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Everolimus Versus Azathioprine in a Cyclosporine and Ketoconazole–Based Immunosuppressive Therapy in Kidney Transplant: 3-Year Follow-up of an Open-Label, Prospective, Cohort, Comparative Clinical Trial

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ABSTRACT

In cyclosporine-based protocols, everolimus is more effective than azathioprine to reduce acute rejection. Ketoconazole may reduce cyclosporine and everolimus requirements. We compared kidney transplant patients treated with everolimus or azathioprine in a ketoconazole- and cyclosporine-based immunosuppressive regimen.

This open-label, prospective trial of low immunologic risk patients. Included one group ($n = 11$) who received everolimus (target blood level, 3–8 ng/mL) and the other ($n = 11$) azathioprine (2.0–2.5 mg/kg/d). Both received steroids, ketoconazole, and cyclosporine with C_0 targets (ng/mL) in the everolimus group of 200–250, 100–125, and 50–65 for months 1 and 2 and thereafter and in the azathioprine group of 250–300 in month 1, 200–250 in month 2, 180–200 until month 6, and 100–125 thereafter.

Their baseline characteristics were similar. Two biopsy-proven acute rejections occurred in each group. Three-year graft and patient survival in both groups was 100%. Creatinine clearances at months 6, 12, 24, and 36 were 63.7 ± 25.4, 58.9 ± 24.9, 56.0 ± 22.9, and 57.0 ± 27.6 in the everolimus group versus 72.6 ± 20, 68.6 ± 21.3, 71.4 ± 23.2, and 68.4 ± 19.2 in the azathioprine group (NS for every comparison).

Major complications were rare and similar in both groups. Five patients in the everolimus group received simvastatin versus 4 in the azathioprine cohort ($P = .53$). The average cyclosporine doses to achieve targets were 0.8–1.2 mg/kg in the everolimus group and 1.6–2.2 mg/kg in the azathioprine group. The average everolimus dose after month 2 was 0.75–0.9 mg/d. We concluded that with cyclosporine, ketoconazole, and steroids, everolimus was as effective and safe as azathioprine. Cyclosporine reduction with everolimus did not influence graft survival or function at 3 years.

LARGE CLINICAL trials in variegated renal transplant populations have demonstrated that patients treated with mammalian target of rapamycin (mTOR) inhibitors are more effective than azathioprine to decrease the incidence of acute rejection episodes in conjunction with cyclosporine-based immunosuppressive therapy.[1–3] Despite this greater efficacy, developing countries have difficulties to add those drugs because of financial constrains. Thus after cyclosporine azathioprine is the most frequently used drug, in solid organ transplantation, especially of the kidney.

The calcineurin inhibitor cyclosporine is metabolized by the liver cytochrome P-450 system.[4] The imidazole antifungal drug ketoconazole inhibits this system, retarding cyclosporine metabolism and, subsequently, increasing its blood level and half-life.[4] At the same time, it has been suggested that the addition of ketoconazole to immunosuppressive

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0041-1345/10/$–see front matter
doi:10.1016/j.transproceed.2009.12.048

schemes may have beneficial immunomodulatory effects to decrease the acute rejection rate.[5]

The mTOR inhibitors, sirolimus and everolimus, are also metabolized by the cytochrome P-450 system. Their combination with ketoconazole also increases blood levels and half-lives, potentially allowing sufficient savings for countries with low budgets for transplantation to use them even in combination with cyclosporine.[6,7]

We designed a comparative protocol between everolimus and azathioprine added to a cyclosporine, ketoconazole, and steroid immunosuppressive regimen in renal transplant patients, seeking, to explore the feasibility of these combinations as effective immunosuppressants in a financially restricted country.

METHODS

Study Design

We performed an open-label, nonrandomized, prospective, cohort, comparative clinical trial among low immunologic risk patients, who were defined as adult males or nonpregnant females undergoing primary deceased donor, living-unrelated or human leukocyte antigen-mismatched living-related donor kidney transplantations. Subjects were required to display a rate of and to undergo grafting with a panel reactive antibodies (PRA) <20%, cold ischemia time of <30 hours and a warm ischemia time less of <45 minutes. All patients signed a written informed consent form approved by the local ethics committee. All participating women consented to use an effective contraceptive method.

