Early steroid withdrawal in pediatric renal transplant on newer immunosuppressive drugs

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Abstract: Steroids have been a cornerstone in renal transplant immunosuppression. New immunosuppressive drugs have led to protocols using early steroid withdrawal or complete avoidance. A prospective protocol in 23 pediatric renal transplant (ages 2–14 yr) who received decreasing steroid doses stopping at day 7 post-Tx, FK, and MMF were compared with a CsA, AZT, historically matched steroid-based control group. Basiliximab was used in two doses. Anthropometric, biochemical variables, AR rates, and CMV infection were evaluated and compared using Student's t-test and regression analysis. A better growth pattern was seen in steroid withdrawal group. GFR rate and serum glucose were similar in both groups. Total serum cholesterol levels were significantly lower in steroid withdrawal group. The incidence of AR at 12 months was 4.3% in steroid withdrawal group vs. 8.6% in steroid-based group (p = ns). No difference in CMV infection was observed. Hemoglobin levels were low during the first months in both groups; reached normal values after six months. SBP became higher at 12 months in steroid-based group. Patient and graft survival was 98% in both groups at one-yr post-transplant. Early steroid withdrawal was efficacious, safe, and did not increase risk of rejection, preserving optimal growth, renal function, and reducing cardiovascular risk factors.

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Steroids have been the central axis of immunosuppression in renal transplantation for 40 yr. However, their action involves cardiovascular risk, infections, arterial hypertension, hyperlipidemia, glucose intolerance, and, especially in children, growth impairment (1, 2). Multiple attempts to taper, withdraw, or eliminate steroids have been tried. Administration on alternating days has demonstrated growth improvement in the prepubertal population, but this has been difficult to maintain due to low adherence to treatment with an increased risk of AR episodes (3, 4). Reports have shown that late steroid withdrawal was associated with increased AR rate in more than 50% of cases (5, 6). Gradual tapering of steroids has been associated with an increase of AR in most solid transplanted organ recipients (Tx). Chronic exposure to steroids could produce dependence making tapering immunologically unsafe for the recipient. The early literature reported in adults with regards to steroid withdrawal in renal transplant recipients showed an unacceptable rate of AR episodes in the first meta-analysis of randomized, controlled trials. The majority of these trials were based on CsA with or without AZT (7–9). The development of new immunosuppressive drugs, like interleukin-2 monoclonal antibodies, FK, and

Abbreviations: AR, acute rejection; AZT, azathioprine; CMV, cytomegalovirus; CsA, cyclosporine A; DBP, diastolic blood pressure; FK, tacrolimus; MMF, micophenolate mofetil; SBP, systolic blood pressure.

MMF have allowed steroid withdrawal or complete avoidance (10–17). Recently, it has been demonstrated that AR rates are not increased if prednisone is completely avoided or if it is stopped during the initial post-transplant period (10, 11). Furthermore, steroid-free recipients have decreased complication rates and improved adherence. The few publications in pediatric kidney transplant that exist to date, support early steroid withdrawal or complete avoidance of steroids with encouraging results (10, 12, 15, 16).

The aim of this study was to evaluate the efficacy and safety of a new immunosuppressive protocol based on FK, MMF, and basiliximab, with early withdrawal of steroids in pediatric renal transplant recipients and compares this group with a historical steroid-based control group.

