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Tacrolimus, cyclosporine and plasma lipoproteins in renal transplant recipients

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Abstract To compare the effect of tacrolimus (FK506) and cyclosporine (CsA) on plasma lipoproteins in renal transplant recipients receiving maintainance therapy, the following prospective study was undertaken. Blood from nineteen recipients on tacrolimus (FK group) and from twenty-one on CsA (CsA group) was collected at baseline, 3-, 6-, and 10-month intervals. Plasma lipids, lipoproteins and oxidation properties of lipoproteins were determined. Plasma total cholesterol, low density lipoprotein (LDL) cholesterol, and apolipoprotein B (apoB) were substantially increased in both groups, although only the CsA group showed significant differences at all time intervals and at the baseline. High density lipoprotein cholesterol, triglycerides, and apolipoprotein A varied in both groups at time intervals from the baseline, but not significantly. The susceptibility to oxidation of LDL isolated from the FK group at all times was uninfluenced by the tacrolimus treatment, and values were comparable to those obtained from LDL isolated from healthy individuals. A significantly higher susceptibility to oxidation as indicated by the shorter time required to start the formation of conjugated dienes was observed in LDL isolated from the CsA group at 3 and at 6 months of therapy. Tacrolimus-treated patients appear to have less hyperlipidemic and have LDL less susceptible to oxidation than patients treated with CsA.

Keywords Tacrolimus · Cyclosporine · Lipoproteins · Cardiovascular disease · Renal transplantation

Introduction

The cause of posttransplant cardiovascular disease (CAD) is not well defined, but it is almost certainly multifactorial. Hyperlipidemia or dislipidemia, which appears to be one of the strongest non-immunologic risk factors for posttransplant CAD, is common after kidney transplantation, and evidence indicates that immunosuppressive agents like cyclosporine (CsA), [6, 8, 15, 16, 21, 23, 29] and corticosteroids [13] play a role in posttransplant atherosclerosis. In addition, recent evidence has shown that low density lipoproteins (LDL) isolated from renal transplant recipients treated with CsA showed an increased tendency to oxidize *in vitro* faster than LDL free of CsA [3, 11, 26, 27]. On the other hand, Chancellere, *et al* [6] showed that CsA-treated heart transplant recipients had increased levels of oxidized lipids circulating in their blood, suggesting that CsA promoted *in vivo* oxidation of circulating lipids. Oxidized lipids and oxidized LDL in particular, have several properties which are potentially atherogenic [25]. There is no reason to assume that oxidized LDL, as a risk factor for atherosclerosis, and CAD in the general population, would be any less in renal transplant recipients who have a higher-than-normal incidence of both hyperlipidemia and CAD. In fact, de Lorgeril, *et al* [9] as well as Cristol, *et al* [7] have demonstrated that the combination of lipid abnormalities and oxidative

stress in renal transplant recipients enhance accelerated transplantation-associated coronary atherosclerosis.

The aim of the present study was to assess lipid abnormalities in renal transplant recipients treated with the immunosuppressant agent tacrolimus or FK506 (Fujisawa Pharmaceutical, Osaka, Japan) during a period of 10 months and to compare results with those obtained from patients treated with CsA and healthy controls. In particular, the study aimed at investigating the susceptibility to oxidation *in vitro* of LDL isolated from renal transplant recipients treated with tacrolimus, and comparing results with patients on CsA and healthy controls.

Materials and methods

Forty patients (30 males and 10 females) were selected from a group of 85 consecutive renal cadaveric allograft recipients. The study cohort comprised patients randomized in a prospective, multicenter comparative trial of FK506 versus Sandimmune-CsA therapy; both groups were supplemented with identical doses of prednisone and azathioprine. In the FK group, tacrolimus was administered at the time of the study at a dosage producing blood levels from 5–15 ng/ml. Sandimmune-CsA was administered to patients in the CsA group in doses calculated to produce trough levels between 200 and 300 ng/ml. Patients with preexisting diabetes, hyperlipidemics, and such receiving cholesterol-lowering medications, antioxidant vitamins, or fish oil supplements were excluded. All patients had a serum creatinine level under 2 mg/dl. Normal subjects (12 males and 4 females) were also included as controls. The experimental protocols, which had been approved by the Human Investigations Institution Review Board of Emory University, were fully explained to the subjects, and their informed consent was obtained.

