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LETTER TO THE EDITOR

LONG-TERM ASSESSMENT OF PLASMA LIPIDS IN TRANSPLANT RECIPIENTS TREATED WITH TACROLIMUS IN RELATION TO FATTY LIVER

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Immunosuppression has improved graft and recipient survival in transplantation but is associated with possible adverse effects including cardiovascular diseases. The impact of tacrolimus on the lipidic profile has been debated for several years. Twenty-nine kidney transplant recipients on tacrolimus treatment were monitored for six years, and multiple laboratory parameters investigating the lipid asset, as well as glucose profile, were carried out. Tacrolimus has been responsible for significant changes in plasma lipid concentrations only for the first six months, but not for the remaining time of observation. Similarly, in the same periods, glycemic imbalance was highlighted. The liver enzyme activity showed a modest derangement during the tacrolimus treatment, suggesting the presence of lipid accumulation in the liver. Fatty liver reversed in the long term follow-up. Tacrolimus, although it is not a completely safe option in the first months of the immunosuppressive protocols in organ transplanted recipients, still retains a certain role in the long-term post-transplantation immunosuppressive approach with high cardiovascular risks

With regard to the conflicting metabolic effects of Tacrolimus (Tac), clinical studies enrolling selected organ transplant recipients, carried out for a long time to better weigh the risk factors for developing cardiovascular disease (CVD), are lacking. The introduction of immunosuppressive agents has improved patient survival rates but it has been reported that the administration of cyclosporine (CsA), sirolimus and everolimus is responsible for hyperlipidemia (1) and consequent early atherosclerosis. On the other hand, Tac is known to affect the behavior of various cytokines such as, interleukin-6 (Il-6) and tumor necrosis factor (2), the overexpression of which contributes to the development of non-alcoholic fatty liver disease (NAFLD), a further expression of the metabolic syndrome that comprehends dyslipidemia. Rise in serum concentrations of triglycerides (TG) is by far the most metabolic imbalance in blood lipids of patients on Tac, most likely due to a decrease of the activity and plasma concentrations of lipoprotein lipase (LPL) (3). Virtually every subject with hypertriglyceridemia is predisposed to suffer from NAFLD, being insulin resistant (IR), high levels of serum Il-6 (4) and mitochondrial dysfunction

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contributors and ultimately leading to exaggerated fat deposition in liver. The link between NAFLD and CVD has been recently established by the fact that the liver is involved in regulating/secreting numerous CVD risk factors (5), notably a cytokine (Tumor Necrosis Factor-alpha), an acute-phase protein (C-Reactive Protein), glucose, lipoproteins, coagulation factors (Plasminogen Activator Inhibitor-1) and substances increasing blood pressure (Angiotensin II).

The intermediate mechanisms of lipid deposition in the liver in patients with NAFLD are controversial. LPL in both plasma and liver biopsies performed in morbidly obese patients presents higher LPL activity than controls, and unlike the controls, this enzyme could be synthesized in the liver because LPL mRNA is also present. The presence of the LPL activity could enable the liver to capture circulating triacylglicerides, thus favoring the typical steatosis observed in these patients (6). TAC, giving place to a sort of LPL deficiency leads to hypertriglyceridemia that, similarly to diets high in refined carbohydrates, which have been shown to cause in contrast tissue-specific overexpression of LPL, induces IR.

What is more, researchers struggle to identify the best ways of attaining optimal combined lipid values, independently and significantly, to decrease the risk of CV events. Dyslipidemia is a cluster of potentially atherogenic lipid and lipoprotein abnormalities that are metabolically interrelated. Recent evidence suggests that a fundamental defect is an overproduction of large, very low-density lipoprotein (VLDL) particles, resulting in higher levels of remnant particles, smaller low density lipoprotein cholesterol (LDL-C), and lower levels of high-density lipoprotein cholesterol (HDL-C) (7). Thus, opportune strategies should focus on the assessment of multiple lipid abnormalities, and not on single lipid risk factor modification. The aim of this study is therefore to investigate the behavior of blood lipids in a well-established population of renal transplant recipients during six years of treatment with Tac.