Patients and methods

Starting in September 2003, a prospective study was performed at the Pediatric Nephrology department of Luis Calvo Mackenna Children's Hospital. Patients aged between two- and 15-yr old, first renal transplant, from a live or deceased donor, with low immunological risk (panel reactive antibody < 10%), who had not previously received steroids for autoimmune diseases were invited to participate in a prospective early steroid withdrawal immunosuppressive protocol. A precedent historical group of patients, with similar characteristics was considered the control group. Acute tubular necrosis, defined by dialysis therapy in the immediate post-Tx period, was an exclusion criteria in both groups because of its well-known negative influence on graft survival (18, 19). In both groups basiliximab (Simulect®) induction immunosuppression was administered in two doses, 20 mg in patients with weight over 30 kg and 10 mg in patients less than 30 kg. The first dose was given at the time of transplant and the second dose four days post-transplant. Group A (steroid withdrawal group) received maintenance immunosuppressive therapy with FK, MMF, and steroids in decreasing doses until their discontinuation on day 7 post-transplantation. Group B (steroid-based historical control group); received CsA – microemulsion, AZT, and steroid daily. The dose of CsA was adjusted to target trough levels and AZT dose was 2 mg/kg/day as indicated in Fig. 1. Prospective data from group A patients were obtained and those from group B by clinical chart review. Information was collected with a
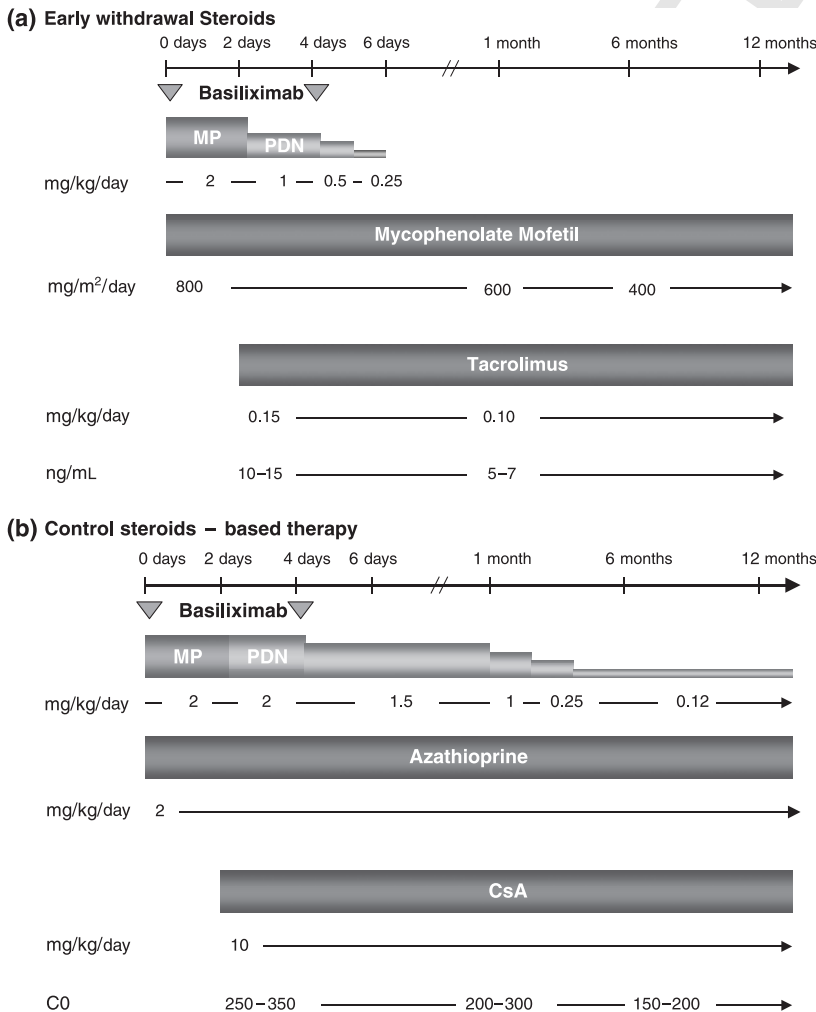

(a) Early withdrawal Steroids

Fig. 1. Immunosuppressive protocol in early steroid withdrawal (a) and steroid-based control group (b). Mean FK trough levels as well as mean daily doses for the steroid withdrawal group (a). CsA, at 12 hours – C0 trough level for steroid-based group and mean daily CsA doses (b) during the first year post-transplant. CsA and FK immunosuppressive drugs were dosed to achieve a target trough level in the total of kidney transplant patients. MP, methylprednisolone; PDN, prednisone; CsA, cyclosporin A; FK, tacrolimus.