Isolation of lipoproteins

Whole blood was obtained at clinic visits and drawn in vacutainers containing 1 mg/l ethylenediaminetetraacetate (EDTA) as anticoagulant and antioxidant. LDL was isolated from plasma using a Beckman TL-100 tabletop ultracentrifuge according to the method of Santanam and Parthasarathy [21]. A single spin gradient isolation was done by adjusting 2 ml of plasma to d = 1.31 with KBr layered with saline and spun at 100,000 rpm for 1 h. The isolated sample was respun at d = 1.21 to concentrate and purify the LDL from any albumin contamination. The isolation was carried out without any EDTA and was completed in less than 3 h. The isolated LDL was dialyzed against PBS at 4 °C for 4–6 h [21]. The purity of the isolated LDL samples was established by agarose and acrylamide gel electrophoresis.

Oxidation of LDL

LDL samples were oxidized immediately after isolating, and each experiment consisted of 4 samples which include two controls [22]. Typically, 100 μg/ml of LDL was incubated in PBS with 5 μM copper ions. The oxidation of LDL was followed continuously by measuring the formation of conjugated dienes at OD 234 nm in SLM-Aminco DB-3500 spectrophotometer equipped with a 12 chamber cuvette changer. Samples and references were measured continuously for periods of up to 6 h. Oxidation was arrested by refrigeration and the addition of 200 μM EDTA. From the kinetic profile of each LDL preparation, several indexes can be determined [3, 18]. The first index is the lag-phase, which is defined as the interval in minutes between the intercept of the linear least-square slope of the curve with the initial absorbance axis. The second index is the maximal rate of oxidation, which can be calculated from the slope of the absorbance curve during the propagation phase by using the molar absorptivity for conjugated dienes (e_{234} = 29,500 l/mol per cm). The molar absorptivity level was used also to determine the third index, the maximal amount of conjugated dienes which were produced during the oxidation.

Biochemical analyses

Cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides in plasma were determined by standard enzymatic procedures on a Dimension AR, Random Access Autoanalyzer (Dupont, Wilmington, DE). Whole blood was obtained at the indicated intervals. The HDL cholesterol level was determined subsequent to precipitation of apolipoprotein B (apoB) containing lipoproteins, according to the procedure of Warnick, *et al* [30], by the dextran-sulfate-magnesium-method, and the LDL cholesterol level was calculated by applying the Friedewald formula [10]. Apolipoprotein A (apoA) and apoB were determined by rate immunonephelometric procedures on a Cobas Mira Autoanalyzer (Roche Diagnostics, Nutley, NJ). Overall coefficients of variation (CV), including interassay and intraassay variability were 2.8%, 3.2%, 4.8%, 5.2%, and 6.8% for plasma cholesterol, plasma triglycerides, HDL cholesterol, calculated LDL cholesterol, plasma apoA, and apoB level, respectively. These measurements were consistently monitored by the lipid program of the Centers for Disease Control and the National Heart, Lung, and Blood Institute. Protein of freshly prepared LDL was determined on the Cobas-Bio (Roche Diagnostics, Nutley, NJ) Automated Centrifugal Analyzer [16]. Concentrations of LDL were calculated on the assumption that the protein part of the lipoprotein was 22.7% (w/w) of the LDL molecule.

CsA assay

Whole blood trough plasma CsA concentrations (12 h after the last dose) were measured with a fluorescence polarization immunoassay, employing monoclonal antibodies on Abbott's TDX Analyzer (Abbott, Abbott Diagnostics, Abbott Park, Illinois), [1]. CsA in LDL preparations was measured similarly. The interassay CV of CsA analyses in whole blood was 5%.

Tacrolimus assay

Whole blood was used with the IMx (Abbott) Tacrolimus Assay, which is a microparticle enzyme immunoassay technology. The intraassay CV is 2.5% and the interassay CV analyses in whole blood was 5%, [2].

Statistical analysis

All values reported are the mean ± SEM. For statistical analysis, the t-test (nominal data), the Mann-Whitney U test for non-parametric data (CsA against FK data), and the Wilcoxon matched

Table 1 Demographic and clinical features

	FK Group	CsA Group
Number of Patients	19	21
Male	15	15
Female	4	6
Age	41.8 ± 19.2	39.2 ± 10.5
% Hypertensive drugs	18	20
Diuretics	2	–
b-Blockers	10	10
Calcium antagonists	16	13
ACE inhibitors	6	6

pairs signed-ranks test (pretransplant against posttransplant data) were used, $P < 0.05$ values were considered significant (Statistical package) [19].

