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Effect of different immunosuppressive therapies on the lipid pattern in kidney-transplanted rats

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Summary

We analyzed the effect of oral administration of cyclosporine–methylprednisone (CsA–MP) and sirolimus (SRL) on the lipid pattern of kidney-transplanted rats after a 7-day survival. A significant increase in plasma cholesterol in CsA–MP group (control: 26 ± 3 mg/dl vs. 59 ± 8 mg/dl, $P < 0.05$) and in triglyceride levels in SRL group (control: 53 ± 4 mg/dl vs. 114 ± 3 mg/dl, $P < 0.05$), was shown. Kidney microsomal membranes from both treated groups showed that cholesterol and triglyceride values and the relative percentage of arachidonic acid in the total amount of n-6 fatty acids decreased. A diminution of linoleic acid occurred in testis (control: 9.4 ± 0.1 mg/dl vs. CsA–MP: 6.0 ± 0.3 mg/dl and vs. SRL: 6.8 ± 0.2 mg/dl, $P < 0.05$), liver (control: 17.7 ± 0.6 mg/dl vs. CsA–MP: 15.1 ± 0.6 mg/dl and SRL: 13.5 ± 0.8 mg/dl, $P < 0.05$) and erythrocyte membranes (control:11.7 ± 0.1% vs. CsA-MP: 10.6 ± 0.2% and SRL: 10.0 ± 0.4%, $P < 0.01$). The immunosuppressive therapies improved the rejection rate of the graft, fact that was remarkable in the SRL-treated group. However, lipid abnormalities still remain in spite of immunosuppressive therapies. (150).

Introduction

Dyslipidemias represent a group of disorders that compromise a variety of lipid abnormalities. These lipid abnormalities are common in patients with renal disease and remain after a successful renal transplantation [1–3]. Moreover, these disorders are key factors in the development of chronic allograft dysfunction, and they are independent factors associated with cardiovascular complications [4].

Although some early studies focused mainly on increased serum cholesterol, recent data emphasized that a high triglyceride concentration predicts coronary heart disease independent of other known factors [5–7].

Essential fatty acids (EFA) are specific polyunsaturated fatty acids (PUFA) for which all animals have an obligatory dietary requirement [8]. EFA of the n-6 and n-3 families, after their release from their membrane phospholipid reservoirs, become eicosanoids, which are important as autacoids and second messengers in signal transduction of endocrine and other kinds of stimuli [9]. EFA deficiency results in biochemical disorders involved in the etiology of coronary vascular diseases [10].

Patients with chronic renal failure exhibit plasma fatty acid patterns which are indicative of EFA deficiency. Thus, plasma PUFA decreased and saturated fatty acids increased in both plasma and red blood membranes, altering the fluidity of the latter [5,11,12]. Moreover, d'Apice *et al.* [13] and Lawen *et al.* [14] showed that kidneys from EFA deficient rats transplanted to normal recipients developed a marked increase in renal interstitial cells and a reduction in allograft survival.

On the contrary, qualitatively and quantitatively, post-transplant secondary dyslipidemias arise as a consequence of the immunosuppressive treatment used to abolish rejection [15]. The extent to which immunosuppressants, directly or indirectly, influence the serum and membranes lipid pattern in renal recipients is still unclear. Post-transplantation steroid therapy in humans has been

correlated with hyperlipidemia which promotes atherosclerosis often prevalent in the hemodialysis population before transplantation [1, 16]. The use of cyclosporine A (CsA) in immunosuppression protocols diminished the incidence of acute rejection episodes without the amelioration of the atherogenic profile [17]. However, the role of CsA on the lipid pattern is not clear as most patients received a combined therapy with prednisone. In previous experiments the influence of both drugs on the biosynthesis of PUFA, serum lipids as well as the fatty acid composition of different tissues has been established in normal rats in which the drugs could be evaluated independently [18]. The dual therapy with CsA–methylprednisone (CsA–MP) increased cholesterol and triglyceride plasma levels and depressed PUFA biosynthesis in liver at the level of both Δ6 and Δ5 desaturation activities. CsA monotherapy produced a significant depression in plasma triglycerides while PUFA biosynthesis remained under the same values obtained for the controls [18].

