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Enhanced LDL oxidation in uremic patients: An additional mechanism for accelerated atherosclerosis?

ELENA MAGGI, ROBERTO BELLAZZI, FRANCESCO FALASCHI, ARTURO FRATTONI, GUIDO PERANI, GIORGIO FINARDI, ANTONIETTA GAZO, MAURIZIO NAI, DINO ROMANINI, and GIORGIO BELLOMO

Department of Internal Medicine, First Medical Clinic, IRCCS Policlinico S. Matteo, University of Pavia, Pavia, Nephrology and Dialysis Unit, USSL 78, Vigevano, and Department of Experimental Medicine and Oncology, University of Torino, Torino, Italy

Enhanced LDL oxidation in uremic patients: An additional mechanism for accelerated atherosclerosis? Since oxidized low-density lipoprotein (LDL) is more atherogenic than native LDL, LDL oxidation was investigated in uremic patients who often develop accelerated atherogenesis. Three groups of uremic patients were studied (10 on predialysis conservative therapy, 11 on repetitive hemodialysis, 13 on peritoneal dialysis) and compared with seventy matched controls. LDL oxidation was evaluated in all patients as: (i) the susceptibility to *in vitro* oxidation (by measuring the resistence to Cu^{++}-induced formation of conjugated dienes), (ii) vitamin E concentration in LDL, and (iii) presence of plasma anti-oxidized LDL antibodies, expressed as the ratio anti-oxLDL/anti-nativeLDL antibodies. The lipid profile was studied in all patients. Vitamin E concentration did not differ between the various groups, although LDL from uremic patients appeared more susceptible to *in vitro* and *in vivo* oxidation (as demonstrated by an earlier generation of conjugated dienes and by the presence of an higher antibody ratio) compared to control subjects. Subclass analysis of the different patients revealed that peritoneal dialysis treatment ameliorated the oxidation markers. However, a prolonged dialytic treatment caused a decrease in vitamin E concentration in LDL and increased their susceptibility to oxidation.

Patients with cronic uremia often develop atherosclerosis and, in addition, more than 50% of these patients die from cardiovascular complications [1, 2]. Extensive investigations have been carried out in order to identify the causes of the accelerated atherosclerosis detected in uremia. Hyperlipidemia is frequently seen in patients with renal failure both in the predialysis and during hemodialysis or peritoneal dialysis [3]. In addition to hypertriglyceridemia [3], which is the most common abnormality detected, an increase in plasma intermediate-density lipoprotein (IDL) concentration and a decrease in high-density lipoprotein (HDL) concentration during renal failure has been frequently reported [4–6].

The most accepted theory of atherogenesis postulates that lipoproteins from the bloodstream are taken up by macrophages in the subendothelial space, leading to the formation of cholesterol-engulfed cells (foam cells), and they trigger a series of biological events ending in the formation of the atherosclerotic plaque [7]. The arterial narrowing that follows impairs the blood supply to several organs such as heart, brain, kidney, etc., and causes the clinical features of the above-mentioned diseases.

Of the various lipoproteins, low-density lipoprotein (LDL) is most avidly taken up by subendothelial macrophages through a specific receptor [8]. Studies performed by Goldstein et al demonstrated that some chemical modifications of LDL resulted in an enhanced uptake of LDL itself by the so-called "scavenger receptor" in macrophages [9]. Among the various modifications that increase LDL uptake by macrophages and that are likely to occur *in vivo*, is the derivatization of the LDL apolipoprotein (apo-B100) by breakdown products of lipid peroxides such as malondialdehyde (MDA) and 4-OH-nonenal (HNE) [10, 11]. Thus free radical- or lypoxygenase-mediated mechanisms could generate oxidized LDL that would be subsequently taken up by macrophages and would precipitate the formation of the atherosclerotic plaque. Subsequent investigations provided evidences that oxidized lipoproteins also cause a series of biological alterations detected in atherosclerosis [12], that oxidative modifications of LDL do actually occur *in vivo* [13] and reported the presence of oxidized lipids in atherosclerotic plaques [14]. This led to the formulation of the "oxidative theory of atherosclerosis" hypothesizing that LDL oxidation would represent a critical step in the developmment and progression of the disease. This hypothesis has been recently validated by Salonen et al [15], who reported that the occurrence of *in vivo* markers of LDL oxidation (presence of autoantibodies to MDA-LDL) was an independent predictor of carotid atherosclerosis progression.

