Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Simultaneous corticosteroid avoidance and calcineurin inhibitor minimization in renal transplantation

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Keywords
calcineurin inhibitors, corticosteroids, immunonutrients, kidney transplantation, steroid avoidance.

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Received: 1 October 2005
Revision requested: 4 November 2005
Accepted: 9 January 2006

doi:10.1111/j.1432-2277.2006.00280.x

Summary

Steroids and calcineurin inhibitors (CNI) have been mainstays of immunosuppression but both have numerous side effects that are associated with substantial morbidity and mortality. This study was carried out to determine if steroids can be eliminated with early discontinuation of cyclosporine A (CsA) and later discontinuation of mycophenolate mofetil (MMF). Ninety-six patients with kidney transplants were entered into four subgroups of two pilot studies. All patients received Thymoglobulin® induction, rapamycin (RAPA), and the immunonutrients arginine and an oil containing ω-3 fatty acids. Mycophenolate mofetil was started in standard doses and discontinued by 2 years. CsA was given in reduced doses for either 4, 6, or 12 months. Follow-up was 12–36 months. Thirteen first rejection episodes occurred during the first year (14%). Combining all patients, 86% were rejection-free at 1 year, 80% at 2 years and 79% at 3 years. No kidney has been lost to acute rejection. Ninety percent of the 84 patients at risk at the end of the study were steroid-free and 87% were off CNI. Fifty-seven percent of 54 patients with a functioning kidney at 3 years were receiving monotherapy with RAPA. We conclude that this therapeutic strategy is worthy of a prospective multi-center clinical trial.

Introduction

Corticosteroids have been a mainstay of immunosuppression in solid organ transplantation since the early 1960s, but they have numerous side effects, the most prominent being hypertension, hyperlipidemia, diabetes mellitus, an increased susceptibility to infection, bone disease, cataracts, dysfigurement, and gastrointestinal complications. The introduction of calcineurin inhibitors (CNI), cyclosporine A (CsA), and tacrolimus (TAC) improved the outcome of transplantation, but nephrotoxicity emerged as a new problem and, in addition, hypertension, diabetes, and hyperlipidemia have become increasingly significant factors leading to adverse outcomes from cardiovascular complications. Recently, mycophenolate mofetil (MMF) and rapamycin (RAPA) have further reduced the likelihood of acute rejection.

Because of the adverse effects of steroids, steroid-sparing regimens have been used widely during the last decade [1–4], and it is now clear that early steroid withdrawal can be accomplished as long as patients can take adequate combinations of MMF, CNI, and/or RAPA. However, in these newer protocols, the CNIs continue to have significant side effects, which lead to atherosclerosis and ultimately increase mortality from cardiovascular events [5–7]. Several studies have been reported in which

either steroids or CNI, but not both, have been avoided completely [4,7]. Rapamycin has also been used successfully in CNI-sparing protocols [8] and elimination of CsA in RAPA-based protocols has been shown to improve renal function [9,10].

Dietary supplementation with the immunonutrients arginine and canola oil (Arginaid®, canola oil; Novartis Nutrition Corp., Minneapolis, MN, USA) (rich in both ω-3 and ω-9 fatty acids) was shown in a previous prospectively randomized trial in kidney transplant patients to significantly reduce post-30-day rejection rates compared with controls (5.4% vs. 23.7%), post-30-day CNI drug toxicity (9.2% vs. 35.3%), new onset diabetes (NODM) (2.3% vs. 14.5%), cardiac events (5.08% vs. 17.1%) and septic episodes (6.5% vs. 18.7%) [11]. Immunonutrients also enhanced graft survival in animals treated with RAPA [12], MMF [13], or CsA [14,15]. The best synergistic effect was seen with RAPA. In none of the animal studies were steroids administered. These experiments suggested that the use of immunonutrients with Thymoglobuin® (SangStat Medical Corp., Fremont, CA, USA) induction to prevent early rejection would allow success for protocols, where steroids were completely avoided and low doses of CNI inhibitors could ultimately be discontinued.

