopsies (in 16 patients the steroids had been withdrawn; four had remained on steroids); of the other two graft biopsies with score I at 12 months, one was inadequate for diagnostic purposes, the other was in category 3, at the sixth month sampling. Eight grade I biopsies at the sixth month showed worsening of the histological picture (three progressed to category 3, two to 4IA and three to 5); six patients had suspended the steroids and two had not. The four inadequate specimens at the sixth month were categorized at 12th month as follows: 1, 3, 4IA and 5I (three with and ome without the suspension of steroids). One graft progressed from category 4IA to 5I, without the suspension of steroids, the 6th month biopsy was not collected in two patients, because of clinical reasons and the remaining five biopsies were not adequate.

Table 2. Banff 97 classification for acute rejection of the sixth month and 12th month biopsies.

Banff 97	Sixth month	Twelfth month
Biopsies performed	47	42
Inadequate specimen	4	6
Normal	37	22
Antibody mediated rejection	–	–
Borderline changes (no intimal arteritis, foci of mild rejection)	2	4
Acute/active rejection		
Int. infiltration + moderate tubulitis	3	3
Int. infiltration + severe tubulitis	–	1
Chronic/sclerosing allograft nephropathy grade I (mild)	1	4
Grade II (moderate)	–	2

Tacrolimus doses and trough levels

There was a great variability in the dosage and in the blood levels of the drug. Some patients needed doses up to 0.7 mg/kg to reach a satisfactory trough level, during the first weeks of treatment. Mean Tac doses and blood levels are reported in Tables 1 and 3, with statistically significant lower dosages (mg/kg/day) in group B.

Infection rates

Eleven patients (21%) developed the following viral infections: three pulmonary infections (CMV and respiratory syncytial virus), five systemic infections (three CMV and two EBV), two PB19 (one graft microangiopathy), one varicella-zoster virus, two BK virus interstitial nephritis; of these infections, one case of ureteral stricture related to BK virus ureteritis, characterized by acute graft failure, without clinical signs of systemic viral infection, has been recently reported [17]; the patient developed subsequently an interstitial nephritis which was the cause of graft loss. The incidence of infections was statistically higher in group A (10/27) than in group B (one of 26) (OR 14.7, 95% confidence interval 1.7–125.7; Pearson's $\chi^2 = 8.871$;

Table 3. Main clinical and biochemical data divided by groups A and B.

	First month		Sixth month		Twelfth month	
Group	A	B	A	B	A	B
Kidney survival	27/27	26/26	24/27	26/26	24/27	26/26
SCr (μmol/l)	104 ± 67	79 ± 25	110 ± 50	86 ± 24	104 ± 37	90 ± 29
CrCl (ml/min/1.73 m^2)	63 ± 23*	76 ± 18*	58 ± 21‡	69 ± 14‡	58 ± 17	66 ± 13
Tac dose (mg/kg/day)	0.36 ± 0.18†	0.25 ± 0.1†	0.21 ± 0.12	0.19 ± 0.19	0.17 ± 0.08	0.13 ± 0.06
Tac trough levels (ng/ml)	14 ± 4.7	13.1 ± 4.1	9.1 ± 2.9§	7.6 ± 1.9§	8.4 ± 2.4	7.8 ± 1.5
Steroid dose (mg/kg/day)	0.54 ± 0.1	0.46 ± 0.1	0.19 ± 0.1	0.18 ± 0.1	0.08 ± 0.1	0.1 ± 0.1
Blood glucose (mmol/l)	4.3 ± 0.8	5.2 ± 2.4	4.8 ± 1.0	5.0 ± 0.7	4.7 ± 0.6	4.8 ± 0.6
Biopsy Banff (% of grade 1)			83%	82%	61%	58%
Steroid withdrawal					17	16

Groups A and B are divided on the basis of Tac dosages (see Patients). Group A, 10/27 infections; group B, 1/26 infections.
*$P = 0.034$; †$P = 0.041$; ‡$P = 0.019$; §$P = 0.019$ group A versus group B.

$P = 0.003$). In this group of patients, as shown in Table 3, Tac dosages ($P = 0.04$) and blood trough levels ($P = 0.02$) were significantly higher than in group B, at 1 and 6 months after transplantation, respectively; kidney function (creatinine clearance) was significantly higher in group B, during the first 6 months after transplantation ($P = 0.034$ and 0.019 at 30 and 180 days respectively); data at the 12th month followed the same trend (58 ± 17 vs. 66 ± 13), although the difference did not reach statistical significance ($P = 0.08$). The reduced Tac dosages resulted in a clear reduction in infections and, contemporarily, in a satisfactory immunosuppression: the percentage of Banff grade I biopsies was equal in the two groups (82% vs. 83%) at the sixth month biopsy.

On the same basis Tac dosage was significantly higher in the 11 patients (ng/ml) who developed a viral infection versus the 42 who did not (0.44 ± 0.20 vs. 0.32 ± 0.11, $P = 0.05$; 0.43 ± 0.19 vs. 0.28 ± 0.12, $P = 0.011$; 14 and 30 days after transplantation respectively).