Oxidant injury has been implicated in the pathogenesis of inflammatory, metabolic and toxic diseases and oxidant-induced alterations of proteins, membranes and DNA have been detected in some renal disorders such as glomerulonephritis, vasculitis, pyelonephritis and acute or chronic renal failure [16]. In addition, stimulated polymorphonuclear leukocytes isolated from uremic patients exhibited an enhanced production of hydrogen peroxide and other oxygen reactive species [17]. Furthermore, Toborek et al [18] have reported an increased lipid peroxidation during hemodialysis and proposed a role for this process in the accelerated progression of atherosclerosis in patients with renal failure. Based on these observations, it can

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Table 1. Biochemical and demographic parameters and serum lipid profile of the subjects under study

Parameter	Control subjects (N = 70)	Total uremic patients (N = 37)	Predialytic conservative therapy (N = 11)	Repetitive hemodialysis (N = 12)	Peritoneal dialysis (N = 14)
Age *years*	61.8 ± 23	56.7 ± 2.3	58 ± 4.7	52 ± 4.6	60 ± 2.7
Sex	28 M, 42 F	20 M, 17 F	4 M, 7 F	7 M, 5 F	9 M, 5 F
Body mass index	24.8 ± 5.2		26.7 ± 6.5	23.5 ± 3	26.9 ± 5.1
Total cholesterol *mg/dl*	163 ± 9.8	198 ± 8.4[a]	184 ± 11	174 ± 18	221 ± 14[a]
LDL cholesterol *mg/dl*	101 ± 7.6	95.3 ± 5.7	88 ± 11	95 ± 13	93 ± 11
HDL cholesterol *mg/dl*	50.5 ± 2.7	43 ± 1.8[a]	40 ± 2[a]	41 ± 3[a]	44 ± 3[a]
Triglycerides *mg/dl*	91.8 ± 37.2	178 ± 15[a]	178 ± 34[a]	167 ± 25[a]	200 ± 6[a]
Apoprotein A1 *mg/dl*	> 110	126 ± 4.5	117 ± 8	117 ± 7	135 ± 6
Apoprotein B *mg/dl*	< 140	142 ± 6.5	136 ± 9	122 ± 14	162 ± 10.5

Results are expressed as mean ± SE.
[a] Significantly different from controls, $P < 0.01$

be assumed that an oxidative stress is likely to take place in the blood of patients with chronic renal failure.

In this work we investigated the process of LDL oxidation in uremic patients who were ending conservative therapy or in dialysis. Data reported indicate that an enhanced oxidation of LDL occurs in uremic patients and that dialysis treatment (and especially peritoneal dialysis) temporarily ameliorates the oxidation pattern. We hypothesize that the increased susceptibility of LDL from uremic patients to oxidation may represent a critical step in the development of the atherosclerotic disease, and we envisage a possible therapeutic approach.

Methods

Patients

Three groups of unselected uremic patients have been investigated: ten predialytic patients on conservative therapy, eleven on repetitive hemodialysis (3 times a week with a HCO_3^- hemodialysis on cuprophane membranes), thirteen on conventional CAPD treatment, and they were compared with seventy control subjects (Table 1). The majority of the patients studied (55%) had a post-inflammatory (glomerulonephritis, pyelonephritis, LES) chronic renal failure; nephrolithiasis, polycystic disease and diabetes mellitus were respectively present in 13%, 13% and 6% of the patients. For three of them, the pathogenesis of the chronic renal failure was unknown. Hypertension was present in 38% of the patients and was clinically well-controlled with an anti-hypertensive therapy using Ca^{2+}-channel blockers. The mean glomerular filtration rate (GFR) for the predialytic patients was 12 ± 5 ml/min. The adequacy of the dialytic treatment was evaluated using the Kt/V (urea) [19] that ranged from 1 to 1.2 for hemodialytic patients and from 1.6 to 1.8 (weekly) for CAPD patients. The Kt/V was calculated using a commercially available computer program. None of the patients was taking drugs directly affecting the antioxidant and the lipidic status.

Evaluation of plasma lipid profile

Plasma obtained from EDTA-supplemented blood (1 mg EDTA/ml blood) was utilized for measuring lipid parameters. Cholesterol was assayed using an enzymatic method as total cholesterol or as HDL-cholesterol in the supernatant obtained after centrifugation of dextran sulphate-precipitated plasma. Triglycerides were measured using an enzymatic-colorimetric method. LDL-cholesterol concentration was calculated from total and HDL cholesterol and triglyceride levels, using Friedewald's formula. Apolipoproteins A1 and B were measured using an immuno-turbidimetric assay.

Measure of LDL oxidation

The experimental protocol described by Esterbauer et al [20] was employed. Briefly, venous blood was taken from each patient, after overnight fasting, in polypropilene tubes containing K-EDTA (final concentration 1 mg EDTA/ml blood), and plasma was collected after centrifugation. The LDL fraction was isolated from the whole plasma by high-speed centrifugation through a KBr discontinuous gradient and collected as the fraction floating at a density of 1.019 to 1.063 g/ml. EDTA was then removed by rapid filtration through disposable desalting columns Econo-Pac 10 DG (Bio-Rad) and LDL was resuspended in oxygen-saturated phosphate-buffered saline (10 mM Pi, pH 7.2) at a concentration of 0.25 mg LDL mass/ml buffer (= 50 μg LDL protein/ml = 0.1 μM).