Materials and methods

The initial pilot study was designed to evaluate the effects of CsA dose and the length of administration in a steroid-free protocol, which was based on the induction with Thymoglobulin®, RAPA, MMF, and the dietary supplements, arginine and canola oil. Sequential reduction in the CsA trough levels and length of administration was achieved among subgroups 1, 2, and 3 with a targeted number of 20 patients per subgroup. In a second prospectively randomized pilot study, the immunosuppressive drug schedule was the same as for subgroup 2, but one-half received arginine (4.5 gm b.i.d.) and canola oil (15 ml b.i.d.) whereas the other subgroup (4) received arginine (4.5 gm b.i.d.) and a fish oil extract (12.5 gm q.d.) high in eicosapentenoic acid (EPA) and docosahexenoic acid (DHA) (Coromega®; ERBL Inc., Carlsbad, CA, USA) rather than canola oil. Inclusion criteria included primary and repeat living donor and cadaveric adult kidney transplant recipients except for those with a fasting serum cholesterol >300 mg/dl or triglycerides >400 mg/dl, or those on chronic steroid therapy. All studies were approved by the Institutional Review Boards of The Christ Hospital, Cincinnati, Ohio and the University of Cincinnati and all participating patients gave informed consent. HIPPA guidelines were followed.

Target immunosuppression is shown in Table 1.

Table 1. Target immunosuppression.

	Subgroup 1 (18)	Subgroup 2 (45)	Subgroup 3 (20)	Subgroup 4 (13)
Thymo	1.5 mg/kg × 3 doses	Same	Same	Same
Rapa	2 mg/d (target level 9–15 ng/dl day 0–90 and 8–12 ng/dl thereafter)	Same	Same	Same
Mycophenolate mofetil	1000 mg b.i.d. 0–9 months; 750 mg b.i.d. 9–12 months; 500 mg b.i.d. 12–24 months discontinue at 24 months	Same	Same	Same
Cyclosporine A (CsA)	4 mg/kg/day (target levels: 0–90 days = 200 ng/ml; 91–180 days = 150 ng/ml; 181–365 days = 100 ng/ml)	2 mg/kg/day (target levels: 0–180 days = 100 ng/ml day 180 = discontinued)	1 mg/kg/day (no target level specified discontinue at 4 months)	2 mg/kg/day (target levels: 0–180 days = 100 ng/ml, day 180 = discontinued)
Arginine	4.5 gm b.i.d.	Same	Same	Same
Canola oil	15 ml b.i.d.	Same	Same	0
Coromega	0	0	0	4–5 packets*

Note: 26 patients from subgroup 2 were in pilot study #1 and 19 were in pilot study 2.
*4–5 packets; each packet containing 190 mg docosahexenoic acid and 290 mg eicosapentenoic acid.

In all patients, Thymoglobulin® was started intraoperatively with the first dose given over 12–24 h to minimize first dose reactions. No steroids were given as a pretreatment for Thymoglobulin®. Subsequent doses were given over a 12-h period. Doses were not given when CD3 counts were <25/mm^3 or if the patient had a severe reaction. Reduced doses were given for platelet counts <100 000/mm^3 or WBC <3000/mm^3. RAPA (Rapammune®; Wyeth Pharmaceuticals, Collegeville, PA, USA) was begun the day of operation at a dose of 2 mg/day and adjusted thereafter to achieve a target trough level of 9–15 ng/ml for the first 90 days and then 8–12 ng/ml indefinitely. MMF (CellCept®; Roche Pharmaceuticals, Nutley, NJ, USA), likewise, was begun at standard doses of 1 g b.i.d. but was incrementally withdrawn beginning at 9 months. CsA (Neoral®; Novartis Pharmaceuticals Corp., East Hanover, NJ, USA) was initiated at 4 mg/kg/day with a target trough level of 200 ng/ml in subgroup 1. The target level was decreased to 150 ng/ml at 90 days and 100 ng/ml at 6 months with discontinuation at 12 months. The target level of CsA was 100 ng/ml in subgroup 2, with discontinuation at 6 months. In subgroup 3, patients got only 1 mg/kg/day of CsA, regardless of blood level and it was discontinued after 4 months. The first patient was entered into the study on May 13, 2000 and the last patient was entered on October 7, 2003. All patients were followed for at least 12 months in pilot study 2 and for 36 months in pilot study 1.

Preliminary analysis of the first study suggested that CNI toxicity was highest in subgroup 1 and more rejections occurred in subgroup 3. Because of this, six additional patients were added to subgroup 2 during a period before the second pilot study could begin. The second pilot study was designed primarily to determine the levels of amino acids and free fatty acids in patients receiving arginine and the two ω-3 containing lipids compared with patients receiving no supplements.

The central protocol was that all patients received the same schedule for Thymoglobulin®, MMF, RAPA, and arginine while subgroups 1 vs. (2 + 4) 3 differed in CsA dosing and subgroups (1 + 2 + 3) vs. 4 differed in the type of ω-3 containing lipids. Steroids were completely avoided except for rejection or recurrent disease.