Cyclosporine A inhibits T-cell activation by blocking the transcription of cytokine genes, including those of IL-2 and IL-4 [19]. Sirolimus (SRL), a novel immunosuppressant, prolongs allograft survival in animal models, inhibiting cytokine receptor-mediated signal transduction instead of a direct inhibition of IL-2 gene transcription [20]. Experimental transplant models and initial clinical data suggest that SRL in combination with CsA reduce the rate of acute rejection episode, and facilitate corticosteroid withdrawal [21,22]. However, SRL seemed to enhance the hypercholesterolemic and hypertriglyceridemic effects of CsA [21–23].

To elucidate the influence of immunosuppressive therapy in producing dyslipidemias after a 7-day survival, we studied serum and membrane lipids of many tissues in kidney transplanted rats that had been treated with CsA-MP or the monotherapy with SRL.

Materials and Methods

Animals

Forty-two outbred male Wistar rats, weighing 280–350 g were used as donors ($n = 18$), recipients ($n = 18$) and controls ($n = 6$). Animals were allowed free access to water and standard laboratory chow *ad libitum*. All animal experiments were performed according to the guidelines set by the National Institutes of Health (NIH publication No. 86-23, revised 1985).

Kidney transplantation and animal treatments

Recipients were divided in three groups of six recipients each. CsA–MP group was given a daily oral dose of CsA (Sandimmune; Novartis Pharma LTD, Basel, Switzerland), 5 mg/kg body weight, in combination with MP, 1 mg/kg body weight; SRL group was administered a daily oral dose of SRL (Rapamycin; Rapamune, Wyeth-Ayerst Laboratories, St David's, PA, USA), 3 mg/kg body weight; recipients without inmunosuppressive therapy were considered as TXR group. Six nontransplanted rats without immunosuppressive therapy served as control group. Doses of immunosuppressants are those required to prevent kidney rejection in the rat [22,24,25].

Blood samples were collected from the tail vein in each recipient (before transplantation) and in controls to obtain the baseline data, and at the end of the experiment by draining off, for cholesterol, triglyceride, creatinine and urea determinations. Animals were fasted 24 h prior to sample collection, surgery procedure, and killing. Rat renal transplants were performed as previously described [26]. Briefly, in anesthetized donor rats, the left kidney [transplanted kidney (TK)] was carefully removed, weighed and stored in cold Ringer lactate solution. The donor's right kidney (DK) was removed, weighed and homogenized in cold homogenizing solution (HS, 1:3 w/v) which consisted of: 0.25 M sucrose, 62 mM phosphate buffer (pH 7.0), 0.15 M KCl, 5 mM $MgCl_2$ and 100 μM EDTA. In the recipient right nephrectomy was performed and the excised kidney (EK) was weighed and homogenized in HS. The donor's left kidney was transplanted into the abdominal cavity of the recipient. The ureter was sutured by an end-to-end anastomosis. The native right kidney (NK) was left *in situ*. The average total ischemic time was 90 ± 15 min. Immediately after surgery rats were allow free access to water and standard laboratory chow *ad libitum* and they were treated with buprinorphine (0.04 mg/kg body weight) as analgesic therapy every 12 h until the third postoperative day. The animals were killed on the seventh postoperative day.

Isolation of kidney, liver and testis microsomes and erythrocyte membranes

One week after surgery rats were killed by decapitation. Blood was drained off and collected into test tubes containing an anticoagulant EDTA solution (Wiener Lab., Rosario, Argentina). Liver, kidney and testis were excised, weighed, and homogenized as indicated before. The microsomal fraction was separated by differential cold (4 °C) centrifugation at 10 000 ***g*** for 20 min and ultracentrifugation at 45 000 ***g*** for 1 h and 40 min.