LDL oxidation was then triggered by the addition of 2 μM $CuSO_4$ and continuously monitored spectrophotometrically at 234 nm, to follow the formation of conjugated dienes. The oxidation curve obtained was characterized by two parameters. The lag-phase (expressed in minutes), that is, the interval between the addition of $CuSO_4$ and the beginning of the extensive oxidation, was measured on the basis of the intercept between the baseline and the tangent to the rapid oxidation phase. The propagation rate, expressed in changes in absorbance (Abs)/min, is the maximal rate of LDL oxidation detected in the kinetic curve.

Vitamin E determination

Vitamin E in LDL was determined as described [21]. Briefly, LDL were precipitated using ethanol and subsequently vitamin E was extracted with hexane. The hexane phase was then evaporated and the residue was dissolved in methanol and separated by HPLC.

Measure of anti-oxidized and anti-native LDL antibodies

The quantitation of anti-oxidized and anti-native LDL antibodies was performed using an ELISA method. Disposable, 96 well polystyrene plates (Corning) were employed. Antigens for this assay included native LDL (protected against oxidation by

EDTA) and oxidized LDL (obtained after extensive oxidation with 2 μM $CuSO_4$). Each well was coated with 10 μg antigen in PBS for four hours. After removal of the antigen, the remaining binding sites were blocked using 3% fetal bovine serum in PBS (coating buffer) for two hours at 37°C.

In the present study, 1:11 dilution of plasma from each subject was prepared and 220 μl were added in duplicate to wells coated with native and oxidized LDL. After two hours incubation at 37°C, wells were decanted and washed four times before an appropriate peroxidase-conjugated monoclonal antibody specific for IgG (diluted 1:2000) was added. After one hour incubation at 37°C and extensive washing, the peroxidase activity was measured using phenylenediamine dihydrochloride and H_2O_2 as revealing reagents.

To calculate antibody titers we used the ratio between the spectrophotometric reading of anti-oxidized LDL and the anti-native LDL wells. Using this approach, the spectrophotometric readings of anti-native LDL wells represent the corresponding blank of anti-oxLDL wells and minimize the possible detection of false positive values due to cross reactivity with both LDL epitopes.

Statistical analysis

All data were statistically analyzed with Student's *t*-test and linear regression analysis using the Micro-Cal Origin program for personal computers. Results are expressed as mean ± SEM.

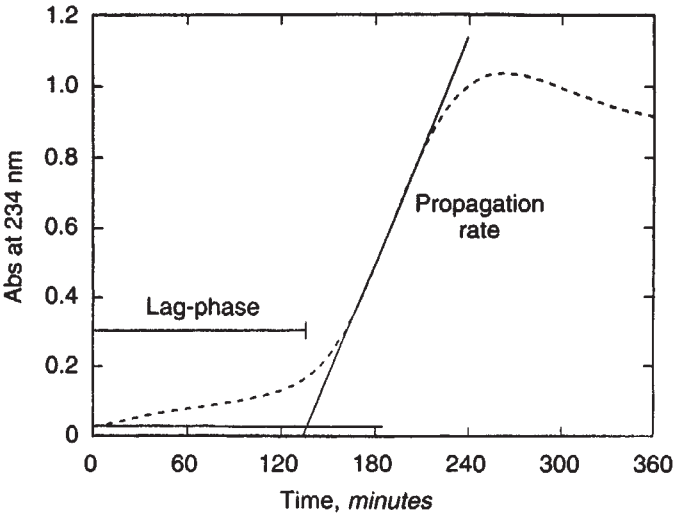

Fig. 1. *LDL oxidation profile. In vitro* oxidation of LDL was continuously monitored spectrophotometrically at 234 nm to follow the formation of conjugated dienes after addition of 2 μM $CuSO_4$ at time zero. The lag-phase is the period in which no appreciable oxidation takes place and is calculated at the intercept between the baseline and the tangent to the propagation phase. The propagation rate is calculated as the maximal rate of increase in the absorbance at 234 nm. One typical curve is shown.

Results

Alterations of plasma lipid profile in uremic patients

The plasma lipid profile of the uremic patients is reported in Table 1. An increase of triglyceride and a decrease of HDL cholesterol levels were typical features of all the groups of uremic patients investigated, in agreement with data reported in literature [1–4]. In addition, patients on peritoneal dialysis had a higher concentration of total cholesterol and of apolipoprotein B100.