Results

Patient demographics are shown in Table 2. The distribution is characteristic for our recipient population.

Immunosuppression

The actual immunosuppression that the patients received is shown in Table 3. Several patients did not receive the full dose of Thymoglobulin® because of adverse reactions or early discharge. Sixteen patients had the immunosuppression changed from CsA to TAC because of drug toxicities (7), rejection (8) or recurrent disease (1). These patients and one other with a decline in renal function received CNI past the time of planned discontinuance. Overall, 87% of patients were eventually off CNIs.

At the termination of follow-up (3 years for study 1 and 1 year for study 2), eight patients were receiving steroids, one for treatment of recurrence of the original disease and seven were on a steroid taper following the treatment of a rejection episode. Thus, 90% were steroid-free. Thirty-one of the 54 patients (57%) who were followed for 3 years were receiving only monotherapy with RAPA and the dietary supplements. The remaining 23 patients continued on another drug because of recurrent disease or prior rejection.

Rejections

Rejections are shown in Fig. 1. Of the 14 first rejections occurring in the 66 patients followed for 3 years, only six were Banff grade 2A or above, but nine patients had more than one rejection, with three patients developing chronic rejection after an initial acute rejection episode. There were a total of 12 acute cellular rejections and two humoral rejections occurring in the first 3 years. Ten of these patients (71%) had less than three doses of Thymoglobulin®. Ten acute rejections were treated with pulse steroids and/or an increase in CNI, eight with a switch to TAC, two were treated with Thymoglobulin®, one was treated with an increased RAPA and one spontaneously resolved without treatment. All acute rejections were reversed.

Combining all four subgroups, four of 31 recipients of cadaveric donors (13%) had a rejection episode in the first year compared with nine of 60 recipients of living donors (15%) and 86% of all patients were rejection-free at 1 year. None of the 12 African–Americans (AA) (0%)

Table 2. Demographics.

	Subgroup 1	Subgroup 2	Subgroup 3	Subgroup 4	Total
Number of patients	18	45	20	13	96
Mean age (years)	45	45	49	49	47
Race (AA/Non-AA)	1/17	7/38	4/16	0/13	12/84
Gender (M/F)	10/8	31/14	12/8	9/4	62/34
LRD/LUD/CAD	11/1/6	22/8/15	7/5/8	6/5/2	46/19/31
Donor age (years)	37	41	42	32	38
HLA MM (AB/DR)	2.1/0.8	2.5/1.1	2.8/1.2	2.4/1.3	2.5/1.1
Pre-TX diabetes	5	11	8	6	30

AA, African–Americans; LRD/LUD/CAD, living related/living unrelated/cadaveric donor; HLA MM, histocompatibility mismatches.

Table 3. Actual immunosuppression.

	Subgroup 1 (18)					Subgroup 2 (45)					Subgroup 3 (20)					Subgroup 4 (13)				
	1 month	6 months	12 months	24 months	36 months	1 months	6 months	12 months	24 months	36 months	1 months	6 months	12 months	24 months	36 months	1 months	6 months	12 months	24 months	36 months
Average dose (mg)/blood level (ng/ml) (for patients receiving drug) (n) = number of patients.																				
Rapamune	2.2/8.9	2.9/8.3	3.3/8.1	4.8/9.5	3.7/9.1	3.1/10.1	2.8/9.8	4.1/10.3	4.0/9.0	3.0/9.1	4.1/9.8	4.7/10.1	5.0/9.9	3.7/8.9	3.6/8.8	3.4/10.1	2.5/9.6	3.2/9.6	*	*
	(17)	(16)	(15)	(14)	(14)	(42)	(37)	(33)	(20)	(17)	(17)	(17)	(14)	(11)	(5)	(11)	(11)	(8)		
Cellcept	1527.8	976.6	966.7	901.8	1020.8	1542.7	1437.5	1145.0	883.3	750.0	1514.7	1514.7	1312.5	1000.0	1250.0	1675.0	1333.3	1500.0	*	*
	(18)	(16)	(15)	(14)	(14)	(43)	(38)	(28)	(17)	(17)	(17)	(17)	(16)	(12)	(2)	(10)	(9)	(2)		
Cyclosporine	290.0/	221.2/	206.3/	0	0	204.3/	158.7/	250.0/	0	0	176.6/	0	0	0	0	147.8/	141.7/	175.0/	*	*
	252.5	132.9	106.1			113.8	109.0	210.0			93.8					92.0	87.3	113.0		
	(15)	(13)	(12)			(36)	(31)	(3)			(16)					(9)	(7)	(1)		
Prograf	2.0/4.7	5.5/5.5	4.0/3.9	3.0/8.0	8.0/4.0	10.0/10.8	6.7/8.1	4.3/8.4	6.0/7.0	3.5/11.5	9.0/8.8	0	6.5/13.3	5.0/9.8	0	5.0/8.5	2.0/3.7	2.0/3.0	*	*
	(1)	(2)	(3)	(3)	(1)	(5)	(4)	(6)	(4)	(3)	(1)		(2)	(1)		(1)	(1)	(1)		
Average dose (mg)/average# of doses																				
Thymoglobulin	104.1 mg/2.6 doses					111.4 mg/2.8 doses					119.7 mg/2.3 doses					128.3 mg/3.3 doses				

