Transpl Int (1996) 9 [Suppl 1]: S54–S57

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Low-density lipoprotein oxidation is increased in kidney transplant recipients

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Abstract Oxidative modification of low-density lipoproteins (LDL) plays an important role in the pathogenesis of atherosclerosis. In addition, there is evidence that chronic vascular allograft rejection may be mediated by oxidised LDL. Plasma lipoprotein concentrations and parameters of LDL oxidation were determined in 19 kidney transplant recipients and 19 healthy controls. Plasma triglycerides and total cholesterol was significantly higher in patients than in the controls. The mean LDL diameter was smaller in patients than in the controls (23.6 ± 0.71 nm vs 27.78 ± 1.16 nm, $P < 0.002$). Furthermore, the lag time of copper-induced in vitro LDL oxidation was shorter in patients than in the controls (101 ± 23 min vs 148 ± 81 min, $P = 0.02$). The titre and concentration of both IgG and IgM autoantibodies against malondialdehyde-modified LDL (MDA-LDL) were higher in the patients. We conclude that there is in vitro and in vivo evidence of increased LDL oxidation in renal transplant recipients. This might facilitate the progression of atherosclerosis and enhance the process of chronic vascular rejection.

Key words Kidney transplantation · Low-density lipoproteins · Atherosclerosis · Chronic vascular rejection

Introduction

The morbidity and mortality due to atherosclerotic cardiovascular disease is high in renal transplant recipients [1, 2]. Besides increased plasma lipoprotein concentrations, alterations in the composition and susceptibility to oxidation of lipoproteins may also play a role in atherosclerosis in kidney transplantation patients [2, 3]. Oxidative modification of low-density lipoproteins (LDL) probably precedes the uptake of LDL by macrophages and accelerates the accumulation of cholesterol in the arterial wall [4, 5]. In addition, oxidised LDL is highly immunogenic and elicits an inflammatory response which often accompanies atherosclerosis and closely resembles chronic vascular rejection [5–7].

The susceptibility of LDL to oxidation can be determined in vitro. A parameter for the susceptibility of LDL to oxidation is the time that elapses before lipid peroxidation products become detectable (lag time) after addition of the oxidant copper chloride [8]. The plasma concentration of autoantibodies against epitopes of oxidised LDL may also reflect in vivo LDL oxidation and appears to be correlated with the progression of atherosclerosis [9, 10].

The lipoproteins in the LDL density range are heterogeneous in size but can be separated by gel electrophoresis. If the LDL fraction contains mainly large LDL, this is designated as the LDL subclass pattern "A". The presence of mainly small LDL is indicated as the LDL subclass pattern "B" [11, 12]. The pattern B is associated with a high plasma triglyceride and a low HDL cholesterol concentration and is, partly, determined by genetic factors [11]. Subjects with the LDL subclass pattern B have an increased risk of coronary heart disease [13]. Small LDL is more prone to oxidative modification than larger LDL [14]. Therefore, the

Table 1 Patient characteristics (*Tx* transplantation, *CsA* cyclosporine A)

	Patients	Controls	*P* value
Male/female	13/6	13/6	NS
Age (years)	42.2 ± 12.3	47.3 ± 9.1	NS
Body mass index (kg/m^2)	24.9 ± 3.5	25.6 ± 3.1	NS
Time after Tx (months)	24 ± 5	–	–
CsA dose (mg/kg per day)	5.5 ± 1.7	–	–
Prednisone dose (mg/day)	9.6 ± 2.1	–	–
Serum creatinine (μmol/l	154 ± 44	76 ± 9	0.05
Systolic pressure (mm Hg)	149 ± 20	139 ± 16	0.05
Diastolic pressure (mm Hg)	97 ± 14	83 ± 13	0.05

Table 2 Plasma lipid profile (mmol/l; mean ± SD)

Lipid	Patients	Controls	*P* value
Total cholesterol	5.91 ± 0.95	5.33 ± 0.50	0.017
Total triglyceride	2.40 ± 0.99	1.43 ± 0.65	0.001
LDL-cholesterol	3.73 ± 0.70	3.44 ± 0.44	0.13
HDL-cholesterol	1.09 ± 0.39	1.21 ± 0.23	0.28

LDL subclass pattern, the susceptibility of LDL to oxidation in vitro and the level of autoantibodies to epitopes of oxidised LDL seem to be indicators of in vivo LDL oxidation. In this study, we determined the LDL subclass pattern, the susceptibility of LDL to oxidation and the level of autoantibodies against oxidised LDL in the plasma of renal transplant recipients and matched controls.

Patients and methods

We studied 19 non-diabetic kidney transplant recipients with stable renal function and 19 matched healthy controls. At the time of blood sampling, none of the patients showed any signs of cardiovascular disease except hypertension. None of the control subjects had overt atherosclerosis or used antihypertensive drugs. Table 1 shows demographic data of patients and controls.

LDL for oxidation experiments were isolated by density gradient ultracentrifugation as described previously [15]. The plasma LDL cholesterol concentration was calculated using the Friedewald formula [16]. The LDL oxidation experiments were carried out as described by Esterbauer and our group [8, 15]. Oxidation was initiated by the addition of copper chloride solution (final concentration 1.66 μM). LDL oxidation was followed by monitoring the change in absorbance at 234 nm every 2 min for 16 h. The lag time was defined as the interval between initiation of the reaction and the intercept of the tangent to the slope of the absorbance curve with the time scale axis expressed in minutes.