Immunosuppressive Therapy

After transplantation, all patients received IV methylprednisolone for the first 3 days and then oral prednisone at doses tapered to reach 15 mg/d at month 6; 10 mg/d at month 12; and 5 mg/d thereafter. From day 0, all patients received oral modified cyclosporine (Neoral, Novartis Pharma AG, Basel, Switzerland), ketoconazole (100 mg/d) and azathioprine (2.0–2.5 mg/kg/d). After day 5, 1 cohort of patients without delayed graft function (defined as a requirement for dialysis), were switched from azathioprine to everolimus with a 0.75-mg loading dose and 0.25 mg once a day thereafter. No induction therapy was allowed.

Immunosuppressant doses were modified according to the following trough blood level targets: Everolimus group: everolimus, 3–8 ng/mL (Innofluor, Seradyn); cyclosporine, 200–250 ng/mL the first month, 100–125 ng/mL the second month, and 50–65 ng/mL thereafter (Axym, Abbott); azathioprine group: cyclosporine 250–300 ng/mL the first month, 200–250 ng/mL the second month, 180–200 ng/mL until the end of the sixth month, and 100–125 ng/mL thereafter.

Table 1. Baseline Characteristics

	Everolimus	Azathioprine	*P*
n	11	11	
Male gender	4	5	
Age (y)	48.5 ± 13.0	37.0 ± 14.4	.062
Deceased donors	8	5	.069
Cold ischemia time (h)	13.4 ± 9.5	9.9 ± 8.3	.433
Warm ischemia time (h)	0.69 ± 0.09	0.60 ± 0.24	.294
MM HLA-A	0.55	0.45	
MM HLA-B	0.59	0.35	.903
MM HLA-DR	0.32	0.25	
PRA (%)	0–13	0–2	

Table 2. Secondary End Points

	Everolimus	Azathioprine	*P*
Clearance creatinine month 1	55.2 ± 22.6	66.1 ± 22.3	.191
Clearance creatinine month 3	61.2 ± 29.6	70.5 ± 21.0	.454
Clearance creatinine month 6	63.7 ± 25.4	72.6 ± 20.0	.403
Clearance creatinine month 12	58.9 ± 24.9	68.6 ± 21.3	.337
Clearance creatinine month 24	56.0 ± 22.9	71.4 ± 23.2	.133
Clearance creatinine month 36	57.0 ± 27.6	68.4 ± 19.2	.277
Total cholesterol (mg/dL) month 12	267 ± 98	209 ± 28	.004
Total cholesterol (mg/dL) month 36	185 ± 45	184 ± 51	.967
Triglycerides (mg/dL) month 12	304 ± 312	183 ± 95	.12
Triglycerides (mg/dL) month 36	241 + 178	221 + 152	.787

Primary End Point

The primary end point was the need for a graft biopsy to exclude the presence of an acute rejection episode, which was clinically suspected.

Secondary End Points

We evaluated biopsy-proven acute rejection episodes (1997 Banff criteria),[8] number of graft losses, calculated by Cockroft–Gault creatinine clearances[9] at 1, 3, 6, 12, 24, and 36 months, and rates of a selected group of adverse events: major infections, cytomegalovirus infection, dyslipidemia, and lymphoceles.

Statistical Analysis

All analyses were performed on an intention-to-treat basis (ITT). Analysis of variance was used for continuous variables; chi-Square and Fisher exact tests for categorical variables.

RESULTS

The baseline characteristics of the 11 patients in the everolimus and 11 in the azathioprine groups were similar with respect to gender, living donor proportion, age, cold and warm ischemia times, and HLA mismatches (Table 1).