LDL oxidation and vitamin E content in uremic patients

The susceptibility of LDL from control subjects and from uremic patients to oxidation was investigated *in vitro*. Figure 1 reports a typical trace of *in vitro* LDL oxidation induced by $CuSO_4$ and monitored as the formation of conjugated dienes, and the parameters (lag-phase and propagation rate) utilized for quantitative estimation. The duration of the lag-phase is a direct measure of the resistance of LDL to oxidation promoted by copper, and is related to both the antioxidant content and some intrinsic properties of LDL, such as the relative abundance of polyunsaturated fatty acids, cholesterol, etc. On the contrary, little is known about the biochemical factors controlling the propagation rate of LDL oxidation. However, based on the analysis performed on more than 400 individuals investigated in our laboratory so far, we found a highly significant inverse correlation between the duration of the lag-phase and the propagation rate ($N = 451$, $r = -0.67$, $P < 0.001$). Thus, it can be conceivably argued that an increase of the propagation rate could be considered as an additional index of enhanced susceptibility of LDL to oxidation.

The lag-phase of LDL oxidation obtained from uremic patients was significantly lower compared to that observed in control subjects (119 ± 3.1 min vs. 142 ± 3.3 min, $P < 0.01$; Table 2). Concomitantly, the propagation rate was markedly and significantly increased (130 ± 5 Abs · 10^4/min vs. 103 ± 3 Abs · 10^4/min, $P < 0.01$; Table 2). It has been recently hypothesized that calcium channel blocking drugs could exert an antioxidant activity on certain tissues and cell compartments [22]. Since a significant proportion of the patients investigated in this study was taking these drugs, a subclass analysis was performed to investigate the influence of this treatment on LDL oxidation parameters. However, neither the duration of the lag-phase (120.8 ± 4.4 min) nor the propagation rate (125.8 ± 5.7 Abs · 10^4/min) differed from those detected in the whole group of patients.

The enhanced susceptibility of LDL to oxidation was even increased when data obtained from patients on ending conservative therapy were analyzed and compared with those obtained from the group of patients on peritoneal dialysis. These patients exhibited a longer lag-phase and a slower propagation rate (135.8 ± 5.1 min vs. 109.6 ± 6.06 min, $P < 0.01$; 118 ± 6 Abs · 10^4/min vs. 135 ± 14 Abs · 10^4/min, $P < 0.01$; Table 2). Surprisingly, no significant differences were detected as concerning vitamin E concentration in LDL obtained from control and from the different groups of uremic patients (2.8 ± 0.3 nmol/mg LDL in control subjects, 2.9 ± 0.1 nmol/mg LDL in uremic patients). Assuming a molecular mass of approximately 2,200,000 for LDL, vitamin E concentration detected in this study in control subjects corresponded to 6.16 ± 0.65 mol/mol LDL, a value comparable to those reported in literature [23]. Taken together, these data suggest that LDL from uremic patients are more prone to oxidation, independently from vitamin E concentration, and that peritoneal dialysis significantly ameliorates this condition.

