Calcineurin inhibitors enhance low-density lipoprotein oxidation in transplant patients

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**Background.** Our objective was to assess the pro-oxidant status of neoral and tacrolimus in renal transplant patients and monitor the protection provided by vitamin C and vitamin E in normalizing low density lipoprotein (LDL) oxidation lag time of tacrolimus-treated patients.

**Methods.** Plasma LDL was isolated by density gradient ultracentrifugation from renal transplant patients receiving neoral, tacrolimus and tacrolimus with vitamin C and vitamin E. Oxidation was initiated by the addition of CuCl2 at 37°C and monitored at 234 nm over 480 minutes and oxidation lag time was computed. Total antioxidant capacity of serum was measured using the enhanced chemiluminescent method.

**Results.** LDL from tacrolimus-treated patients had significantly lower oxidation lag time and serum antioxidant activity in comparison with neoral-treated patients, and this was particularly significant during the first four months after transplantation. Vitamin C and E supplementation in tacrolimus-treated patients provided protection against oxidation and normalized their oxidation lag time.

**Conclusion.** Calcineurin-inhibiting drugs, CsA and tacrolimus, have pro-oxidant activity and they increase the susceptibility of LDL to oxidation. Neoral formulation is fortified with DL-α-tocopherol and therefore provides protection against oxidation. The present study clearly demonstrates the benefit of giving vitamin C and E supplements to patients taking tacrolimus and this seems to be particularly important during the early period after transplantation.

The potential causes of hyperlipidemia in renal transplantation include dietary indiscretion and the use of atherogenic immunosuppressive drugs; it is possible to ascribe an hierarchical status for the atherogenicity of immunosuppressive drugs: steroids > cyclosporine > sirolimus > tacrolimus > azathioprine > mycophenolate mofetil [1]. The interleukin-2 (IL-2) inhibiting immunosuppressive drugs, cyclosporine and tacrolimus form complexes with their respective immunophilins and these complexes then inhibit a key enzyme serine/threonine phosphatase, calcineurin, which is involved in the dephosphorylation of the transcription factor NFA-T. Calcineurin-mediated dephosphorylation of NFA-T is a pivotal step in the transcription and translation of the IL-2 gene. The susceptibility of low density lipoprotein (LDL) to oxidation is increased by cyclosporine (CsA) [2, 3]. Oxidized LDL (Ox-LDL) may be of critical importance in triggering a cascade of cellular processes that leads to the formation of fatty streak and eventually atherosclerotic lesions in the artery wall [4, 5]. The susceptibility of LDL to oxidize can be determined in vitro [6] and the lag phase (the time elapsed before the oxidation chain reaction starts) has been found to correlate with the extent of atherosclerosis in patients with coronary artery disease. Previously such studies were done in Sandimmune (CsA)-treated renal transplant patients [2, 3]. Therefore, a pilot study [7] was undertaken to compare the ability of LDL collected from neoral- (N = 23) and tacrolimus-treated renal transplant patients (N = 10) to oxidize with the ability of LDL obtained from normal subjects (N = 15) to oxidize. This study established that the LDL oxidation lag time of neoral-treated patients were comparable to normal subjects and the difference between our results and those previously published for Sandimmune treated patients [2, 3] were due to the fortification of neoral formulation with DL-α-tocopherol. LDL collected from tacrolimus patients had significantly lower oxidation lag time in comparison with neoral-treated patients. There was also a significant deficiency of total serum antioxidant concentration in tacrolimus-treated patients. These findings suggest a proxidant status for calcineurin-inhibiting drugs. Therefore, the present study was undertaken to compare the ability of LDL from tacrolimus-treated renal transplant patients to oxidize before and after giving them with vitamin E and C supplements.

Key words: cyclosporine, neoral, tacrolimus, oxidation, renal transplantation.

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RESULTS

METHODS

Patients

We studied the oxidizability of LDL from 20 stable kidney transplant patients (10 female, 10 male, with a mean age of 48 ± 12 years) treated with tacrolimus before and four weeks after giving vitamin C 500 mg b.i.d. together with vitamin E 200 mg b.i.d. These patients were also treated with steroids and azathioprine and had been transplanted for 23 ± 5 months. None of the patients in the study were taking lipid-lowering drugs before or during the study nor antioxidants before the study.

Serum cholesterol, triglycerides and renal function tests were carried out on blood samples collected at 12 hours after taking tacrolimus or tacrolimus with vitamins C and E using standard methods on a Hitachi 747 analyzer. Whole blood (EDTA) tacrolimus levels were measured using IMX Tacrolimus II assay (Abbott Diagnostics, Maidenhead, Berks, UK). LDL oxidizability was measured using the method of McDowell et al [7, 8]. Total antioxidant capacity of serum was measured using the enhanced chemiluminescent method [7, 9].