*Data collected only through 12 months.

had a rejection episode in the first year compared with 13 of 84 non-AA patients (15%). The mean serum creatinines at 1, 2 and 3 years were 1.4, 1.6, and 1.7 mg/dl.

Death and graft loss

Three of the 96 patients (3%) have died. Two of these died of cardiac events on post-transplant days 2 and 9 (subgroup 3) and one patient died of post-transplant lymphoproliferative disease (PTLD) on day 108 (subgroup 1). There were four additional graft losses at 1 year: one on day 1 from vascular thrombosis and infarction of the allograft (subgroup 4), one on day 30 from infection transmitted with a cadaveric donor kidney (subgroup 1), one on day 58 from recurrence of original disease (subgroup 3) and one from discontinuance of immunosuppression on day 241 because of PTLD (subgroup 3). Two graft losses from chronic rejection (subgroups 1, 2) and one from membranoproliferative glomerulonephritis occurred in the second year (subgroup 3) and three grafts were lost during the third year: one from recurrent disease (subgroup 3), one from chronic allograft nephropathy (subgroup 3) and one from chronic CNI toxicity (subgroup 1). There were no graft losses from acute rejection. Death-censored graft survival at 1 year was 96% (89 of 93), at 2 years was 89% (56 of 63) and at 3 years was 86% (54 of 63) for patients at risk for that time period.

Other complications

Other complications during the 3 year period of observation are shown in Table 4. Of the three patients with PTLD, two were Epstein–Barr virus (EBV) positive donor to EBV-negative recipient, and one was an EBV-positive donor to EBV-positive recipient. Only one patient had a cardiac event after day 9 (coronary artery bypass surgery P.O. day 100). Blood pressure and blood lipids were easily controlled, suggesting a benefit of withholding steroids and reducing CNI.

Discussion

Because of multiple adverse effects, withdrawal of steroids from immunosuppressive regimens in 'low-risk' renal transplant patients has been tried for many years. However, in earlier studies, it was found that there was an increased incidence of rejection after complete withdrawal, even when performed late in the patient's course. With more modern immunosuppression, steroid withdrawal has been achieved at a much earlier time and numerous studies have now shown that administration of corticosteroids for only a few days is not associated with an increased rejection or loss of the kidney. These studies have been

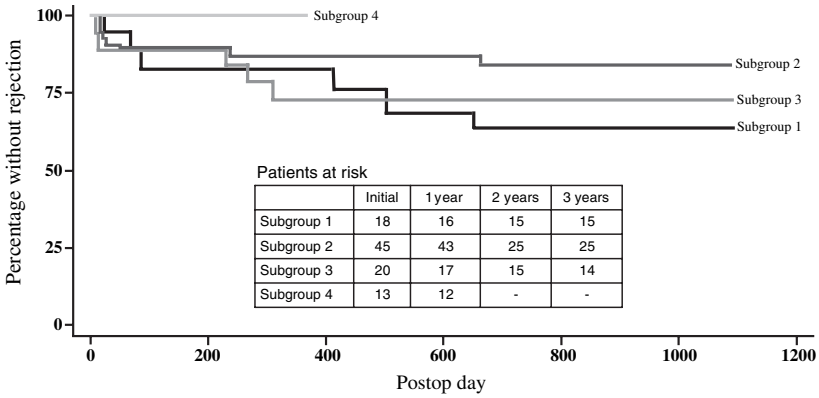

Figure 1 Kaplan–Meier analysis for first rejection episode.