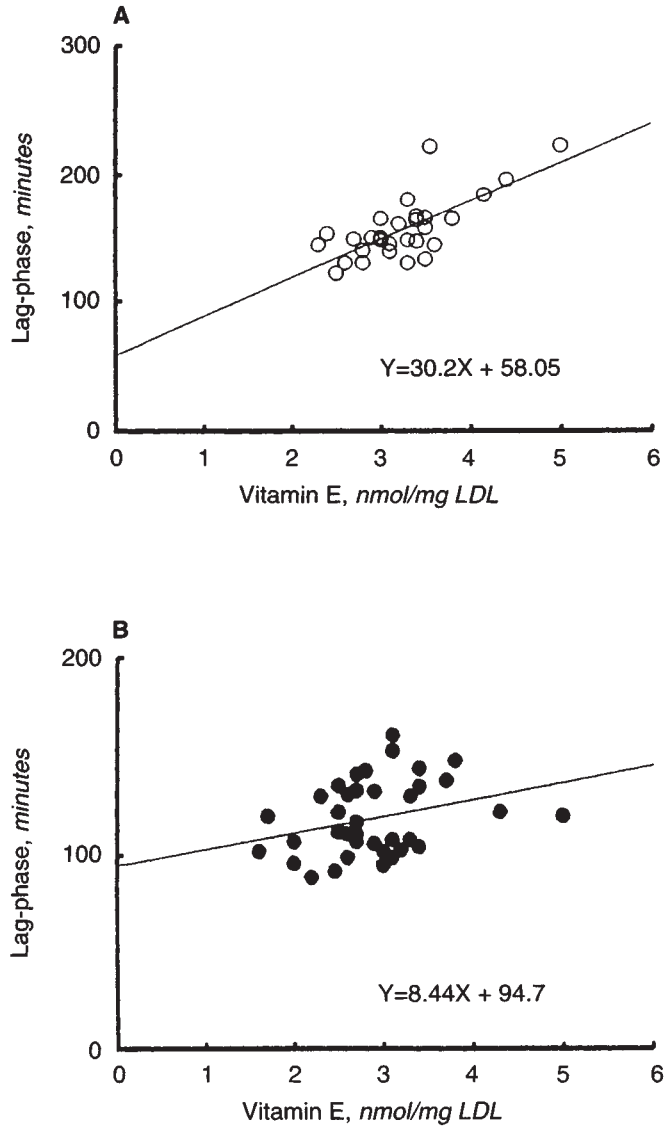

Fig. 2. *Statistical correlation between lag-phase and vitamin E content of LDL from control subjects (A) and uremic patients (B).* LDL were isolated from control and uremic patients and processed as described in **Methods** to measure the lag-phase and to quantitate vitamin E concentration. Equations on the bottom right corner describe the relationship observed (Y = kX + a). Y: lag-phase (in min); k: efficacy constant of vitamin E; X: vitamin E (nmol/mg LDL); a: vitamin E-independent variable (in min).

As stated above, the resistance of LDL to oxidation is related to both the antioxidant content of LDL (mainly vitamin E) and some antioxidant-independent, intrinsic properties of LDL. Esterbauer et al have recently adressed the problem of quantifying the relative contribution given by vitamin E content to the resistance of LDL to oxidation (lag-phase) [20]. This can be experimentally approached by loading LDL from individual subjects with various amounts of vitamin E and by subsequently measuring the resistance of vitamin E-loaded LDL to oxidation. A linear relationship is obtained and the curve fitting is described by the equation

$$y = kx + a$$

Table 2. Alterations of *in vitro* LDL oxidation parameters and vitamin E content in uremic patients

	Lag-phase *min*	Propagation rate *Abs · 10⁴/min*	Vitamin E *nmol/mg LDL*
Control subjects	142 ± 3.3	103 ± 3	2.8 ± 0.3
Uremic patients	119 ± 3.1[a]	130 ± 5[a]	2.9 ± 0.1
Group A	109 ± 6.06[a]	135 ± 14[a]	2.91 ± 0.1
Group B	119 ± 4.4[a]	137 ± 11[a]	3.12 ± 0.2
Group C	125.8 ± 5.1[a,b]	118 ± 6[a,b]	3.0 ± 0.2

LDL were isolated from control and uremic patients and processed as described in **Methods** to measure the lag-phase and propagation rate and to quantitate vitamin E concentration. Group A refers to uremic patients on conservative therapy; group B refers to uremic patients on repetitive hemodialysis; group C refers to uremic patients on peritoneal dialysis.

[a] Significantly different ($P < 0.01$) from controls
[b] Significantly different ($P < 0.01$) from group A

where y is the lag-phase in minutes, x is vitamin E concentration, k is the efficacy constant of vitamin E and a is the vitamin E-independent variable, in minutes.

In the present study, a similar procedure was employed to analyze the data obtained in control subjects and in uremic patients, with the assumption that the general formula developed by Esterbauer et al [23] for LDL from single individuals loaded with different amounts of vitamin E could also apply to a LDL isolated from a rather homogeneous group of patients and containing variable amounts of endogenous vitamin E. As reported in Figure 2, the two parameters investigated (lag-phase and vitamin E content) exhibited a linear correlation for both control subjects and uremic patients. However, the correlation parameters obtained from curve fitting were markedly different. The slope of the correlation line (k) was higher in controls (30.2 ± 7.6 vs. 8.44 ± 4.5 min/nmol vitamin E) and the intercept of the correlation line with the y axis (a) was higher in the uremic group (94.7 vs. 58.05 min). In other words, the relative contribution of vitamin E in protecting LDL from oxidation was lower in uremic patients as compared to control subjects.

Presence of plasma anti-oxidized LDL antibodies in uremic patients

The oxidation parameters investigated and the data reported in the previous section refer to the susceptibility of LDL to *in vitro* oxidation but they do not give any reliable information on the oxidation process that is actually occurring *in vivo*. Since oxidative modifications of LDL and the formation of MDA-LDL or HNE-LDL adducts results in a marked change in the antigenic properties of the LDL molecule [15, 24, 25], it can be conceivably assumed that a prolonged exposure to oxidized LDL *in vivo* could result in the production of specific autoantibodies. The detection of anti-oxidized LDL antibodies in serum or plasma has been taken, indeed, as the biological signature of the *in vivo* LDL oxidation [15].