No major bacterial or other infectious complications developed in our patients, except 11 urinary tract infections (in one case *Candida* was the etiological agent) and two cases of gastroenteritis (one sustained by *Salmonella* Typhi and one by criptosporium).

In 38 patients, in whom we were able to monitor viral status closely, the following asymptomatic infections were noted: three CMV, seven EBV, and two PB19.

Adverse effects

No hypersensitivity reactions to any of the immunosuppressive drugs utilized were noted. One patient was switched from Tac to CyA because of a biopsy-proven nephrotoxicity. No malignancies developed during the first 12 months.

Three patients (5.6%) developed insulin-dependent diabetes; insulin was utilized for 2, 3 and 4 months, respectively, with normalization of blood glucose levels, probably related to the reduction in steroids and Tac dosages. Moderate and transient hyperglycemia, with the need of dietary recommendations, occurred in 11 patients, exclusively during the first months post-transplantation.

Arterial hypertension was common with 70% of patients needing at least one antihypertensive drug versus 47% of the patients pretransplantation. The majority of the children ($n = 30$) were taking one or two antihypertensive drugs, three and one patients were taking three and four antihypertensive drugs, respectively.

Discussion

Tacrolimus has proven to be an effective immunosuppressive drug in terms of patient and graft survival in children in a large prospective trial [18] and in small, single center, nonrandomized trials [19, 20]. Efficacy was maintained in renal adult transplant recipients, after total withdrawal of corticosteroid therapy, the incidence of acute rejection episodes being similar with or without steroids [21,22]. The rationale of our immunosuppressive protocol was to keep the incidence of acute rejections, in the early post-transplant phase, as low as possible, using the chimeric monoclonal antibody basiliximab, in order to switch from corticosteroids to MMF, during the second half of the first post-transplant year.

Our protocol showed very satisfactory results: all patients are alive and the overall graft survival after 1 year is 94%; during the first 6 months, rejection rate was low and protocol biopsies showed the absence of subclinical events in the majority of biopsies (Banff grade 1 in 86% of biopsies at 6 months).

We agree with what has been previously reported [23]: the combination of basiliximab and Tac, in addition to steroids, represents a safe and effective initial treatment of kidney transplant recipients. We must emphasize the

necessity to pay attention to the total daily doses of Tac, together with the monitoring of blood levels, as side effects, mainly viral infections, appear at high dosages/kg of the drug, especially during the very first months after transplantation. In our study we found a significant difference between group A and group B in the daily dosage of Tac at 14 and 30 days, but not in Tac blood levels; in addition, the incidence of infections was significantly higher in group A (OR 14.7), whereas the degree of immunosuppression was satisfactory in both groups (Banff 97 grade I in 80% of routine kidney biopsies in both groups). Kidney function of the graft appears better in group B; it is tempting to speculate that the worst glomerular filtration rate of group A could be partly related to the use of the antiviral drugs, which have a well-known nephrotoxic effect, and also to a nonspecific negative effect on the kidney by the etiologic agent itself, which could trigger clinical or subclinical rejections [24,25]. Another possible explanation is the nephrotoxicity of higher levels of Tac. Furthermore viral infections (one microangiopathy secondary to PB19 and one nephropathy secondary to BK virus) represent the primary cause of the loss of two of the three kidneys lost during the first year of follow-up. It is our feeling that currently available therapeutic agents to prevent acute rejections are very effective; a major goal in the future is represented by the necessity to reduce side effects and ameliorate chronic/sclerosing allograft nephropathy via better modulation of immunosuppression, which, in our series, has yield satisfactory results in terms of infection reduction.

Steroid withdrawal has been repeatedly attempted in the past with variable results [9–14]; our protocol used a combination of new and potent immunosuppressors, such as MMF and Tac, in patients monitored with routine kidney biopsies to minimize the risk of graft loss. We stopped steroids in 32 patients; some degree of immunologic activation of the Banff 97 categories was present in some of the 12th month biopsies. As our series lacks a control group, the follow-up after steroid withdrawal is too short to permit a complete evaluation of the efficacy and safety of this therapeutic regimen.

In conclusion, the combination of Tac and basiliximab, in association with steroids, is a safe and efficient immunosuppressive regimen, associated with a low number of acute rejection episodes and a high percentage of normal biopsies at the 6th month biopsy. We recommend Tac doses lower than 0.3–0.35 mg/kg/day during the first 2 months of treatment, when an induction therapy (in our case basiliximab) is added; this Tac dosage allows a good balance between immunosuppression and side effects, especially infections, to be achieved. The 12th month biopsy showed an increase in immunologic activation and in the appearance of chronic allograft nephropathy, albeit to a mild degree. The feasibility and the effects (especially on growth) of steroid withdrawal needs a longer follow-up period.

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