Table 4. Complications and renal function.

	# of Pts	CMV	PTLD	Reccurrent disease	Mouth ulcers	Wound complications	NODM*	Sepsis	Pneumonia	UTI	CsA toxicity	SCr 1&3 years
Study 1–3 years follow-up												
Subgroup 1	18	1	1	0	5	6	0	3	5	8	7	1.5, 1.5
Subgroup 2	26	1	0	1	3	2	1	0	1	3	5	1.4, 2.1
Subgroup 3	20	1	1	1	6	2	1	0	0	0	4	1.4, 1.2
Study 2–1 year follow-up												
Subgroup 2	19	1	1	1	1	3	0	1	0	2	1	1.4, –
Subgroup 4	13	0	0	0	5	2	0	0	0	1	2	1.6, –
Total	96	4	3	3	20	15	2	4	6	14	19	1.4, 1.7

*As defined by requiring insulin >30 day [16]. Five additional patients received oral agents for hypoglycemia. CMV, cytomegalovirus; PTLD, post-transplant lymphoproliferative disease; NODM, new onset diabetes mellitus; SCr, serum creatinine; UTI, urinary tract infection.

summarized recently by Hricik [1], Lerut [2], Matas [3], and Marsh [4]. The strategies for minimizing immunosuppression have recently been summarized in a comprehensive paper by Kirk *et al.* [7]. These investigators emphasize the importance of early lymphocyte depletion strategies to avoid steroids as was accomplished in our study. They also emphasized the importance of reducing maintenance therapy after 6 months with target of rapamycin (TOR) inhibitors playing a role in protolerant adaptation.

Complete avoidance of steroids

Complete avoidance of steroids in immunosuppressive protocols has only recently been achieved. Birkeland [17] recorded a series of 100 consecutive renal transplant patients, who were treated with a steroid-free immunosuppressive protocol that included a 10-day course of antithymocyte globulin (ATG) induction and maintenance therapy with CsA and MMF. Only 13 patients had acute rejection episodes and 10 of these were in the first 3 months. One-, 2-, 3-, and 4-year graft survivals were 97%, 96%, 90% and 82%, respectively. These results are especially impressive considering that 67% received cadaveric grafts and 17% were second grafts. Seven of their patients were children. This report differs from our own in that CsA was continued with its potential adverse effects, and immunosuppression with ATG was very aggressive during an early therapy. Sarwal *et al.* [18] reported a small series of 10 pediatric patients, where steroids were substituted with extended daclizumab use in combination with TAC and MMF. Their results were also excellent with graft and patient survival of 100%. Patients who became intolerant to MMF were switched to RAPA, but the patients remained on TAC. This group subsequently reported their experience with 57 pediatric transplant recipients and showed that only 8% had acute clinical rejection at 1 year [19]. Cantarovich *et al.* [20] also reported a series of 11 patients who received immunosuppression with ATG, CsA, and MMF. Ten days of ATG were given to all patients. Acute rejection occurred in 27% (three patients), compared with 14% in our series. Two patients received steroids for recurrence of glomerulonephritis and two patients for rejection episodes. Cole *et al.* [21] reported the results of a multicenter controlled trial in Canada. In their study, they used daclizamab induction with a CsA and MMF-based

immunosuppression. Fifty-seven patients were treated, 29 from cadaveric donors. At 1 year, patient and graft survival were 95% and 89%, respectively, but 25% experienced rejections compared with 14% in our series. Steroid-free protocols have also been reported in patients following the liver transplantation [22–24], islet transplantation [25–26], and simultaneous kidney/pancreas transplants [27]. None of the above studies, however, were designed to discontinue CNI.

Minimization or elimination of CNI

Several recent studies have examined CNI-sparing regimens [28]. Vincenti *et al.* [29] enrolled 98 patients in a CNI-avoidance study. Immunosuppression was with daclizumab, high dose MMF, and conventional corticosteroids. Forty-eight percent of patients had rejection during the first 6 months and 62% of patients were given CNI for more than 7 days. A more recent study by Flechner *et al.* [30] reported a randomized, prospective trial in 61 adult patients who were treated with basiliximab and MMF. Half the patients received RAPA 5 mg/day after a loading dose, and half received CsA. Biopsy-proven acute rejection occurred in 6.4% in the RAPA group compared with 16.6% in the CsA group. All patients received steroids in standard doses. Oberbauer *et al.* [10] evaluated early CsA withdrawal from a RAPA/CsA/steroid-based protocol. Eligible patients were randomly assigned at 3 months to remain on triple drug therapy or to have CsA withdrawal with RAPA trough concentrations targeted to 20–30 ng/ml during the first year. Acute rejection after randomization occurred in 5.1% of the triple therapy vs. 9% of double therapy. Serum creatinine was better and blood pressure was lower in patients who had CsA withdrawn. Grinyo *et al.* [31] reported a series of 30 patients treated with Thymoglobulin®, MMF, and steroids without CsA. However, CsA was started subsequently in 16 patients and rejection occurred in 24% of patients.