As reported in Figure 3A a marked increase in anti-oxidized LDL antibody (IgG) titer was detected in plasma of uremic patients as compared to control subjects (1.6 ± 0.1 vs. 1.2 ± 0.1, $P < 0.01$). In patients assuming calcium channel blocking drugs, the anti-oxidized LDL antibody ratio was similar to that

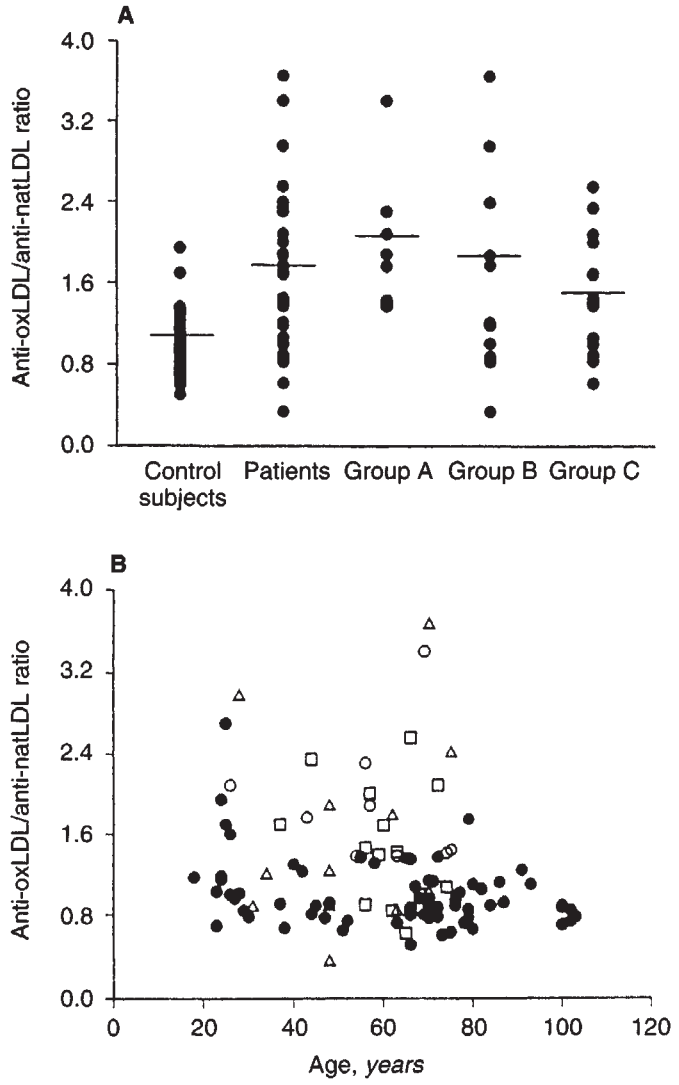

Fig. 3. *Presence of anti-oxidized LDL antibodies in plasma of uremic patients.* Plasma was taken from uremic patients and age-matched control subjects (●) and processed as described in Methods to measure anti-oxidized LDL antibodies. The antibody titre is expressed as the ratio between anti-oxidized LDL and anti-native LDL antibodies. Group A (○) refers to uremic patients on conservative therapy; group B (△) refers to uremic patients on repetitive hemodialysis; group C (□) refers to uremic patients on peritoneal dialysis.

detected in the whole group of patients (1.67 ± 0.17). Moreover, dialysis treatment (hemodialysis and peritoneal dialysis) did not significantly modify the antibody titer.

We have recently reported that the anti-oxidized LDL antibody ratio in a group of control subjects progressively declines during aging [25]. This did not seem to occur in uremic patients, in which no correlation was found between the antibody ratio and the age (Fig. 3B).

Effects of the duration of dialytic treatment on LDL oxidation and vitamin E content

Dialysis, and particularly hemodialysis, represents a continuous inflammatory stimulus and hence a continuous source of oxygen reactive species generated by inflammatory cells. It thus appears rather suprising that LDL from patients on dialytic treatment could exhibit an enhanced resistance to oxidation as compared to LDL from patients on conservative therapy. We then investigated the effects of dialytic treatment duration on LDL oxidation and vitamin E content. As reported in Figure 4, no correlation was found between the lag-phase of *in vitro* LDL oxidation or vitamin E content and the length of repetitive hemodialysis. However, a highly significant negative correlation was found with the duration of peritoneal dialysis, thus indicating that long-term peritoneal dialysis progressively decreased vitamin E content and increased the susceptibility of LDL to oxidation. On the contrary, short-term peritoneal dialysis (< 10 months) markedly increased the length of the lag-phase. Preliminary results obtained on individual uremic patients before and after short-term peritoneal dialysis revealed a marked amelioration of LDL susceptibility to oxidation (the lag-phase increase from 119.5 ± 13.8 to 149.6 ± 19.3 min, $P = 0.02$; vitamin E concentration increased from 2.77 ± 0.14 to 3.36 ± 0.26 nmol/mg LDL, $P = 0.01$; $N = 4$), further supporting a direct effect of this dialytic treatment. No correlation was found between the anti-oxLDL antibody titer and the duration of peritoneal dialysis or hemodialysis (not shown).