It was necessary to perform 6 graft biopsies in the azathioprine group compared with 2 in the everolimus group ($P = .058$). Two of the 6 biopsies in the azathioprine group and 2 of the 2 biopsies in the everolimus group showed a 1997 Banff classification borderline acute rejection ($P = .1$). Because of histological evidence of nephrotoxicity, one Azathioprine patient was switched from Cyclosporine to sirolimus. Another 2 patients in the azathioprine group changed immunosuppression, one owing to a rejection episode (cyclosporine to tacrolimus), and another due to full house HLA compatibility (azathioprine stopped).

Selected secondary end points are shown in Table 2. No graft losses occurred until month 36. There was 1 major infection and 1 deep venous thrombosis (DVT) in each group; all resolved with appropriate therapy. The patient

with DVT in the everolimus group was switched to azathioprine to avoid a drug interaction with oral anticoagulants. There was 1 lymphocele in each group, both requiring resolution through laparoscopic surgery. No cytomegalovirus infections were observed. Six patients in the everolimus and 2 in azathioprine group received a hydroxy-methyl-glutaryl coenzyme A inhibitor by month 12 ($P = .002$), but by month 36, 5 patients in the everolimus group and 4 in the azathioprine group were receiving a statin ($P = .53$). No liver function abnormalities were observed. The use of antihypertensive drugs was also not significantly different between the groups.

The average everolimus dose to achieve the target blood level was 075–0.90 mg/d. The average cyclosporine doses to achieve targets were 0.8–1.2 mg/kg in the everolimus group and 1.6–2.2 mg/kg in the azathioprine group. Although not significantly different, creatinine clearances tended to be lower in the Everolimus group over the follow-up.

DISCUSSION

Our results showed that the combination of cyclosporine, ketoconazole, steroids, and everolimus was feasible and as effective and safe as cyclosporine, ketoconazole, steroids, and azathioprine for low-risk kidney transplant patients.

We selected the primary end point of a graft biopsy because, after ruling out obstruction, this procedure clarifies the clinical problem of graft dysfunction after kidney transplantation, namely, discussing cyclosporine toxicity from an acute rejection episode as the main differential diagnoses. Despite theoretical considerations, it is technically possible to combine several drugs that compete within the hepatic CYP3A4 enzyme of the cytochrome P-450 system, thus approaching the objectives of potentially improving immunosuppressive quality without an unusual rate of adverse events. Moreover, the low dose of ketoconazole seemed to be safe, because we did not observe any case of liver dysfunction.[10]

Because of the small cohort, it was not possible to demonstrate immunologic superiority of the everolimus scheme. In this regard, the primary end point rate difference of 30% between groups, suggested that a statistical type I error occurred. In contract, it was reassuring that we did not observe more infectious complications among the experimental group, although it is necessary to accumulate more experience and a greater number of treated patients to be confident on this particular issue.

Recently it has been suggested that at 1 year after kidney transplantation, cyclosporine nephrotoxicity is the main finding among protocolized graft biopsies.[11] This constitutes the rationale for decreasing or even withdrawing calcineurin inhibitors to improve graft function and survival.[12,13] Our immunosuppressive protocol used low trough levels of cyclosporine blood levels after the third month posttransplantation, so we can speculate that graft function may be better preserved in the everolimus than in the azathioprine group after the first year, but this contention was not reinforced by our study, due to the tendency for lower creatinine clearances in the everolimus group.

Certainly, it is necessary to follow serum lipids. In this regard, both total cholesterol and triglycerides were initially higher in everolimus group, and so then was a need to prescribe an HMG-CoA inhibitor, confirming previous reports of hyperlipidemia associated with mTOR inhibitors,[14] but this side effect seemed to vanish in the longer term. Due to budget constrains we used simvastatin without problems, although in theory, if a HMG-CoA inhibitor is prescribed, we should use pravastatin or fluvastatin, because the other statins are also metabolized by CYP3A4.[4]

A formal economic analysis is underway, but it is possible that switching azathioprine to everolimus within a cyclosporine- and ketoconazole-based therapy did not significantly increase expenditures compared with the traditional scheme with azathioprine. Because we could avoid hospitalizations, diagnostic procedures, biopsies, rejection therapies, and retesting of ambulatory patients, the mTOR inhibitor everolimus may be a more cost-effective drug for low-budget transplant centers.

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