Early Steroid Withdrawal in Pediatric Renal Transplantation at a Single Center: Preliminary Report

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ABSTRACT

Steroids have been a cornerstone in renal transplant immunosuppression despite cardiovascular risk and growth impairment in children. New immunosuppressive drugs have allowed early withdrawal or even complete avoidance of steroids. To evaluate a new immunosuppressive protocol with early withdrawal of steroids in a pediatric renal transplant population, we initiated a prospective study in recipients >1 year old who showed low immunologic risk was started. Group A ($n = 12$) received decreasing doses of steroids until day posttransplant 7 under a regimen of Tacrolimus (FK) and mycophenolate mofetil (MMF). Group B ($n = 11$) were controls treated with steroids, cyclosporine and azathioprine. In both groups, induction therapy included basiliximab. We evaluated anthropometric and biochemical variables, acute rejection episodes (ARE), and cytomegalovirus (CMV) infection. Mean values and variations for continuous variables were calculated at months 1 and 3 for comparison at the same time using student's *t*-test and regresion analysis. We obtained mean values at months 1, 3, and 6 for groups A and B of creatinine clearance (mL/min): 86.2 versus 107.4; 76.9 versus 96.6; 73.3 versus 97.9 ($P < .05$); hematocrit (%) was 27.4 versus 31.8; 29.3 versus 33.9; 32.9 versus 34.3% ($P < .05$); total cholesterol (mg/dL), 148 versus 195, 139 versus 85, 142 versus 174 ($P < .05$); creatinine clearance decreased in both groups during follow-up with a smaller slope among group A ($P < .05$). No differences were observed between the groups in Z height, diastolic and systolic blood pressures at 6 months of follow-up. Serum total cholesterol mean levels at months 1, 3, and 6 were significantly lower among the group withdrawn from steroids ($P < .05$). Plasma bicarbonate levels were lower among group A than B; there was no difference in blood glucose levels. No AREs and no difference in CMV infections were observed. In conclusion, early withdrawal of steroids with FK and MMF was not associated with a higher incidence of either ARE or CMV infection. Lower levels of cholesterol could imply a reduced cardiovascular risk. Longer follow-up is needed to evaluate the impact of this therapy on renal function and linear growth.

STEROIDS HAVE BEEN the cornerstone of immunosuppressive therapy in renal transplantation for the last 40 years. However, they have important side effects, including cardiovascular risk, infections, hypertension, hyperlipidemia, glucose intolerance, diabetes, bone mass loss, overweight, cataracts, Cushing syndrome, and growth impairment, which are particularly important in children.[1] Multiple trials have been performed to space, reduce or avoid steroid therapy. Administration on alternate days has shown a better outcome on height in the prepuberal pediatric population,[2,3] but it has been difficult to use this schedule because of reduced adherence.[4] Depending on the immunosuppressive protocol and the timing of steroid withdrawal, the first reports in adult patients have shown that steroids had to be reintroduced in more than 50% of the cases after acute rejection episodes (ARE).[4–6] The first meta-analysis of randomized, controlled trials in adults with regard to steroid withdrawal in renal transplant recipients showed an unacceptable rate of ARE. The majority of these

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trials were based on cyclosporine with or without azathioprine.[7–9] Recently, new, more potent immunosuppressive drugs have led to an important decline in the incidence of ARE providing a substrate to attempt steroid withdrawal.[10] To date, there is no meta-analysis of randomized, controlled pediatric patients. These experiences in adults cannot be extrapolated to the pediatric population. The development of new immunosuppressive drugs, including interleukin 2 receptor blockers, daclizumab and basiliximab, mycophenolate mofetil (MMF) and tacrolimus (FK), have lead to new immunosuppressive protocols for steroid withdrawal and even their total avoidance. However, most pediatric reports include only small numbers of patients, which are not controlled prospective trials and also have only short follow-up.[1,11,12] The purpose of this trial was to prospectively evaluate a new immunosuppressive protocol using FK, MMF, and basiliximab with early steroid withdrawal in pediatric renal transplantation using a comparison with historical controls to prevent the adverse effects of steroids without affecting allograft survivals.