Whole blood was centrifuged, plasma was immediately separated, and packed red blood cells were washed four times, at 4 °C, with a buffered solution containing NaCl (140 mM), KCl (5 mM), $NaHSO_4$ (1 mM), Tris buffer (10 mM), pH 7.4. After agitation they were kept at 4 °C for 10 min, and centrifuged at 16 000 ***g*** for 15 min. This

procedure was carried out twice, leaving a substantially hemoglobin-free pellet of erythrocyte membranes, which were resuspended in a small amount of supernatant, and stored (−70 °C) until assayed.

Lipid extraction and analysis

Lipids from erythrocyte membranes and liver, kidney and testis microsomes were extracted with chloroform–methanol (2:1 v/v). After conversion of the fatty acids to their corresponding methyl esters they were analyzed using a Hewlett-Packard Model 5840-A gas-liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a flame-ionization detector. An aliquot from the organic phase of kidney microsome extraction was separated to determine phospholipid and neutral lipid content through flame ionization detector (FID) of an Iatroscan apparatus model TH 10 (Latron Laboratories, Tokyo, Japan). Lipids were separated on previously activated chromarods type S-III, under a double-development system. The first mobile phase was hexane–benzene (70:30 v/v) whereas the second was benzene–chloroform–formic acid (70:25:2 v/v/v). Lipidic species were quantified by comparison with known amounts of pure standards run under the same conditions. The signals from the FID were registered on a Hewlett-Packard model HP-3396 A integrator.

Chemical determinations

To measure plasma cholesterol and triglyceride levels, blood was collected by puncture of the tail vein 1 week before surgery and at the time of killing. Both variables were assayed by commercial enzymatic methods (Sera-Pak Bayer Corporation, Tarrytown, NY, USA). In the same blood samples creatinine and urea plasma levels were also determined by enzymatic methods (Bio Systems, S. A. Barcelona, Spain; Wiener Lab., Rosario, Argentina, respectively).

Histopathologic analysis

Histopathologic analysis was performed on graft and native kidney removed on seventh postoperative day. Biopsies were fixed with Bouin's solution and embedded in paraffin. For this routine 5-μm (paraffin) sections were stained with hematoxylin–eosin and periodic acid-Schiff standard procedures. Samples were studied by light microscopy at 40×.

Statistical analysis

Results were tested statistically using either the Student *t*-test compared with the respective control, or the one-way ANOVA as appropriate.

Results

Plasma cholesterol, triglyceride, creatinine and urea levels

Cholesterol and triglyceride levels in nontransplanted (control) and transplanted rats as well as in the transplanted ones treated with CsA–MP and SRL are shown in Table 1. A significant increase was noted in cholesterol in TXR group compared with control. A further enhancement in sterol was shown in transplanted rats under CsA–MP treatment. A slight increase was observed in the SRL group, compared with the data obtained before the surgery. Triglyceride values showed no changes in either transplanted nontreated rats or CsA–MP-treated animals compared with the control. A twofold increase in triglycerides values was shown in the SRL group compared with either the control or those rats without immunosuppressant therapy.

Creatinine plasma levels increased significantly in the TXR group compared with nontransplanted rats (Table 1). In the animals with immunosuppressive therapy the values of creatinine were similar to those of controls. Urea plasma levels showed no significant changes among the groups.

Membrane lipids

Fatty acid profile of plasma, erythrocyte membrane, and liver, testis and kidney microsomal membranes.