Results

There were no significant differences between the demographical and clinical features of the groups. Table 1 shows that the FK group had 15 males and 4 females, and the CsA group had 15 males and 6 females. The mean age was 39.2 ± 10.5 for the FK group, and 41.8 ± 9.2 for the CsA group, respectively. The number of patients taking antihypertensive drugs was 18 in the FK group, and 20 in the CsA group.

Table 2 shows serum lipid concentration in both groups and at all time intervals. Healthy control levels were as follows: 175 ± 15 mg/dl for total cholesterol, 82 mg/dl ± 8 for triglycerides, 52 ± 7 mg/dl for HDL cholesterol, 95 ± 10 mg/dl for LDL cholesterol, 120 ± 13 mg/dl for apoA, and 98 ± 9 mg/dl for apoB.

Baseline values of serum lipids were within the normal range according to NIH criteria, and there was no hyperlipoproteinemia. Compared with healthy subjects, cholesterol, LDL cholesterol, and apoB values were significantly higher in recipients receiving CsA treatment. Triglyceride values at baseline were significantly higher in renal recipients in both groups compared to controls. Compared to baseline values, there was an increase in serum cholesterol in the FK group (5–7%) as well as in the CsA group (15–18%) during therapy. That increase reached significance ($P < 0.01$) only in the CsA group. The increase in the cholesterol level that occurred during treatment varied from patient to patient and ranged from 0–50%. There was also an increase in the mean LDL cholesterol values in the FK group (3–5%), and a significant increase of LDL cholesterol in the CsA group (11–16%) at 6- and 10 month intervals. HDL cholesterol levels and triglycerides did not change in a remarkable way during treatment either with tacrolimus or CsA. Levels of apoA showed a slight increase (4–5%), compared to the baseline values in both groups. In contrast, apoB levels showed an increase of 5–9% compared to baseline values during treatment with tacrolimus, and a significant increase (10–18%) during treatment with CsA at 6- and 10-month intervals.

In a preliminary study, conjugated diene curves obtained during copper oxidation of LDL from twelve healthy subjects (average age 28 ± 9 years) gave the following oxidation parameters: lag time was 99.8 ± 12.3 min; maximum rate of oxidation was 2.8 ± 1.1 mol/min per g LDL; and maximum production of dienes 105 ± 18 mol/g LDL. Reproducibility with pooled LDL was within and among assays, 4.8% and 6.7% for lag time, 6.8% and 10% for maximum oxidation rate, and 5.9 and 7.8% for maximum diene production, respectively. In this study, we use the terms "susceptibility or resistance to oxidation" to refer to the delay or onset of LDL oxidation (lag time) and "oxidizability" for the maximum rate of oxidation.

Table 3 summarizes data obtained from the oxidation of isolated LDL by copper ions in both groups. At baseline levels, the lag time in conjugated diene formation was significantly shorter in renal transplant recipients, compared with healthy subjects. Patients receiving CsA had significantly shorter lag times than patients receiving tacrolimus and healthy individuals. The maximum rate of oxidation and maximal amount of conjugated dienes at baseline were lower than controls, but not at a significant level. There was an insignificant increase in lag time in the FK group at the 3-, 6-, and

Table 2 Plasma lipids and lipoprotein (mg/dl) concentrations[a]

	Cholesterol		Triglycerides		HDL-Cholesterol	
Time interval	FK-506	CsA	FK-506	CsA	FK-506	CsA
Baseline	176.4 ± 5.5	180.3 ± 7.9	164 ± 14.5	160 ± 10	44.8 ± 3.5	45.2 ± 3.4
3 Months	189.2 ± 5.2	207.3 ± 6.09[b]	160 ± 14.8	171 ± 11	48.6 ± 3.8	45.8 ± 4.1
6 Months	185.3 ± 5.8	210.5 ± 6.2[b]	172 ± 14.2	168 ± 11	44.2 ± 4.0	50.2 ± 4.2
10 Months	183.8 ± 5.4	213.2 ± 10.3[b]	168 ± 15.2	158 ± 14	50.3 ± 3.9	49.2 ± 4.0
	LDL-Cholesterol		APO A		Apo B	
Time interval	FK-506	CsA	FK-506	CsA	FK-506	CsA
Baseline	116 ± 17.6	118 ± 18	127.8 ± 19.6	129.8 ± 16.4	109.8 ± 21.4	112 ± 18.2
3 Months	118 ± 18.2	124 ± 20	134.7 ± 19.2	136.6 ± 17.3	116.4 ± 20.6	124 ± 16.3
6 Months	122 ± 17.6	132 ± 24[b]	138.6 ± 17.3	134.7 ± 20.4	118.2 ± 20.8	132 ± 17.4[b]
10 Months	122 ± 18.2	131 ± 22[b]	132.4 ± 18.4	136.6 ± 14. 8	121.4 ± 21.4	136 ± 16.8[b]