Statistics

Data are presented as means ± standard error of means (SEM) and were tested for statistical significance using the unpaired (group I vs. group II) and paired (group II vs. group III) Student’s t-test. A P-value of <0.05 was considered significant.

RESULTS

Demographic data and quantitative results are given in Table 1.

The susceptibility of LDL to copper-induced oxidation was continuously monitored by the measurement of conjugated diene formation at λ = 234 nm. In a pilot study involving 23 neoral-treated and 10 tacrolimus-treated patients, there was a significant difference between the mean oxidation lag times (neoral 67.5 ± 2.5 min and tacrolimus 45.1 ± 16 min, P < 0.002). The total antioxidant concentration, measured as micromoles per liter of trolox equivalent (283 ± 77), was significantly lower (P < 0.03) in tacrolimus-treated in comparison with neoral-treated patients (368 ± 22).

However, the tacrolimus-treated patients had been transplanted for a shorter period of time (4 ± 4 months) compared with neoral-treated patients (94 ± 43 months), and this difference was significant (P < 0.001). Therefore, we selected a group of patients who had been on tacrolimus for a relatively longer period of time (23 ± 5 months) for the vitamins C and E supplementation study. Before vitamin supplementation the mean oxidation lag time was 56.5 ± 3 minutes, and there was a significant improvement in the mean oxidation lag time measured four weeks after starting vitamin supplementation (77.3 ± 5.1 min, P < 0.02). The mean total antioxidant concentration also increased significantly (P < 0.006) from 504 ± 47 μmol/liter before vitamin supplementation to 623 ± 67 μmol/liter after vitamin supplementation. However, it is important to note that there was an improvement in the mean oxidation lag time with time after transplantation in the tacrolimus-treated patients even before starting vitamin supplementation (45.1 ± 16 at 4 months to 56.5 ± 3 at 23 months, P < 0.03). This could be due to general improvement in the nutritional status of patients and this was reflected in their total serum antioxidant measurements, which rose from 283 ± 77 to 504 ± 47 μmol/liter, P < 0.001; Fig. 1).

DISCUSSION

Studies into macrovascular disease have highlighted the role of oxidation of plasma lipoproteins as well as
the cytotoxicity of these oxidation products. An association has been demonstrated between susceptibility of LDL to oxidation in vitro and the extent of atherosclerosis of coronary vessels [10]. Very low concentrations of Ox-LDL can activate T lymphocytes [11], and enhance proliferation of smooth muscle cells by increasing the expression of platelet-derived growth factor [12]. Furthermore, mildly oxidized LDL can induce the expression of genes of cellular adhesion molecules [13] and monocyte chemotactic protein-1 [14] in endothelial cells. The monocytes undergo activation and differentiation to become tissue macrophages and take up Ox-LDL through scavenger receptors and become foam cells. Similar mechanisms are also thought to be involved in the development of glomerulosclerosis. Ox-LDL may also influence the generation of nitric oxide (NO) by vascular wall endothelial cells and macrophages. NO reacts with superoxide to form peroxynitrite, a potent agent of LDL oxidation [15]. Rahman et al demonstrated an Ox-LDL-induced vasoconstriction in an isolated perfused rat kidney model that is mediated by decreased activity of NO, and probably is due to the inactivation of NO by reactive oxygen species [16]. Ox-LDL is also highly immunogenic, and antibodies that bind to it are found in humans [3, 17].

Kidney transplant patients have been shown to have higher levels of small, dense LDL, which are thought to be easily oxidizable [3], and patients on CsA have been shown to have lower lag times than normal controls and transplant patients not on CsA [2]. However, LDL separated from patients taking neoral, a new formulation of CsA with the addition of DL-α tocopherol, did not show an increased susceptibility to copper induced LDL oxidation [7]. The increased susceptibility reported [7] in tacrolimus-treated patients was thought to be due to the decreased antioxidant activity. With time after transplantation antioxidant activity increased in patients taking tacrolimus, so the susceptibility to oxidation decreased. A protective role for antioxidants in large-vessel disease has been postulated [18], and Rabl et al demonstrated that antioxidant treatment with vitamins could be important in reducing reperfusion damage in renal transplantation [19]. Therefore, the present short-term, limited study demonstrates the benefit of giving vitamin C and E supplements to patients taking tacrolimus, and this is particularly important during initial stages of transplantation when there is an adequate intake of antioxidants.

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**Fig. 1. Low density lipoprotein (LDL) oxidation curves of tacrolimus treated patients.** LDL was isolated from the blood of tacrolimus treated patients > 12 hours post-dose, before (○) and one month after (▼) taking vitamin E and C supplements. Oxidation of LDL (50 mg/l) was initiated by adding Cu²⁺ (2 µmol/liter) and oxidation monitored continuously by recording the absorbance at λ = 234 nm. Lag times were calculated as described in the Methods section. The graph shows the mean oxidation curves of 20 patients before (○) and after (▼) vitamin E and C treatment.