The fatty acid composition of kidney microsomal total lipids of CsA–MP and SRL groups is shown in Figs 1 and 2, respectively. No changes were seen in the relative percentages of the different fatty acids between the donor's remaining kidney (DK) and the recipient's EK when compared using Student *t*-test. A decrease in the relative percentage of arachidonic acid (20:4 n-6) was shown after comparing the fatty acid composition of controls versus NK and TK of the rats treated either with CsA–MP or SRL. These alterations were more pronounced in the graft than in native kidneys. In both groups (treated with CsA–MP and treated with SRL) an increase in 18:1 n-7 acid

Table 1. Cholesterol, triglycerides, urea and creatinine levels.

	Pretransplant values ($n = 24$)	TXR ($n = 6$)	CsA–MP ($n = 6$)	SRL ($n = 6$)
Cholesterol	26 ± 3	39 ± 2*†	59 ± 8*	35 ± 4
Triglycerides	53 ± 4	57 ± 5	51 ± 4	114 ± 3*†
Urea	40 ± 4	49 ± 3	50 ± 4	54 ± 4
Creatinine	1.5 ± 0.05	2.4 ± 0.2*	1.2 ± 0.05	1.4 ± 0.08

Data are the mean ± SEM expressed as mg/dl.
*Significantly different from pretransplant values at $P < 0.05$.
†Significantly different from transplanted rats without immunosuppressive therapy at $P < 0.05$ using ANOVA.

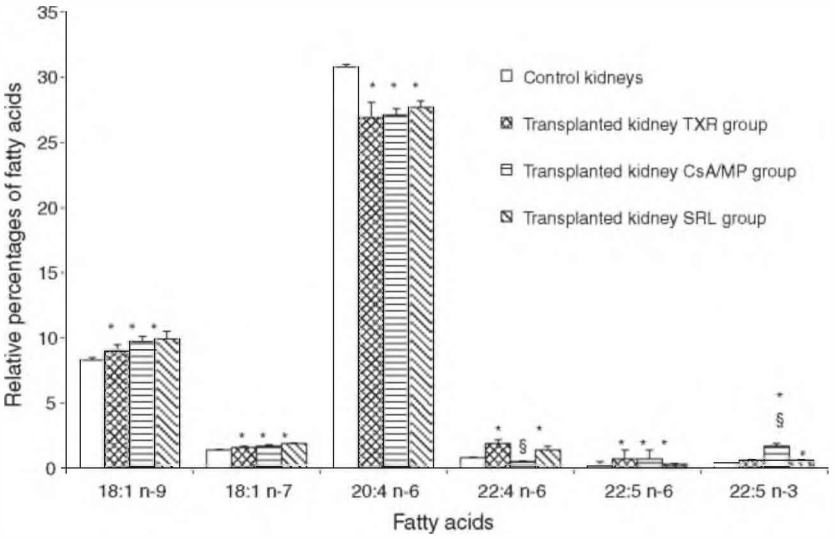

Figure 1 Fatty acid composition of kidney microsomal total lipids from control and transplanted kidneys of recipients. Fatty acids are identified by: number of carbon atoms in the chain is given first, value following the double point represents number of double bonds (zero means saturated fatty acids); number following 'n' indicates the position of the last double bond counting the double bond from the terminal methyl group. Data are the mean of six determinations ±SEM expressed as % of total fatty acids. *Significantly different from control at $P < 0.05$. §Significantly different from transplanted rats without immunosuppressive therapy at $P < 0.05$ using ANOVA.