Discussion

The results obtained in this study provide compelling evidence that an increased *in vitro* and *in vivo* oxidation of plasma LDL occurs in uremic patients. This is demonstrated by the increased susceptibility of isolated LDL to Cu^{++}-induced formation of conjugated dienes and by the detection of a high anti-oxidized LDL antibody titer in plasma.

The methodology employed to promote LDL oxidation *in vitro* utilizes low concentrations of $CuSO_4$ that would initiate peroxidation of LDL lipids through the formation of LO· according to the reaction:

$$LOOH + Cu^{+} \rightarrow LO\cdot + OH^{-} + Cu^{++}$$

In the experimental conditions employed here Cu^{++} should be associated with critical binding sites in apo-B100 [23] and reduced to Cu^{+} by an unknown reducing agent, that has been proposed to be a preformed LOOH itself [26]. Although this reaction is thermodynamically unfavored, experimental evidence supports the absolute requirement of preformed LOOH in initiating Cu^{++}-induced peroxidation of LDL lipids [24]. Whichever is the mechanism involved in the initiation of lipid peroxidation, the subsequent propagation and decomposition of lipid peroxides will depend on the abundance of polyunsaturated fatty acids and on the concentration and efficiency of chain-breaking antioxidants. LDL isolated from plasma contain a wide variety of antioxidants, including α- and γ-tochopherol, carotenoids and ubiquinol-10 that are sequentially consumed during the lag-phase of the oxidation process triggered by Cu^{++} [20]. Vitamin E, however, is the most abundant antioxidant present (approximately 6 molecules per LDL molecule), and Halliwell has suggested that is also the only significant antioxidant in LDL [27]. Additional evidence to this assumption comes from the demontration that *in vitro* or *in vivo* supplementation with vitamin E increases the resistance of LDL to

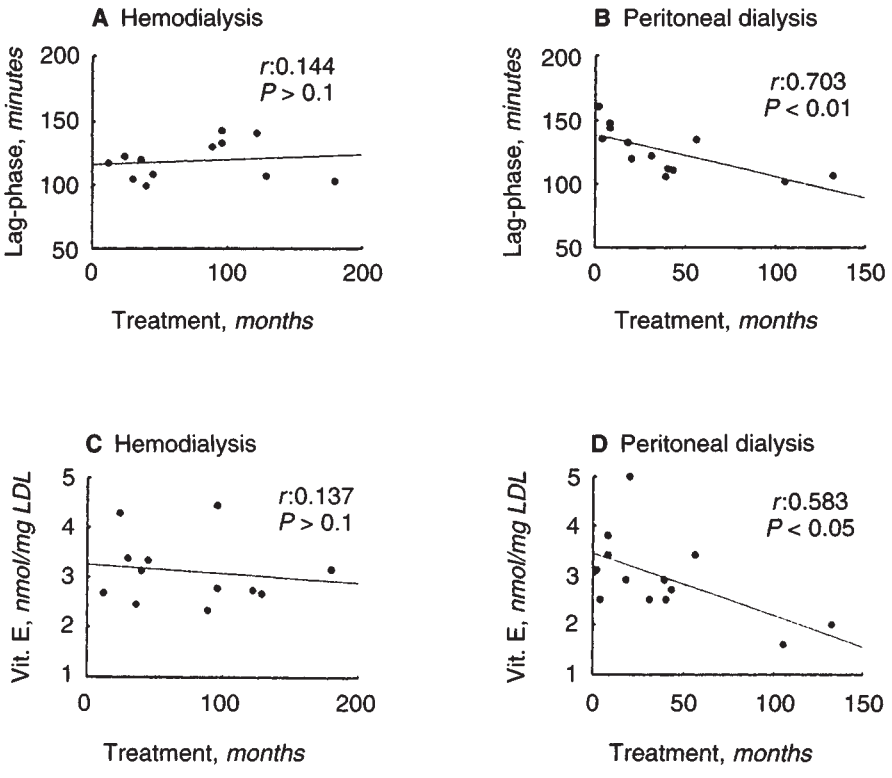

Fig. 4. *Correlation between the duration of dialytic treatment and the lag-phase of in vitro LDL oxidation or vitamin E content.* The length of the lag-phase and the concentration of vitamin E in LDL is plotted against the duration of dialytic treatment in patients undergoing repetitive hemodialysis or peritoneal dialysis.

Cu^{++}-induced oxidation and prolongs the length of the lag-phase [21].

The decrease of the lag-phase observed in uremic patients could then theoretically arise from a reduced concentration of vitamin E in LDL. This, however, does not seem to be the case since vitamin E level in LDL from uremic patients is similar to that present in LDL from control patients. In addition to the actual concentration of vitamin E, the enhanced susceptibility of LDL to oxidation could also depend on: (i) a decreased efficiency of vitamin E, (ii) a selective depletion of other antioxidants and (iii) alterations of physicochemical properties of LDL.