MATERIALS AND METHODS

Twenty-nine kidney transplant recipients, 14 female, mean age±SD: 44.3±7.8 years, mean time ± SD since transplantation: 6.6 ± 3.2 years, selected from a cohort of 164 subjects on Tac treatment and routinely followed-up were enrolled in the study. Exclusion criteria are reported in Table I. The patients gave their informed consent and the study was approved by the Local Ethics Committee. Detailed data concerning immunosuppressive regimens during the follow-up study are reported in Table II. Thirteen patients were switched from CsA to Tac treatment because of cosmetic complications and sixteen owing to the initial impairment of graft function. Before Tac treatment (basal) and at 3, 6, 12, 18, 24, 36, 48, 60, 72 months of Tac treatment, the following biochemical parameters were monitored: total cholesterol (TC), TG, HDL-C, LDL-C, VLDL-C, glucose, alanine aminotransferase (ALT), gamma-glutamiltransferase (γ-GT). All the laboratory data were measured by using commercial kits except LDL-C and VLDL-C that were calculated according to the following formulas: LDL-C = TC – (HDL-C + TG/5) and VLDL-C = TG/5 (8). At the same time points, Tac blood concentrations were also measured by using the microparticle enzyme immunoassay on IMx instrument (Abbott, North Chicago, IL) (9). Data were expressed as mean ± SD. The values obtained at the considered times were compared to basal values by applying the ANOVA test for repeated measures. For each subject, the same variable was measured several times and the basal value was used as covariate using the Wilks's Lambda multivariate test. The correlations between Tac concentrations or Tac doses and all biochemical parameters were evaluated by the linear regression analysis. P values <0.05 were considered statistically significant.

RESULTS

During the period of follow-up, Tac concentrations fell into the recommended therapeutic range of 5-15 ng/mL, Table II. During the first three months of treatment with Tac, there was a rapid increase of TG (W-L = 0.146, p= 0.007) as well as VLDL-C (W-L = 0.118, p= 0.03) and LDL-C (W-L = 0.196, p= 0.004), a decrease of high density HDL-C for both men and women (W-L = 0.188, p= 0.004 and W-L = 0.192, p= 0.004, respectively), and finally a rise of glucose (W-L = 0.112, p= 0.02), all of which reached stable concentrations between six and twelve months and then decreased during the following months, showing a clear bi-phasic trend. TC levels remained practically unchanged (W-L = 0.72, p= 0.9). Both lipids and glucose profiles obtained at the end of the entire period of Tac treatment did not significantly differ from those observed at the basal time. In addition, significant

Table I. *Exclusion criteria of selected patients.*

Diseases	Patients	Drugs
HCV-related-chronic hepatitis	41	
HBV-related-chronic hepatitis	13	
Alcohol abuse	28	
	19	Statins
	11	Clofribrate
	17	Oral antidiabetics
	6	Insulin

Table II. *Immunosuppressive regimens during the six-year follow-up period.*

Dosage	Basal	3m	6m	12m	18m
TAC, mg/day (29)	6.5±4.1	6.4±4.3	6.3±3.7	5.4±2.7	5.1±2.1
CCS, mg/day (29)	10.4±3.7	10.0±3.7	8.3±3.4	7.4±2.3	6.2±1.8
MMF, g/day (5)	1.7±0.6	1.7±0.6	1.7±0.3	1.7±0.3	1.7±0.3
AZA, mg/day (12)	68.7±17.7	68.7±17.7	62.50±18.9	53.1±16.0	53.1±16.0
TAC level, ng/mL	9.2±3.9	8.8±3.4	8.4±2.7	7.9±2.3	9.3±2.8

Dosage	24m	36m	48m	60m	72m
TAC, mg/day (29)	5.0±2.2	5 .1±2.2	4.2±2.5	4.5±3.6	4.2±4.2
CCS, mg/day (29)	5.7±1.9	4.8±1.8	4.3±1.7	4.4±2.1	4.2±1.6
MMF, g/day (5)	1.7±0.3	1.5±0.5	1.6±0.5	1.6±0.5	1.2±0.3
AZA, mg/day (12)	50.0±13.4	37.5±16.1	21.8±6.2	18.7±8.8	18.7±8.8
TAC level, ng/mL	7.5±1.6	7.3±1.6	7.7±2.2	7.0±2.1	7.0±2.0

Tac: tacrolimus; CSS=corticosteroids; MMF: mycophenolate mofetile; AZA: azathioprine; m.: months. In parenthesis the number of treated patients.

changes in enzymatic activity of ALT and γ-GT were also found (W-L = 0.189, p= 0.003 and W-L = 0.162, p= 0.005, respectively), Table III. No significant correlations between all the biochemical parameters investigated and Tac concentrations or Tac dose were observed, Table IV. No patient needed treatment with lipid lowering agents. Finally, no noteworthy cardiovascular events were observed in the course of follow-up period.

DISCUSSION

Tac is able to inhibit insulin gene transcription leading to a decline of insulin synthesis (10). In addition, the immunosuppressive agent is responsible for hyperlipidemia by the reduction of the lipoprotein lipase activity (11). On the basis of this "experimental" evidence, the drug has a potential for IR and hyperlipidemia. Associated elevated LDL cholesterol levels and hypertriglyceridemia observed in the first period following Tac treatment may be due to the increase in cholesteryl ester transfer protein (CETP) activity and suppression in hepatic LPL (11). The same mechanisms undermine the development of hepatic fat accumulation, well-documented by the contextual, specific increase of

Table III. *Biochemical profiles of 29 kidney transplant recipients before (basal) and during tacrolimus treatment.*