[a]Mean ± SEM
[b]$P < 0.01$

Table 3 Oxidation parameters of LDL isolated from patients receiving FK-506 and cyclosporine[a]

	Lag phase (*min.*)		Maximum rate of oxidation (μmol/min/mg LDL)		Maximal amount of conjugated dienes (μmol/g LDL)	
Time interval	FK-506	CsA	FK-506	CsA	FK-506	CsA
Baseline	96.9 ± 15.2	88.9 ± 14	1.9 ± 0.3	1.8 ± 0.5	76 ± 18	81 ± 15
3 Months	96.1 ± 16	71.4 ± 10.1[b]	1.7 ± 0.3	1.8 ± 0.5	78 ± 14	78 ± 13
6 Months	92.3 ± 13.5	76.3 ± 10[b]	1.8 ± 0.4	1.7 ± 0.4	83 ± 12	81 ± 13
10 Months	97.2 ± 12.8	85.6 ± 13.5	1.9 ± 0.3	1.7 ± 0.4	86 ± 13	80 ± 14

[a]Mean ± SEM
[b]$P < 0.02$

Table 4 Blood concentration[a] of immunosuppressants at times of sampling to test oxidation susceptibility of LDL

Time Interval	FK-506 ng/ml	CsA ng/ml
Baseline	8.2 ± 0.8	300 ± 17
3 Months	7.3 ± 0.4	262 ± 16
6 Months	7.5 ± 0.7	240 ± 16
10 Months	6.4 ± 0.4	248 ± 14

[a]Mean ± SE

10 month intervals. In contrast, there was a substantial decrease in lag time values in the CsA group, which at 3- and 6 months became significant ($P < 0.02$). At 10-month intervals, lag phases were shorter than those of the baseline at a marginal level of significance. The maximum rate of oxidation and the maximal amount of conjugated dienes remained unchanged from the baseline values at all times.

Table 4 shows the mean ± SEM, CsA trough values at baseline and during treatment. Values ranged from 300–240 ng/ml, and differences were significantly different among the baseline at 6- ($P < 0.013$) and at 10-month ($P < 0.027$) intervals. CsA levels correlated poorly with total cholesterol values ($r = 0.47$), or with changes in cholesterol ($r = 0.38$) during the period of the study. Tacrolimus levels are also shown in Table 4 at baseline and time intervals. The values ranged from 8.2–6.4 ng/ml, and there were no significant differences among time intervals.

Discussion

Our study demonstrates that: 1) In kidney-transplant recipients, serum levels of total cholesterol increased during tacrolimus and CsA treatment. The increase in total cholesterol, LDL cholesterol and apoB reached significantly higher levels only during the CsA treatment, compared to the levels of healthy individuals or patients receiving the tacrolimus treatment. 2) The lag time in conjugated diene formation during oxidation by copper ions was significantly shorter in LDL derived from renal transplant recipients under CsA treatment. LDL isolated from patients receiving tacrolimus showed no influence of the treatment on the LDL susceptibility to *in vitro* oxidation. Values remained the same and were similar to those of healthy controls. In contrast, LDL isolated from plasma of recipients treated with CsA, showed significantly higher susceptibility to *in vitro* oxidation at 3- and at 6 months treatment. At 10 months of CsA treatment, however, we observed a decrease in the susceptibility of LDL to *in vitro* oxidation, although the CsA blood levels were maintained at the same range as the CsA blood levels in previous months. It should be emphasized, however, that in our study we used Sandimmune-CsA, a formulation of CsA which is free of antioxidants and other supplements. In contrast, Varghese and coworkers in their recent study [28] used the formulation of CsA, known as neoral, which is CsA fortified with dl-tocopherol. They reported that LDL from tacrolimus-treated patients had a significantly lower oxidation lag time compared with neoral-treated patients. The presence of the antioxidant tocopherol most likely explains the relative normality of oxidation lag time in neoral treated patients.