PATIENTS AND METHODS

A prospective study was performed on patients aged 1 and 15 years who received first renal transplants from a living or cadaver donor and were of low immunologic risk (panel reactive antibody <10%). They had not previously received steroids and did not have autoimmune diseases. Acute tubular necrosis immediately after transplantation, was defined as requiring dialysis, an exclusion criterion for the study. The control group was a historical group of patients, with similar characteristics. In both groups, basiliximab (Simulect) was administered in two doses: one at transplant time and the other on day 4. Group A received FK, MMF, and steroids in decreasing doses until their discontinuation on posttransplant day 7. Group B used historical immunosuppression: cyclosporine, azathioprine and daily steroids. Prospective data were obtained from group A patients and those for group B from the clinical chart. Evaluated variables included epidemiologic characteristics, anthropometric measurements, donor data, cold ischemia time, blood and urine biochemical parameters, cytomegalovirus (CMV) infections, and AREs. Renal function was estimated by the Schwartz formula. Independent of the clinical condition, CMV infection was diagnosed when more than 10 nuclei among 400,000 cells showed positive antigenemia. Height for age as diastolic and systolic blood pressure were compared using Z score, according to a National Child Health Study and the last Task Force Report, respectively.[13] Patient follow-up was performed according to a preexistent transplant protocol. Anthropometric, clinical, and laboratory results were recorded monthly. Acute rejection was suspected when creatinine increased more than 20% of the basal value on two successive controls, necessitating renal biopsy with light microscopy, immunofluorescence, and electron microscopy. Acute rejection was confirmed according to the Banff criteria.[14] All biopsies were analyzed by the same pathologist. Patients in group A, with ARE less than grade II according to Banff criteria and a favorable response to steroids were returned to their previous immunosuppression. When a second ARE or a first ARE scored higher than grade II by Banff classification ocurred, steroids were added to the immunosuppressive schedule. In contrast, conversion of the immunosuppressive protocol from FK to cyclosporine owing to hyperglycemia or glucose intolerance, was not an indication for protocol exclusion. Patient and allograft survivals at 1 year posttransplantation were evaluated using Kaplan–Meier curves. Our center Institutional Review Board approved the study before inception; informed consent was required before inclusion in the protocol.

Statistical Analysis

Variables at 1, 3, and 6 months were compared between groups by means of the Student's *t* test. Tendency analysis along time utilized regression repeated measures (generalized estimating equations).[15] Significance was assigned to P values $< .05$ with a confidence interval of 95%.

RESULTS

Among 12 patients in group A, 2 were withdrawn by month 2; one of whom at transplantation underwent extirpation of a supranumeric hepatic lobule, requiring digestive tract surgery presenting as untreatable diarrhea, causing MMF to be suspended and steroids to be initiated. The second was a toddler of 2.7 years, with recurrent bronchial obstructive syndrome and segmental lung hypoplasia, who developed severe adenoviral bronchopneumonia, *Pseudomona aeroginous* bacterial and *Aspergillus* mycotic septicemia resulting in death. Table 1 shows the demographic characteristics of the groups.

Renal Function, Growth, and Incidence of Acute Rejection

A decreased creatinine clearance was observed in both groups, which was significant for group A at months 3 and 6 ($P < .05$). Both groups showed a negative variation of creatinine clearance during follow-up, but it was minor in the steroid withdrawal group ($P < .05$). No differences were observed in height/age, or diastolic and systolic blood pressure Z score at 6 months follow-up. Mean serum total cholesterol levels at 1, 3, and 6 months were significantly lower among the group withdrawn from steroids ($P < .05$).