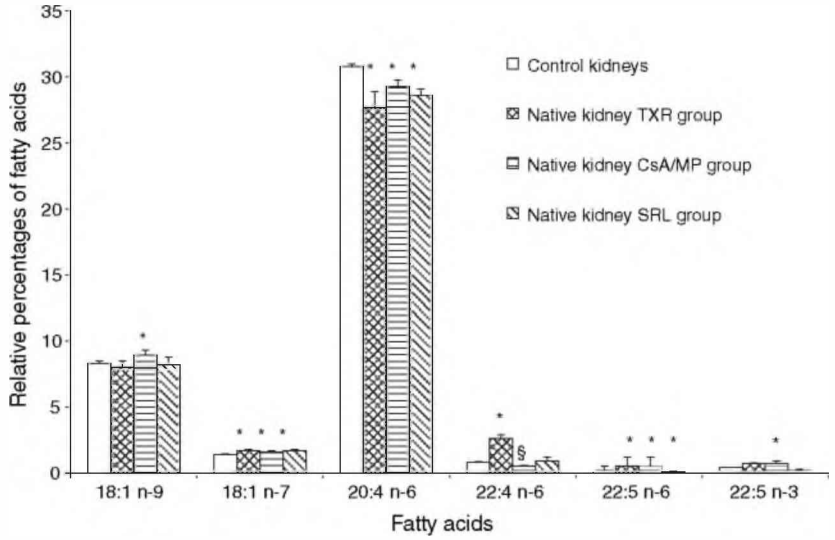

Figure 2 Fatty acid composition of kidney microsomal total lipids from control and native kidneys of recipients. Identification of fatty acids as in Fig. 1. Data are the mean of six determinations ±SEM expressed as % of total fatty acids. *Significantly different from control at $P < 0.05$. §Significantly different from transplanted rats without immunosuppressive therapy at $P < 0.05$ using ANOVA.

was observed. In the CsA–MP group an enhancement of 18:1 n-9 and in 22:5 n-3 was observed in TK and NK compared with control (18:1 n-9: mean ± SEM were 7.4 ± 0.2% in control group vs. 9.7 ± 0.4% TK and 8.6 ± 0.3% NK in CsA-MP group $P < 0.05$ for both comparisons). In addition, in NK and TK of rats treated with SRL a significant increase in palmitic (16:0) acid was shown (mean ± SEM were 18.7 ± 0.2% in control group vs. 20.8 ± 0.5% NK in SRL group and 20.7 ± 0.4% TK in SRL group $P < 0.05$ for both comparisons). Both treatments produced a decrease in the n-6 fatty acid family ($\sum$ n-6 CsA-MP group: control 47.8, NK: 43.7 and TK: 43.0; SRL group, control: 46.3, NK: 43.3 and TK: 42.4) as well as in the unsaturation index (calculated as $\sum n_1x_1$/FA where n_1 is the number of double bonds in each fatty acid; x_1, moles of each fatty acid; FA, total moles of fatty acids) (CsA–MP group, control: 1.87, NK: 1.86 and TK: 1.82; SRL group, control: 1.88, NK: 1.79 and TK: 1.78). An increase in n-3 fatty acids was observed in kidneys obtained from the CsA–MP-treated rats ($\sum$ n-3 CsA–MP group: control 2.7, NK: 4.9 and TK: 4.7).

The relative amount of the different lipid species in kidney microsomal membranes of control and recipient rats is shown in Table 2. A significant increase in phospholipid levels and a decrease in cholesterol and in free fatty acids were found for both treatments. In SRL group a reduction of triglyceride values was also noted in the graft.

Table 3 shows the fatty acid composition of liver microsomal membranes. The main change observed in these membranes obtained from the rats treated with the immunosuppressants was a decrease in linoleic acid (18:2 n-6) (15% decrease in CsA–MP group and 24% in SRL

Table 2. Relative percentages of the lipid species in kidney microsomal membranes.

		TXR		CsA/MP		SRL	
	CONTROL	NK	TK	NK	TK	NK	TK
TG	3.2 ± 0.3	3.6 ± 0.3	4.3 ± 0.2	3.0 ± 0.3*†	3.6 ± 0.6	2.1 ± 0.4*†	1.5 ± 0.1*†
FFA	5.0 ± 0.4	4.4 ± 0.4	4.8 ± 0.2	1.5 ± 0.2*†	2.0 ± 0.4*†	1.6 ± 0.1*†	1.0 ± 0.2*†
C	26.4 ± 1.2	23.5 ± 1.5	26.0 ± 0.9	14.5 ± 0.6*†	17.3 ± 1.6*†	15.6 ± 1.0*†	14.8 ± 0.9*†
PL	65.4 ± 1.4	67.8 ± 1.7	64.9 ± 1.0	81.0 ± 0.4*†	77.1 ± 1.3*†	80.3 ± 1.4*†	83.3 ± 1.1*†