All these results suggested to us that the interplay between tacrolimus and lipoproteins in transplant patients as well as in other patients is completely different from the interplay between CsA and lipoproteins. Tacrolimus, like CsA, is lipophilic. It suppresses interleukin-2 production and receptor expression on T-cells [24]. However, it may differ in other properties, including the formation of complexes with lipoproteins, which determine the susceptibility to oxidative modification of plasma lipoproteins *in vitro* and presumably *in vivo*. It is of interest to note, that Warty *et al* [31] reported that tacrolimus and CsA show significantly different binding and distribution among plasma lipoproteins and proteins. The majority of plasma CsA is associated with LDL and HDL, [12, 22] where the average percent distribution of cholesterol is highest. Plasma tacrolimus, on the other hand, is mainly associated with plasma proteins, such as albumin and/or alpha-1 acid glycoprotein [26].

Abnormalities in circulating lipoproteins are common after renal transplantation, and they have been reported in the past [5, 8, 12, 14, 15, 20, 22, 29]. These abnormalities include elevations in total cholesterol, LDL cholesterol, apoB [17], as well as increases in VLDL and triglycerides. Vathsala, *et al* [29] studied 500 CsA-

treated renal transplant recipients and found a 37% incidence of cholesterol levels over 300 mg/dl, which occurred within 6 months in the majority of patients. HDL cholesterol concentrations, on the other hand, were usually normal, although the composition of HDL may not be normal. In addition to increased levels of commonly measured lipoproteins, other more subtle abnormalities may make lipoproteins particularly atherogenic in renal transplant recipients.

It has become quite apparent that the atherogenicity of LDL, quite apart from plasma concentrations, may be dependent on molecular modifications of LDL size and -structure [25]. Oxidatively modified LDL is taken up by macrophages via scavenger receptor leading to formation of lipid-laden foam cells, which are the hallmark of early atherosclerotic fatty streaks. Oxidatively modified LDL may promote atherosclerosis by other mechanisms as well, such as recruitment and retention of monocyte-macrophages in the arterial wall and cytotoxicity toward endothelial cells. The "oxidative hypothesis of atherosclerosis" is supported by a number of *in vivo* findings [25]. However, until we can assess the course of kidney-grafted patients over several decades, the clinical implications of these findings would remain unclear. It is of interest to note however, that it is not only the incidence but also the duration of lipoprotein abnormalities that has important implications for renal transplant recipients. Most studies show that lipoprotein abnormalities persist into the very late posttransplant period [17]. Others report increased lipid levels after more than 10 years of follow-up [4].

Because LDL levels and properties of LDL have been shown to correlate directly with risks of atherosclerosis and CAD [25], changes in the lipoprotein profiles and in their properties observed here are likely to contribute to atherosclerosis in posttransplant patients in the long term incidence of atherosclerosis and CAD.