Selection of RAPA and MMF for therapy during the first 2 years

These two drugs were selected not only because of their efficacy in suppressing immune response, but also because of their long-term effects on vascular dysfunction and atherosclerosis. Jolicoeur *et al.* [32] showed that a combination of MMF and RAPA could prevent chronic renal allograft rejection in rats. Also, Klupp *et al.* [33] showed that blood levels of MMF were correlated with the degree of inhibition of intimal hyperplasia. The ability of RAPA to inhibit cell proliferation is well known [34], and there was indeed a high incidence of wound complications in our series of patients. Importantly, sirolimus-eluting coronary artery stents were able to elicit persistent inhibition of neointimal hyperplasia for up to 2 years [35].

Unique features of the current study

The above-mentioned studies clearly demonstrate that either steroids or CNI can be eliminated from immunosuppressive protocols, but this is the first study known to us that completely avoids steroids (in most patients) and is associated with an early discontinuance of CNI. The lack of allograft loss from acute rejection and the ability to discontinue MMF in some cases (42% at 2 years) are also unique features. Whether it will be possible to also stop RAPA is a question that remains to be answered.

In this present study, immunonutrients were also given as, in our previous study, they were associated with a reduced rejection and CNI toxicity [11]. However, their role needs to be established in larger well-controlled trials. Rejection was 0% in AA vs. 14% in non-AA in this study, and the low incidence in AA is consistent with findings in our previous study, which used supplementation with arginine and canola oil to reduce rejections [11]. The reasons for this are not clear, but it is well established that both arginine and oils containing ω-3 fatty acids will enhance the immunosuppressive effects of CsA, MMF, and RAPA in experimental animals [12–14].

Another important finding in our study is the low incidence of NODM. Diabetes mellitus is a major risk factor for cardiovascular disease following the kidney transplant and is often associated with steroid administration. Kasiske *et al.* [36] examined data on 11 659 medicare beneficiaries who received their first kidney transplant between 1996 and 2000. NODM occurred in 9.1% and factors which increased the relative risk of NODM included AA race (relative risk 1.68), Hispanic ethnicity (relative risk 1.35), and body mass index >30 (relative risk 1.73). Use of a CNI increased the relative risk to 1.53. Conversely, NODM was decreased by the use of MMF. NODM was associated with an increased overall graft failure, death-censored graft failure, and mortality. It is of interest that NODM was also decreased in our previous randomized study in patients who received immunonutrients, but not in controls [11].

One of the major potential benefits of the current protocol is reducing the risk of ischemic heart disease. Cardiovascular disease is now the leading cause of death in patients with end stage renal disease (ESRD) and transplantation [5,7,37,38]. TAC results in less elevation of serum cholesterol and mean arterial blood pressure compared with CsA, but there is a higher incidence of elevated blood glucose. The 10-year risk of coronary artery disease was significantly lower in men treated with TAC than with

CsA, but the risk was unchanged in women. The possibility of protection from cardiovascular disease with the current protocol is extremely promising as only one cardiovascular event has occurred after the first 9 days, despite the fact that 30% of the patients already had diabetes mellitus at the time of entry into the study. Other studies show that both arginine [39] and ω-3 fatty acids [40] reduce intimal hyperplasia. Reduction of intimal hyperplasia by sirolimus-eluting coronary artery stents is associated with a significant reduction in major cardiac events [41].

We emphasize that no definitive conclusion regarding the long-term outcome can be made from these pilot studies and that the role of the immunonutrients needs to be further defined. However, it is evident that the overall therapeutic strategy is promising. An expanded study with longer follow-up will be needed to determine the precise benefits on the cardiovascular system in transplant patients in protocols that eliminate steroids, spare CNI, and provide immunonutrients. Modifications of the protocol which might be considered could include more aggressive induction therapy with antilymphocyte antibodies.

Acknowledgements

This study was supported by USPHS Grant RO1 AI42743.

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