NK, native kidney; TK, transplanted kidney; TG, triglycerides; FFA, free fatty acids; C, cholesterol; PL, phospholipids.
Data are the mean of six determinations ±SEM expressed as relative mg% of total lipid species.
*Significantly different from pretransplant values at $P < 0.05$.
†Significantly different from transplanted rats without immunosuppressive therapy at $P < 0.05$ using ANOVA.

Table 3. Fatty acid composition of total lipids of liver microsomes.

Fatty acid	Control	TXR	CsA/MP	SRL
16:0	16.2 ± 0.3	17.4 ± 0.2	12.8 ± 1.2*	17.4 ± 0.4
16:1	0.3 ± 0.05	0.3 ± 0.02	0.2 ± 0.03	0.5 ± 0.05*
18:0	24.7 ± 0.6	25.2 ± 0.4	25.3 ± 0.4	25.4 ± 0.5
18:1 n-9	4.2 ± 0.3	4.0 ± 0.3	4.1 ± 0.1	5.6 ± 0.4*
18:1 n-7	1.0 ± 0.04	1.1 ± 0.1	1.5 ± 0.06	1.5 ± 0.2
18:2 n-6	17.7 ± 0.6	15.3 ± 0.4*	15.1 ± 0.6*	13.5 ± 0.8*
20:3 n-6	0.3 ± 0.04	0.3 ± 0.05	0.4 ± 0.05	0.7 ± 0.08*
20:4 n-6	27.5 ± 0.3	27.9 ± 0.3	29.3 ± 0.9	25.5 ± 0.9
22:4 n-6	0.4 ± 0.03	0.5 ± 0.02*	0.3 ± 0.03	0.4 ± 0.03
22:5 n-6	0.1 ± 0.05	0.3 ± 0.03	1.3 ± 0.1*†	0.4 ± 0.03*†
22:5 n-3	1.3 ± 0.1	1.0 ± 0.1	0.4 ± 0.02*†	0.9 ± 0.07*†
22:6 n-3	6.4 ± 0.2	6.7 ± 0.3	9.4 ± 0.5*†	8.2 ± 0.4*†

Fatty acids are identified by: number of carbon atoms in the chain is given first, value following the double point represents number of double bonds (zero means saturated fatty acids); number following 'n' indicates the position of the last double bond counting the double bond from the terminal methyl group.
Data are the mean of six determinations ±SEM expressed as % of total fatty acids.
*Significantly different from control at $P < 0.05$.
†Significantly different from transplanted rats without immunosuppressive therapy at $P < 0.05$ using ANOVA.

group), compared with controls. In the CsA–MP group a decrease in palmitic (16:0) and 22:5 n-3 together with an increase of 22:5 n-6 and 22:6 n-3 was observed compared with the control and nontreated recipient rats. In the SRL group an increase in 16:1 and 18:1 n-9 was observed.

Fig. 3 shows the fatty acid composition of testis microsomes. In the treated groups a significant decrease of palmitoleic (16:1) acid was found compared with controls. In this tissue the decrease of 18:2 n-6 acid was 32% in CsA–MP and 28% in SRL group. In the CsA–MP group an increase of arachidonic, 20:3 n-6 and 22:5 n-6 acids, as well as a decrease of palmitic acid, were observed compared with the remaining groups.

The most important change in erythrocyte membranes was the decrease in linoleic acid which was about 12%, either in the rats treated with CsA–MP or with SRL (mean ± SEM were 11.7 ± 0.1% in control group vs. 10.6 ± 0.2% in CsA-MP group and 10.0 ± 0.4% in SRL group, $P < 0.01$ for both comparisons). There were no differences in plasma fatty acid composition of transplanted rats treated with either CsA–MP or SRL, compared with the controls (data not shown).