The LDL subclass patterns were identified by electrophoresis on 2–16 % PAGE gels, as described by Austin et al. [13]. Concentrations of autoantibodies against malondialdehyde-modified LDL (MDA-LDL) were determined by ELISA as described previously [15]. Plasma cholesterol and triglycerides (Boehringer Mannheim, Mannheim, Germany) and creatinine (Sigma Diagnostics, St. Louis, USA) were determined using commercially available test kits. Creatinine clearance was estimated from the plasma creatinine concentration by the Cockcroft-Gault formula. Data are presented as means ± SD. Statistical analysis was performed using Student's *T*-test or ANOVA followed by Bonferoni where appropriate. Correlations between variables were calculated using the Pearson correlation test. The level of significance was set at $P < 0.05$.

Results

Patient characteristics are shown in Table 1. Plasma triglyceride and cholesterol levels were significantly higher in renal transplant recipients than in controls (Table 2). LDL cholesterol tended to be higher in patients than in the controls. The mean size of the most prominent LDL fraction was significantly less in patients than in the controls (Table 3). The size of the LDL was inversely correlated with the plasma triglyceride ($r = -0.66$, $P < 0.001$) and weakly positively with HDL cholesterol ($r = 0.34$, $P < 0.05$). The LDL subclass pattern B was more frequently found in patients than in the controls (Table 3). The LDL subclass pattern A (mean particle diameter 25.09 ± 0.92 nm) was more frequently found in the control subjects. In kidney recipients, LDL was more susceptible to oxidation as reflected by a shorter lag time. In subjects with an LDL subclass pattern B, the lag phase was significantly shorter than in subjects with an LDL subclass pattern A (Table 3). In patients and controls with the same subclass pattern, the lag time tended to be shorter in the patients but the differences were not statistically significant (Table 3). Plasma concentrations of autoantibodies against MDA-LDL were significantly higher in renal transplant patients than in controls (Table 3). LDL subclass pattern B was associated with a higher IgM antibody concentration (pattern B vs. A 48.3 ± 17.6 mg/ml vs 26.2 ± 10.0 mg/ml ($P < 0.001$). Pattern A and pattern B subjects did not differ in IgG autoantibodies. There was no correlation between LDL cholesterol and any of the antibody parameters nor between the plasma cyclosporine A content and any of the determined variables (not shown).

Discussion

Kidney transplantation is associated with an increased occurrence of atherosclerotic cardiovascular disease. Several factors are associated with atherosclerosis, hypercholesterolemia being one of them [3, 4]. Oxidation of LDL is considered to be a major event in the development of atherosclerosis [4, 5]. We found that parameters of in vitro (lag phase of LDL oxidation) and in vivo (autoantibodies against MDA-LDL) LDL oxidation, consistently indicate the LDL oxidation is increased in these patients. This finding may be an additional cause

Table 3 Parameters of LDL oxidation

	Patients	Number	Controls	Number	*P* value
Lag time (min)	101 ± 23	–	148 ± 81	–	–
LDL size (nm)	23.65 ± 0.73	–	24.78 ± 1.16	–	< 0.05
Subclass pattern					
Lag time pattern A	122 ± 30	6	169 ± 80	13	–
Lag time pattern B	92 ± 11[a]	13	102 ± 21	6[b]	–
Anti-oxLDL Ab					
IgM (mg/l)	49.4 ± 15.9	–	24.4 ± 8.7	–	< 0.0001
IgG (mg/l)	5.6 ± 3.8	–	3.2 ± 1.9	–	< 0.02

[a] $P < 0.05$ lag time patients, pattern A vs B
[b] $P = 0.0502$, patients vs controls. Fisher's exact test

of the accelerated atherosclerosis in renal transplant patients. Increased lipid peroxidation has also been reported in cyclosporine-treated heart transplant recipients [17]. Apanay et al. found that high plasma cyclosporine levels were correlated with increased susceptibility to LDL oxidation in kidney transplant recipients [18].

Oxidative modification of lipoproteins has been suggested as a mediator of both acute and chronic vascular rejection [19]. Histologically, graft vascular disease closely resembles accelerated atherosclerosis. Oxidised LDL may act as a chemoattractant, trigger T lymphocyte activation and proliferation, and induce the expression of class II MHC antigens and cell adhesion molecules [20, 21]. There is also evidence that oxidised LDL leads to endothelial damage, enhanced platelet-derived growth factor expression and subsequently vascular smooth muscle cell proliferation [5, 11]. These features point towards a pathogenetic role of oxidised LDL in chronic transplant rejection [11]. Supposing that increased LDL oxidation contributes to atherosclerosis, and possibly chronic rejection, in the transplant patients, a lowering of plasma triglycerides (leading to a shift of the LDL subclass pattern and subsequently less LDL oxidation) or administration of antioxidants may attenuate atherosclerosis and chronic rejection in renal transplant patients.

In conclusion, our results show that, in renal transplant recipients, there is increased incidence of the LDL subclass pattern B and an increased susceptibility to oxidative modification. Probably, LDL oxidation in vivo is also increased in the transplant patients as indicated by increased values of autoantibodies against MDA-LDL. These factors may play a role in the accelerated atherogenesis and graft vascular disease occurring after renal transplantation.

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