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References

1. Abbott Cyclosporine Package Insert (1990) Abbott Laboratories, Diagnostics Division, Abbott Park, IL 60064
2. Abbott Tacrolimus II Package Insert (1993) Abbott Laboratories, Diagnostics Division, Abbott Park, IL 60064
3. Apanay DC, Neylan JF, Ragab MS, Sgoutas DS (1994) Cyclosporine increases the oxidizability of low-density lipoproteins in renal transplant recipients. Transplantation 58: 663–669
4. Arnadottir M, Berg AL (1997) Treatment of hyperlipidemia in renal transplant recipients. Transplantation 63: 339–345
5. Ballantyne CM, Podet EJ, Patsch WP, Harati Y, Appel V, Gotto AM Jr, Young JB (1989) Effects of cyclosporine therapy on plasma lipoprotein levels. JAMA 262: 53–56
6. Chancerelle Y, de Lorgeril M, Viret R, Chiron B, Dureau G, Renaud S, Kergonou JF (1991) Increased lipid peroxidation in cyclosporin-treated heart transplant recipients. Am J Cardiol 68: 813–816
7. Cristol JP, Vela C, Maggi MF, Descomps B, Mourad G (1998) Oxidative stress and lipid abnormalities in renal transplant recipients with or without chronic rejection. Transplantation 65: 1322–1328
8. de Groen PC (1988) Cyclosporine low-density lipoproteins, and cholesterol. Mayo Clinic Proc 63: 1012–1021
9. de Lorgeril M, Richard MJ, Arnaud J, Boissonnat P, Guidollet J, Dureau G, Renaud S, Favier A (1993) Lipid peroxides and antioxidant defenses in accelerated transplantation-associated coronary arteriosclerosis. Am Heart J 125: 974–980
10. Friedewald WT, Levy RI, Fredrickson DS, (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18: 499–502
11. Ghanem H, van den Dorpel MA, Weimar W, El-Kannishy MH, Jansen H (1996) Increased low density lipoprotein oxidation in stable kidney transplant recipients. Kidney Int 49: 488–93
12. Gurecki J, Warty V, Sanghvi A (1985) The transport of cyclosporine in association with plasma lipoproteins in heart and liver transplant patients. Transplant Proc 17: 1997–2002
13. Hricik DE, Mayes JT, Schulak JA (1991) Independent effects of cyclosporine and prednisone on posttransplant hypercholesterolemia. Am J Kidney Dis 18: 353–358
14. Kasiske BL, Tortorice KL, Heim-Duthoy KL, Awni WM, Rao KV (1991) The adverse impact of cyclosporine on serum lipids in renal transplant recipients. Am J Kidney Dis 17: 700–707
15. Kuster GM, Drexel H, Bleisch JA, Rentsch K, Pei P, Binswanger U, Amann FW (1994) Relation of cyclosporine blood levels to adverse effects on lipoproteins. Transplantation 57: 1479–1483
16. Clifton PM, Chang L, Mackinnon AM (1988) Development of an automated Lowry protein assay for the Cobas-Bio centrifugal analyzer. Anal Biochem 172: 165
17. Macri L, Thibert RJT, Adeli K (1997) Effects of the immunosuppressive agent cyclosporine A on the production of apolipoprotein B containing lipoproteins (abstract). Clin Chem 43: 5261
18. Noble BP (1968) Electrophoretic separation of plasma lipoproteins in agarose gel. J Lipid Res 9: 693–700
19. Norusis MJ (1988) Statistical package for the social sciences (SPSS/PC) base manual. SPSS Inc, Chicago, IL
20. Raine AE, Carter R, Mann JI, Morris PJ (1988) Adverse effect of cyclosporine on plasma cholesterol in renal transplant recipients. Nephrol Dial Transplant 3: 458–463
21. Santanam N, Parthasarathy S (1995) Paradoxical actions of antioxidants in the oxidation of low density lipoproteins by peroxidases. J Clin Invest 95: 2594–2600

22. Sgoutas D, MacMahon W, Love A, Jerkunica I (1986) Interactions of cyclosporine A with human lipoproteins. J Pharm Pharmacol 38: 583–588
23. Sharma AK, Myers TA, Hunninghake DB, Matas AJ, Kashtan CE (1994) Hyperlipidemia in long-term survivors of pediatric renal transplantation. Clin Transplant 8: 252–257
24. Starzl TE, Fung J, Jordan M, Shapiro R, Tzakis A, McCauley J, Johnston J, Iwaki Y, Jain A, Alessiani M (1990) Kidney transplantation under FK506. JAMA 264: 63–67
25. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL (1989) Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 320: 915–924
26. Sutherland WH, Walker RJ, Ball MJ, Stapley SA, Robertson MC (1995) Oxidation of low density lipoproteins from patients with renal failure or renal transplants. Kidney Int 48: 227–236
27. van den Dorpel MA, Ghanem H, Rischen-Vos J, Jansen H, Weimar W (1997) Conversion from cyclosporine A to azathioprine treatment improves LDL oxidation in kidney transplant recipients. Kidney Int 51: 1608–1612
28. Varghese Z, Fernando R, Turakhia G, Psimenou E, Brunton C, Fernando ON, Davenport A, Burns A, Sweny P, Powis SH, Moorhead JF (1998) Oxidizability of low-density lipoproteins from neoral and tacrolimus-treated renal transplant patients. Transplant Proc 30: 2043–2046
29. Vathsala A, Weinberg RB, Schoenberg L, Grevel J, Dunn J, Goldstein RA, Van Buren CT, Lewis RM, Kahan BD (1989) Lipid abnormalities in renal transplant recipients treated with cyclosporine. Transplant Proc 21: 3670–3673
30. Warnick GR, Benderson J, Albers JJ (1982) Dextran sulfate-Mg2 + precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 28: 1379–1388
31. Warty V, Venkataramanan R, Zendehrouh P, McKaveney T, Chao J, Todo S, Starzl T (1991) Distribution of FK506 in plasma lipoproteins in transplant patients. Transplant Proc 23: 954–955