Histopathology

The histopathologic study of nontreated transplanted kidneys revealed a moderate to severe degree of rejection, presenting at interstitial level dense inflammatory infiltrates which developed to mononuclear predominance, hemorrhage and slight to moderate edema. Glomerules were observed with moderate vasocongestion and hypercellularity. Moderate tubulitis, focal tubular necrosis, moderate hyalinization and intimal vascular thickening of arteries and arterioles were also evident. CsA–MP therapy provoked a decrease in the interstitial, tubular and vascular abnormalities compared with the nontreated group. In

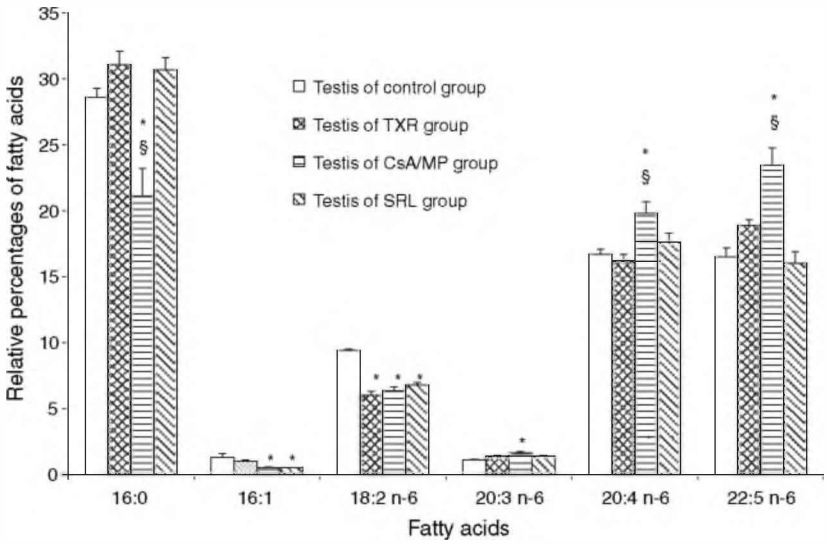

Figure 3 Fatty acid composition of testis microsomal total lipids in control and transplanted rats. Identification of fatty acids as in Fig. 1. Data are the mean of six determinations ±SEM expressed as % of total fatty acids. *Significantly different from control at $P < 0.05$. §Significantly different from transplanted rats without immunosuppressive therapy at $P < 0.05$ using ANOVA.

addition, a slight to moderate proliferation of the glomerular capsular epithelium was observed. SRL therapy markedly improved morphological alterations, evidencing only a moderate focal leukocyte infiltration of perivascular preferential location with preservation of vascular and glomerular structures.

Discussion

Adverse effects on serum lipids have been reported concerning the immunosuppressive therapy in transplanted patients as well as in experimental models of organ transplantation [27]. The type and level of immunosuppressive agents used can also adversely produce post-transplant hyperlipidemia. However, in a previous study we observed 50% increment in plasma cholesterol levels as well as modifications in the lipid profile of kidney, testis and erythrocyte membranes in transplanted rats without immunosuppressive therapy [26]. In a previous study, where the effect of CsA and MP administered to normal nontransplanted rats could be evaluated independently, it was demonstrated that cholesterol values were significantly and substantially increased under the dual immunosuppressive therapy [18]. In the same report, CsA monotherapy decreased plasma triglyceride levels, but it did not modify plasma cholesterol levels. However, the CsA monotherapy in nontransplanted patients produced an increase in total plasma cholesterol and low-density lipoprotein cholesterol [1,28,29]. In transplanted rats studied here the hypercholesterolemia obtained from CsA–MP group was higher than that from transplanted nontreated rats (Table 1); effect that could be ascribed mainly to corticoid therapy.