Table 1. Patient Characteristics: Groups A and B

Characteristics	Group A (n = 12)	Group B (n = 11)	P
Recipient mean age (range)	7.8 (2.8–12.2)	6 (1.1–14.2)	NS
Male, n (%)	2 (17)	5 (42)	.05
N° PD/HD/preemptive	7/4/1	9/1/1	NS
End-stage renal disease etiology			
Renal dysplasia	5	5	
Reflux nephropathy	0	1	
Glomerulopathy	1	2	
Unknown/others	6	3	
Cadaver donor	9	6	NS
Cadaver donors mean age (range)	36 (1.7–46)	21.5 (2.2–40)	NS
Donor gender (male/female)	7/5	4/7	NS
Cold Ischemia hours, mean (SD)	19.8 (3.5)	18.5 (8.9)	NS
"En bloc" transplants	1	1	NS

Mean glucose blood levels were significantly lower in group A at month 3 (Table 2). There were no AREs in either group. Three renal biopsies were performed during the follow-up period in group A: one each showed renal toxicity owing to FK, recurrence of focal and segmental glomerulosclerosis, and normal findings with negative immunofluorescence for C4d. No difference in CMV infection was observed, only an increased antigenemia in five patients in group A and three in group B without evidence of CMV symptomatic disease.

Hematologic Profile and Acid–Base Metabolism

Both groups developed anemia during the initial posttransplant months. Mean hematocrit values for group A were lower at 1, 3, and 6 months. Patients in the steroid withdrawal therapy group displayes significantly lower white cell and absolute neutrophil counts during the first month ($P < .01$), which normalized at month 6. There was no difference in platelets numbers. Plasma sodium bicarbonate levels were lower among the steroid withdrawal group during the initial period post transplant.

DISCUSSION

Current immunosuppressive therapy has improved allograft survival among pediatric recipients in our centers. A recently published experience showed a 5-year 72% graft survival rate among 100 allografts.[16,17] Steroids have adverse effects on cardiovascular disease risk factors and growth impairment. Pediatric patients, especially infants, displayes greater immune responses than adults.[18] New immunosuppressive protocols have been developed with fewer adverse effects, but similar or greater immunosuppressive potency. Recent reports of immunosuppression without or with early steroid withdrawal in renal transplantation are more optimistic.[19,20] Steroids inhibit effector molecules of cytotoxic cells, which are involved in adaptative immune responses leading to early graft acceptance.[21,22] This experience is the first preliminary report of a prospective protocol in our center, wherein the number of boys was less than the number of female patients. We plan an intake of 30 patients per group.

The mean creatinine clearance was lower among the group with early steroid withdrawal at the beginning of the study in opposition to other reports.[23] This observation could not be explained by differences in age or cold ischemia time. Cornelis et al[24] reported that the group without steroids initially showed a lower creatinine clearance, but by 12 months posttransplantation it was similar for both groups. This observation could be explained by an effect of steroids to increase glomerular filtration rates (GFR). The creatinine clearance in this protocol was estimated by the Schwartz formula, which normalizes GFR to body surface. Thus, although the historical controls were not age or size matched, the GFR in this case was comparable. However, GFR does vary by gender, group A had significantly fewer boys. This factor would tend to artificially lower the GFR, particularly in a small sample. No differences in height/age Z scores between the groups were observed at 6 months follow-up; however, this period might not be sufficient to show differences in growth rate. Also, metabolic acidosis may influence the lack of growth in the group with early steroid withdrawal. No differences were observed in arterial blood pressure, although we had expected blood pressure to be higher among the steroid group. This protocol showed significantly lowered total cholesterol levels when compared with the control group; this finding may imply a decrease incidence of early vascular disease.