common template and the same variables were evaluated in both groups: demographics (gender, age, original disease leading to chronic renal failure, and type of dialysis), transplantation characteristics (donor source, cold ischemia time, biochemical parameters, graft function, AR episodes and CMV infection, and graft and patient survival), and anthropometric measurements (height, weight, and blood pressure) with a 12 month of follow-up. Renal function was determined by Schwartz formula. Our immunosuppressive protocol does not include anti-CMV prophylaxis. Antigenemia (pp65) was monitored weekly after kidney transplantation for the first two months and once a month for another 12 months. CMV seronegative recipients were treated preemptively for the first positive antigenemia. Seropositive recipients were treated only when their antigenemia count reached a threshold of ≥10 positive cells per 400 000 leukocytes (20, 21). The height/age index as well as SBP and DBP was compared using Z-scores according to the NCHS and the last report of the Task Force (22). Recipients with an increase of more than 20% in serum creatinine level underwent percutaneous allograft biopsy, which was read by the same pathologist. The histological analysis included C4d immunofluorescence and Banff classification (23). When grade I or II AR was confirmed and a satisfactory response to methylprednisolone pulses occurred, children continued with the same protocol. If a second AR or the initial rejection was vascular or steroid-resistant, steroids were started and the patient was removed from the protocol. AR episodes were treated with boluses of intravenous methylprednisolone at doses of 1 g per square meter of body-surface area, administered in three successive days; thymoglobulin was used in steroid-resistant episodes. The need to switch from FK to CsA, due to hyperglycemia or from MMF to AZT due to gastrointestinal intolerance, was not a reason to start steroids or for the patient to be removed from the protocol. The institutional review board on human research approved the study, and written informed consent was obtained from all patients or legal guardians.

Statistical analysis

Continuous variables were expressed as mean ± s.d. Categorical and continuous variable measures serially during the study were compared using either a paired t-test or analysis of variance for repeated measures. Patient and graft survival rates were estimated using Kaplan–Meier methods and compared by log-rank test. Significance was assigned with a p value <0.05, with a confidence interval of 95%. Statistical analysis was performed using STATA version 7.0.

Results

A total of 23 pediatric kidney transplant recipients, aged 2–15 were in each group. In goup A (mean age 7.2 ± 3.8 yr), 15 were prepubertal (age 4.7 ± 2.5 yr) and eight pubertal patients (age 12.1 ± 2.7 yr). In group B (mean age 8.6 ± 3.8), 14 were prepubertal (age 5.1 ± 2.0 yr) and seven pubertal patients (age 12.3 ± 2.9). Two patients in group A were withdrawn at two months after the study. The first patient at the time of transplant underwent a supranumeric hepatic lobule extirpation,

requiring a repair of the intestinal tract, MMF was discontinued and steroids were initiated. The second patient, a toddler 2.7-yr old, with recurrent bronchial obstructive syndrome and segmental lung hypoplasia hospitalized with fever and abdominal complaints underwent abdominal surgery for an intestinal perforation. Later he had an adenovirus infection, developed bacterial (*Pseudomona aeroginous*) and mycotic (*Aspergillus*'s) septicemia, resulting in death. At the time of the analysis, 19 patients from group A and 20 patients from group B had completed 12 months of follow-up. Demographic characteristics in both groups are shown in Table 1.

AR incidence and graft outcome

Seven biopsies were performed during the follow-up period in group A. In one (4.3%) cellular AR was confirmed at eight months after transplant, non-adherence of treatment was documented, resulting in loss of the graft. The other six biopsies showed: toxicity due to FK in three and the other three were histologically normal with negative immunofluorescence for C4d. In group B four biopsies were performed. AR showed in two (8.6%), attributed to non-adherence of immunosuppressive treatment and classified as late cellular AR after eight months of follow-up, resulting in graft loss in one of them. The other two biopsies showed: IgA nephropathy in one and the other patient had a chronic allograft nephropathy. One patient died in each group, in group A; two months after the transplant due to

Table 1. Demographic data in the steroid withdrawal group (Group A) and steroid-based group (Group B)

Demographic characteristics	Group A (n = 23)	Group B (n = 23)	p-value
Recipients mean age (yr)	7.2 ± 3.8	8.6 ± 3.8	NS
Male number (%)	14 (60)	12 (65)	NS
N° PD/HD/preemptive	18/4/1	21/1/1	NS
End-stage renal disease etiology			
Renal dysplasia	14	11	
Reflux nephropathy	2	2	
Glomerulopathy	7	6	
Unknown/others	6	3	
Living/deceased donors	3/20	7/16	NS
Deceased donors mean age (range)	36 (1.7–46)	2.5 (2.2–40)	NS
Donor's sex M/F	7/5	4/7	NS
Number of HLA mismatches	2.5 ± 0.8	2.8 ± 0.9	NS
Cold ischemia hours mean	19.8 ± 3.5	18.5 ± 8.9	NS
``en bloc'' graft	1	1	NS
CMV (+) donor/recipient	10/5	11/6	NS

Results are expressed as mean ± s.d.
NS, not significant; HLA, human leukocyte antigen; CMV, cytomegalovirus; PD, peritoneal dialysis; HD, hemodialysis.