Cyclosporine has been also associated with high triglyceride levels through the inhibition of lipoprotein lipase [1]. Triglyceride levels remained unaltered when the dual therapy CsA–MP was administered to normal nontransplanted rats (18). Similar results were obtained in this work in recipient rats treated with both drugs. When SRL was administered in combination with corticosteroids a significant increase in triglyceride levels was shown [30]. Moreover, the hypertriglyceridemia returns to its pretransplantation values after reduction or discount SRL [30]. The European Study comparing SRL and CsA triple therapy reported that SRL treatment caused a more frequent and severe hypercholesterolemia and hypertriglyceridemia than those of CsA (44% vs. 51% and 51% vs. 12%, respectively). In our transplanted rats, we found that the monotherapy with SRL produced a considerable elevation of triglyceride values without producing any change in cholesterol levels (Table 1). In contrast to the increase in cholesterol and triglyceride plasma levels produced by the administration of CsA and SRL, respectively, both lipids were considerably reduced in the graft and in native kidney membranes (Table 2). From these data along with the significant increase in membrane phospholipids we can infer that kidney membranes tend to be more fluid when the rejection damage is attenuated by the immunosuppressive therapy.

Related to the fatty acid profile of kidney microsomes, the decrease of arachidonic acid observed in the graft of rats under the dual therapy could be ascribed to both, the production of metabolites of the cyclo-oxygenase and 5-lipoxigenase pathways and the production of lipocortin induced by corticoids [1]. CsA nephrotoxicity is associated with specific alterations in renal arachidonic acid metabolism [31]; arachidonic acid metabolites are considered important mediators of renal dysfunction and acute rejection [1,32]. However, lipocortin directly inhibits the

activity of phospholipase A_2 which catalyzes the release of arachidonic acid from membrane phospholipids. However, in the present work a similar fatty acid profile of kidney microsomes was demonstrated with SRL which inhibits cytokine receptor-mediated signal transduction (20). The decrease in arachidonic acid was partially compensated by an enhancement of the fatty acids of 22 C belonging to n-3 series in the CsA group. In the fatty acid pattern of liver and testicular microsomes as well as in erythrocyte membranes, the depression in the relative concentration of linoleic acid was the most important change in treated-transplanted rats. This fact together with minor changes in other PUFA led to a depression in the total amount of n-6 fatty acids in SRL group in liver microsome membranes. This effect was not evident in CsA-MP group. Then, an increase in the 22:6 n-3 was observed. Steroid therapy produced a significant decrease in the desaturation and thioesterification of linoleic acid in liver as shown elsewhere [18]. From the analysis of the fatty acid pattern in liver of rats treated with both immunosuppressive drugs, we cannot infer the aforementioned effect. The relative percentages of PUFA belonging to linoleic acid remained similar to those of controls in spite of the depression observed in linoleic acid. A similar fatty acid pattern in nontreated transplanted rats has been published [26]. However, the enhancement observed in 16:1/16:0 and 18:1/18:0 ratio in SRL group is an indirect evidence of the increment in $\Delta 9$ desaturation activity.

In conclusion, CsA–MP produced an additional increment in total cholesterol plasma levels when compared with those obtained in nontreated transplanted rats. The immunosuppressants improved the relative composition of the lipid species in kidney microsomes increasing phospholipids and decreasing cholesterol and triglycerides. SRL deteriorates triglyceride plasma composition which showed 67% increase over the controls. However, this effect was not detected in kidney membranes where triglyceride and cholesterol levels were reduced compared with control kidneys. The fatty acid profile of the different tissues in transplanted rats treated with either CsA–MP or SRL was not different from that occurring in nontreated transplanted rats [26]. Moreover, both treatments improved the rejection of the graft evident through plasma creatinine levels and histopathological studies. This fact was remarkable in SRL-treated group. Currently, we are studying the effect of the immunosuppressive drugs at longer periods.

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