Parameter	Basal	3m	6m	12m	18m	24m
TC (mg/dL)	187±38	181±33	181±39	183±34	180±36	180±32
TG (mg/dL)	133±45	225±44	240±31	129±36	127±37	123±34
HDL-C (mg/dL♂)	44±8	35±7	37±6	42±5	44±6	44±5
HDL-C (mg/dL♀)	55±6	39±5	41±8	57±6	50±8	49±5
LDL-C (mg/dL)	116±37	210±30	213±37	115±32	112±33	111±29
VLDL-C (mg/dL)	26±9	75±9	86±6	26±7	26±7	25±6
Glucose (mg/dL)	97±33	159±14	198±11	94±21	92±17	93±16
ALT (U/L)	22±13	87±15	92±13	24±12	19±6	18±6
γ-GT (U/L)	29±18	63±26	71±9	35±18	37±14	36±11

Parameter	36m	48m	60m	72m	W-L value	P
TC (mg/dL)	182±33	175±36	174±36	167±32	0.72	0.9
TG (mg/dL)	126±29	135±53	133±43	126±4	0.146	0.007
HDL-C (mg/dL♂)	42±4	45±11	47±8	46±8	0.188	0.004
HDL-C (mg/dL♀)	52±5	50±13	56±8	55±8	0.192	0.004
LDL-C (mg/dL)	113±31	100±33	100±31	99±26	0.196	0.004
VLDL-C (mg/dL)	25±5	27±11	26±8	24±7	0.118	0.03
Glucose (mg/dL)	101±28	96±22	104±27	99±25	0.112	0.02
ALT (U/L)	21±7	22±9	22±7	19±7	0.189	0.003
γ-GT (U/L)	29±13	29±11	29±8	31±12	0.162	0.005

m: months; W-L value: Wilk's lambda value; TC: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; VLDL-C: very low density lipoprotein-cholesterol; ALT: alanine aminotransferase γ-GT: gamma-glutamiltransferase

Table IV. *Correlation degrees (r) between lipids and glucose levels and tacrolimus (Tac) dose, Tac concentrations, corticosteroids (CCS) dose.*

	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)	Glucose (mg/dL)
vs Tac (mg/day)	0.17	-0.06	0.07	0.17	-0.08	-0.14
vs Tac (mg/Kg/day)	0.17	-0.06	0.03	0.19	-0.07	-0.12
vs Tac (ng/mL)	0.05	0.02	0.00	0.05	0.00	0.04
vs CCS (mg/day)	-0.02	0.08	-0.10	0.01	-0.06	-0.18

TC: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; VLDL-C: very low density lipoprotein-cholesterol

ALT and γ-GT. How are there such opposing results regarding the metabolic side-effects of TAC, when analyzing literature data? In fact, hyperlipidemia was found by several authors (12), but these finding disagree with the results of others (13). Likely, the observation time plays a key role because in our patients a bi-phasic profile is clearly shown that is outlined by dietetic restriction after serum lipid and glucose elevation.

In the present study Tac was temporarily burdened by a modification of the glucose and lipid profiles in kidney transplant recipients, independently from its doses and serum levels as confirmed by the absence of correlations between doses or levels of

Tac and serum concentrations of the investigated biochemical parameters. Our results support the idea that Tac has precocious and short-lasting effects on the glucose and lipids profiles. Furthermore, the absence of prolonged hyperlipidemia observed during the following months of Tac treatment can be advantageous for an optimal setting of the immunosuppressive regimen.

This study has some limitations: the relatively low number of enrolled patients; the stringent criteria of patients' inclusion (no individual and/or family positive history for diabetes and hyperlipidemia, no HCV positivity and no co-administration of other drugs potentially able to influence glucose and lipid profiles) associated with a long follow-up partially justified this drawback. Another flaw could be represented by the lack of features of abdominal ultrasonography to detect NAFLD, even though it was consequential to imagine the presence of this disease in dyslipidemic patients. Finally, some roles of steroids on the induction of the liver steatosis could not be excluded, even though it is difficult to explain why their effects lasted only for a relatively short period of time.

Discussing possible mechanisms concerning the role of the liver to explain our findings, we pinpoint the following: Tac is known to affect the expression of various cytokines, such as interleukin-6 and tumor necrosis factor, and having a long-term action on these cytokines probably reduces the exposure of the liver to lipid excess by decreasing the chronic inflammation that increases the IR in a vicious circle.

In conclusion, the Medical Heart Associations have repeatedly emphasized CVD prevention, offering evidence-based recommendations, mainly based on lipid management. This study shows a good lipid-level attainment on long term, differently from CsA and mTOR inhibitors. As the current challenge in choosing the best immunosuppressive regimen is the balance between the efficacy and the safety to optimize graft and patient survival rates, long term Tac treatment can be a reliable option in the immunosuppressive protocols of organ transplanted recipients.

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