There was an important degree of anemia in the steroid withdrawal group according with Sarwal report,[1,19] probably owing to MMF immunosuppression and the lack of steroids stimulus to the bone marrow. Also the bioavailability of MMF is greater without concomitant steroid use,[25] suggesting that lower dosing is important to limit MMF toxicity usually evidenced by gastrointestinal toxicity and leukopenia. These findings suggested that we should lower the MMF dose from 800 mg/m^2 per day to 600 mg/m^2 per day during the first month and to 400 mg/m^2 per day afterward. The higher incidence of metabolic acidosis among children with steroid withdrawal had not been previously described. It could be related to the absence of mineral corticoid effect as described with calcineurin inhibitor drugs.[26] Currently, our protocol does not include anti-CMV prophylaxis; we only monitor the CMV antigen levels (pp65). There was no difference in CMV infection observed between groups and no patient presented symptomatic CMV disease. The choice of an induction agent for steroid elimination is still a matter for debate; we used

Table 2. Biochemical Parameters and Growth at Months 1, 3, and 6

	Month 1			Month 3			Month 6		
Variable	A (12)	B (11)	*P*	A (9)	B (11)	*P*	A (5)	B (11)	*P*
Creatinine clearance	86.2 ± 18.3	107.4 ± 21.1	*	76.9 ± 20.6	96.6 ± 20	*	76 ± 19.5	97.9 ± 17.8	*
Hematocrit	27.4 ± 6.9	31.8 ± 3.4	*	29.3 ± 3.6	33.9 ± 3.6	*	32.9 ± 7.8	34.3 ± 3.0	*
Glucose blood	88 ± 13	83 ± 11		82 ± 10	93 ± 13	*	93 ± 9	90 ± 9	
Total cholesterol	148 ± 34	195 ± 47	*	139 ± 29	185 ± 38	*	142 ± 174	174 ± 49	*
Bicarbonate	19.6 ± 1.97	22.2 ± 2.4	*	19.8 ± 3.32	22.7 ± 1.93	*	20.7 ± 4.9	20.9 ± 2.27	
Height/age Z score	−2.5 ± 1.2	−2.4 ± 1.5		−2.3 ± 1.0	−2.2 ± 1.6		−2.6 ± 0.97	−2.1 ± 1.47	

Values are presented as means ± standard deviation.
*$P < .05$.

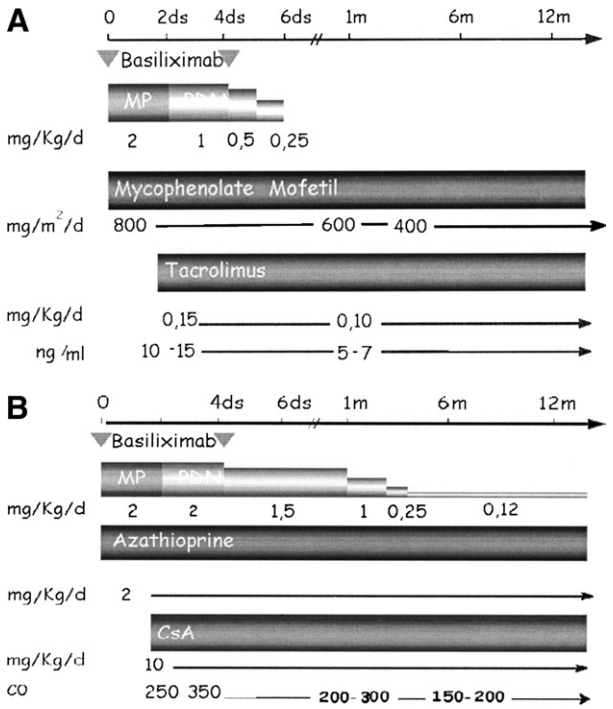

Fig 1. Induction therapy: basiliximab in both groups. Maintenance immunosuppressive therapy. **(A)** Group A: early withdrawal of steroids. **(B)** Group B: historical controls.

basiliximab in two doses. The most important benefit provided by basiliximab is its lower cost compared with daclizumab.

Although the number of patients is small and the follow-up period is still short, our results lead us to conclude that early steroid withdrawal in association with FK and MMF was efficacious and safe in pediatric renal transplant recipients. It did not increase the risk of rejection or CMV infections. The normalization of plasma lipids could help to reduce the cardiovascular risk. Long-term follow-up is needed to evaluate the real impact of this protocol on renal function and growth in pediatric renal transplant recipients. Fig 1.

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