Regarding vitamin E efficiency, this can be calculated from the slope of the correlation between the length of the lag-phase and the concentration of vitamin E in LDL. In uremic patients, indeed, the slope is markedly reduced compared to controls. In other words, much more vitamin E is required in order to obtain a comparable increase in the length of the lag-phase. Thus vitamin E in LDL from uremic patients is less efficient than in LDL from control subjects. Moreover, the concentration of antioxidants such as carotenoids and ubiquinol-10 in LDL is about one order of magnitude lower than that of vitamin E [23], and based on simple stoichiometric calculations they would be present in only one-tenth of the LDL particles. Thus, it can be conceivably argued that a selective decrease in their concentration would not play a major role in decreasing the resistance of LDL from uremic patients to oxidation. In addition, the measurement of β-carotene and lycopene in LDL from uremic patients failed to reveal any appreciable decrease in their concentration (H. Esterbauer, personal comunication). Further support of this conclusion comes from the evidence obtained in this study that the antioxidant (vitamin E)-independent component of the lag-phase in LDL from uremic patients is significantly higher than in controls. Taken together these findings suggest that in uremia, major modifications could occur in the chemical properties of LDL, and these modifications would increase their susceptibility to oxidation. In agreement with this view are the demonstrations that a change in protein/lipid ratio affects the oxidation of LDL from control subjects [28] and that an increase in PUFA concentration reduces the resistance of LDL to oxidation [29]. Interestingly, studies performed in coronary disease patients have revealed the existence of a negative correlation between the duration of the lag-phase and the triglyceride concentration in LDL [30]. The presence of high concentrations of triglycerides is one typical feature of LDL from uremic patients [31] and this would, in part, explain their increased susceptibility to oxidation.

In addition to kidney transplantation, hemodialysis and peritoneal dialysis represent the treatments that definitely ameliorate the life expectancy of uremic patients even if, as concerning the LDL oxidation parameters, only peritoneal dialysis seems to afford a slight although significant improvement. This could result from: (i) a normalization of the altered chemicophysical features of LDL that enhance their susceptibility to oxidation, (ii) an increased efficiency of vitamin E, and (iii) an increase in vitamin E content. However, none of these hypotheses is validated by experimental data obtained considering the whole group of patients under peritoneal dialysis. On the other hand, by itself a prolonged peritoneal dialysis treatment decreases both the length of the lag-phase and vitamin E concentration. Thus, the apparent efficiency of a peritoneal dialytic treatment observed in the whole group would result from the balance between a short-term improvement of LDL oxidation parameters and a progressive consuption of vitamin E itself.

Little is known about the inefficiency of hemodialysis in providing significant protection against LDL oxidation, although the enhanced oxidative stress detected in this conditions could play a role. Clinical and experimental investigations are in progress in our laboratory. Evidence indicates that LDL oxidation may play a role in atherogenesis. It includes, for instance, the detection of oxidized lipids in the atherosclerotic plaque [32] and the demonstration of oxidized LDL [13] and anti-oxidized LDL autoantibodies [15] in plasma of atherosclerotic patients. Furthermore, a treatment with an antioxidant drug (probucol) has been reported to reduce the extent of the atherosclerotic lesions and their progression in rabbits [33]. Based on these observations, we can hypothesize that, in addition to the detected disorders of lipid metabolism, the enhanced *in vitro* and *in vivo* LDL oxidation observed in uremic patients could play a relevant role in the overall process of atherosclerosis.

Since LDL oxidation results from the balance between the pro-oxidant stimuli on one hand and antioxidant defences on the other, it could be theoretically possible to interfere with the oxidation process by increasing the antioxidant defenses. Two major classes of antioxidants are now pharmacologically available for medical treatment, namely lipid-soluble and water-soluble antioxidants. Among the former is vitamin E, whose oral supplementation has been demonstrated to increase the resistence of LDL to oxidation in a group or normal volunteers [21]. However, the efficiency of vitamin E in uremic patients is decreased as compared to control subjects (this study), and thus relatively high doses of vitamin E are likely to be necessary to get a significant protection of LDL. Alternatively, water-soluble antioxidants, such as vitamin C, could be given, due to the effect of ascorbate in re-reducing oxidized vitamin E [34]. In conclusion, it can be postulated that, in addition to the pharmacological correction of lipid disorders, an antioxidant regimen with drugs efficiently protecting against LDL oxidation would provide a powerful strategy for delaying the onset of the irreversible atherosclerotic disease in uremic patients.

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Reprint requests to Giorgio Bellomo, M.D., Clinica Medica I, Policlinico S. Matteo, I-27100 Pavia, Italy.

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