Aspergillus septicemia and in group B, one yr after transplant due to ovarian teratoma with peritoneal metastasis. Patient and graft survival was 98% in steroid withdrawal group at one-yr post-transplant and no differences with control steroid group were found.

Incidence of CMV infection

No difference in CMV infection was observed, only an increase in antigenemia titers occurred in 10 patients from group A and in six patients from group B. One patient of group A, developed symptoms of hepatitis and hemolytic anemia, MMF dose was lowered and gancyclovir was started. Infection resolved and there was no detrimental effect on graft function.

Renal function

Renal function was not different between groups; however, the historical control group showed a major decrease of delta creatinine clearance after 12 months of follow-up compared with the early withdrawal steroid group, although the difference was not significant (Table 2).

Growth

The growth was evaluated only in prepubertal patients. The mean values of height Z-score between months 1 to 12, in prepubertal steroid withdrawal patients increased compare with steroid-based group, although the difference was not significant. However positive changes in delta of height Z-score between months 1–6 and 1–12 were only statistically significant for

group A compared with group B in every period of follow-up (Table 2).

Cardiovascular risk

The mean value of SBP and DBP Z-scores were normal in both groups. Blood pressure was similar in the two groups at the beginning of the study, but SBP became slightly higher after nine months of follow-up in steroid-based group. A decrease in mean serum cholesterol levels was seen during the follow-up period in both groups, but they were significantly lower in group A (Table 2). The mean blood glucose levels were normal during the follow-up for both groups except for one patient in each group. These patients were switched from FK to CsA, and the serum glucose levels became normal.

Hematological profile and acid-base metabolism

Anemia was seen in both groups during the first months post-transplant, hemoglobin levels increased in both afterwards, but only patients belonging to group B reached normal values after 12 months of follow-up. White cell count and platelet mean values were normal during follow-up in both groups and no difference was observed in calcium–phosphorous metabolism. During the first month after transplant, a lower level in bicarbonate was observed in group A, with no difference afterwards.

Discussion

New immunosuppressive therapy has improved allograft survival in pediatric recipients at our

Table 2. Biochemical variables, growth rate, and renal function in the two groups

Month	1			6			12		
Group (N°)	A (23)	B (23)	p-value	A (21)	B (23)	p-value	A (19)	B (20)	p-value
Age	7.2 ± 3.8	8.7 ± 3.8	NS	8.0 ± 3.7	9.1 ± 3.8	NS	8.4 ± 3.4	9.5 ± 3.7	NS
Height Z score	−2.7 ± 1.1	−2.5 ± 1.4	NS	−2.26 ± 0.96	−2.34 ± 1.43	NS	−1.8 ± 0.88	−2.3 ± 1.42	NS
Delta height Z score				0.6 ± 0.4	0.17 ± 0.3	*	1.1 ± 0.6	0.28 ± 0.4	*
SBPZ score	0.8 ± 1.2	1.2 ± 1.5	NS	0.60 ± 1.35	0.56 ± 1.5	NS	−0.11 ± 0.94	0.77 ± 1.26	*
DBP Z score	0.4 ± 0.9	0 6 ± 1 1	NS	0.17 ± 1.08	0.13 ± 0.17	NS	0.14 ± 0.85	0.52 ± 0.7	NS
Creatinine clearance	87.6 ± 19.4	94 0 ± 28 3	NS	80.5 ± 21.2	86.4 ± 1.81	NS	88.0 ± 20.9	82.7 ± 16.8	NS
Delta creatinine clearance				−7.3 ± 26	−5.3 ± 21.6	NS	−3.8 ± 31.3	−9.7 ± 25.6	NS
Blood glucose	86 ± 16	88 ± 20	NS	89.4 ± 10.5	91.6 ± 11.7	NS	90 ± 8.6	86 ± 7 5	NS
Total cholesterol	148 ± 27	206 ± 43 2	*	137 ± 15	178 ± 40	*	136 ± 20	192 ± 43	*
Triglyceride	134 ± 63	317 ± 338	*	88 ± 34	218 ± 165	*	98 ± 53	127 ± 21	NS
Bicarbonate	20.2 ± 2.3	22 3 ± 2 74	*	20.47 ± 3.39	20.93 ± 2.33	NS	22.2 ± 2.0	21.9 ± 2.7	NS
Hemoglobin	9.43 ± 1.36	10 5 ± 1 45	*	10.20 ± 1.58	11.4 ± 1.33	*	10.6 ± 1.3	12.0 ± 0.95	*
White cell count	9487 ± 2840	10448 ± 2931	NS	9745 ± 5715	8267 ± 3703	NS	8139 ± 3372	7820 ± 3613	NS

A, Early steroid withdrawal group and B, Steroid-based group.
*Statistical significant, p = p-value.
Results are expressed as mean ± s.d.
SBP, systolic blood pressure; DBP, diastolic blood pressure; NS, not significant.

centre; recently published experience has shown that graft survival rates from 100 grafts at five yr was 72% (19). Cardiovascular disease is an important cause of mortality in renal transplant patients, especially in young adults. Patients, who receive transplants at younger ages, are exposed for long time period to a greater risk, when compared with adults (25). New immuno-suppressive protocols have been developed searching for fewer adverse effects as well as for avoidance of AR. Late steroid withdrawal after transplant has been associated with an increase of AR episodes; chronic exposure to steroids could result in a dependence, which makes steroid withdrawal immunologically unsafe for the allograft recipient. Experiences in steroid-free or early steroid withdrawal protocols have been published with promising results (6, 10, 15). These protocols intend to promote immunologi-cal tolerance, which can only be developed during the first contact between the host and the allograft. Steroids inhibit effector molecules of cytotoxic cells involved in an adaptive immune response, which is responsible for early graft acceptance, through immunological tolerance (26, 27).

As reported by others (14, 16), we did not find an increase in AR rates and no differences in graft survival were seen, which makes our pro-tocol safe. Even though differences between the groups in renal function were not found, a tendency toward a negative increase of delta creatinine clearance in the steroid-based group was seen after 12 months of follow-up. Longer follow up may confirm this initial tendency, which may be associated to CsA/AZT in the historical control group vs. FK/ MMF, as has been reported by others (28).

Improvement in growth rate was seen in both groups but it was significantly higher in the steroid withdrawal group. A normalization of growth patterns at month 12 was observed in the study group. The total cholesterol levels were significantly lower in the group without steroids, which is a favorable factor diminishing the risk of early vascular damage described in transplanted patients. This could be not only due to the absence of steroids but also to the use of FK instead of CsA, which has been associated with dyslipidemia in transplanted patients (29). Although, FK increases the resistance to insulin, only one case of hyperglycemia was reported in each group with a good response to switching to CsA and diet modification.

One of our concerns was the presence of anemia in the group without steroids, which was an indication for blood transfusion in some cases. We believe that this anemia could be due to bone marrow suppression from MMF or immunosuppression and the lack of stimulus of the steroids in the bone marrow. Also the bioavailability of MMF is greater without con-comitant steroids use (30), suggesting that lower dosing may be important to limit MMF toxicity usually evidenced by gastrointestinal toxicity and leucopenia. These findings suggested that we lower the MMF dose from 800 to 600 mg/m^2/day during the first month and to 400 mg/m^2/day afterwards. We may reduce the MMF dose even more in the future, while screening MMF blood levels. Our current protocol does not include CMV post-transplant prophylaxis, because of its high costs; we only do follow-up with antigene-mia. Although the antigenemia titers were higher in the study group the majority of patients were asymptomatic at this time, only one patient had symptomatic disease when we used high doses of MMF, resolution was achieved with antiviral treatment and lowering the MMF dose. Graft deterioration was not seen in this patient and nor in patients with positive antigenemia. The choice of induction agent for steroid elimination is still a matter for debate at the moment. We used basiliximab in two doses as did Meulen et al. (31); the most important benefit provided by basiliximab is its lower cost compared with daclizumab as well as lower immunosuppression. Experiences with steroid-avoidance or steroid withdrawal protocols include daclizumab induc-tion in multiples doses (10, 15); Oberholzer et al. report the use of thymoglobulin (16). In our study, as well as that of Meulen et al. (31), we used only two doses of basiliximab; the reason was lower cost and lower immunosuppression. In consideration of our financial constraints, results are comparable with those of developed coun-tries. It is important to emphasize that there has been a significant incidence in the adverse effects of steroid-based treatment protocols; however, the deleterious effects of the introduction of new therapy were not observed. Our results showed that early withdrawal of steroids associated with FK and MMF in the pediatric renal transplant recipient was effective and safe. The risk of AR and clinical CMV infection did not increase and this produced a favorable impact on growth, preservation of graft function, and reduced cardiovascular risk factors.

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