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The Relation Between Low-Density Lipoprotein (LDL) Oxidation and Hemodialysis with Respect to Membrane Types

Protective Effects of Vitamin E-bonded membrane

Abstract: Plasma total antioxidant (TAO) levels, LDL (low density lipoprotein), antioxidant defense potential (AOP) and the resistance to oxidation of LDL fractions were studied in chronic hemodialysis patients. The differences with respect to dialyzer membrane types before and after hemodialysis were also assessed. After isolation of LDL from plasma by precipitation, AOP was measured based on the determination of TBARS (thiobarbituric acid reactive substance), and resistance to oxidation was assessed by the metal-catalyzed peroxidation of LDL followed by the determination of the values of the peroxidation product malondialdehide (MDA) before and after peroxidation. The assay principle of TAO was based on the suppression of ABTS+ radical cation (2,2'-Azino-di- [3-ethylbenzthiazoline sulfonate]) by the sample. AOP and the resistance to oxidation of LDL were expressed as 1/nmol/ml.h. Plasma TAO levels of patients (n=22) were significantly lower than those of the control group (n=20). In the patient group, the decrease in TAO levels was also significant and continued to the end of the single dialysis session. The rate of decrease was similar in the two types of conventional membranes used (polysulfone and hemophan). The antioxidant defense potential of LDL significantly decreased after hemodialysis with the polysulfone type dialyzer but resistance to oxidation values of LDL showed no difference. When the same group of patients were hemodialyzed with a vitamin E bonded membrane no difference was observed in the AOP of LDL, but resistance to oxidation of the LDL fraction significantly increased during the hemodialysis session. The results suggest that the vitamin E-bonded dialysis membrane strengthens the LDL antioxidant defense potential and protects the LDL from oxidation.

In conclusion, the decreased antioxidant capacity in these patients may be attributed to several factors such as elimination via the dialyzer of enzymatic or nonenzymatic antioxidants or deformation of these compounds by the increased oxidant stress. Improving LDL resistance to oxidation by using a vitamin E-bonded membrane appears to be an attractive alternative to prevent accelerated oxidative processes in hemodialysis patients.

Key Words: Oxidative stress, hemodialysis, antioxidants, LDL oxidation

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Introduction

Chronic renal failure (CRF) is associated with an increased risk of atherosclerotic cardiovascular disease (1,2), which represents the major cause of mortality in patients on long-term hemodialysis (2,3). A number of factors contribute to accelerated atherogenesis in the presence of renal failure, including hypertension, diabetes and dyslipidemia. However, after taking the increased prevalence of conventional risk factors into account, the exact role it plays in the evolution of atherosclerosis remains unclear partly due to the limitation of the methodologies available to measure free-radical generation in vivo (4).

Several reports suggest that uremia is a state of oxidative stress. These include findings such as an increase in lipid peroxides in uremic red blood cell membranes (5) and a decrease in serum antioxidant activity (6). The accumulation of lipid peroxidation products in uremia may be the result of a number of different factors. One possibility is uncontrolled radical mediated lipid peroxidation during the hemodialysis procedure. Neutrophils are activated at the surface of the dialyzer membrane, which may cause the subsequent release of oxygen radicals capable of oxidizing lipids.

Lipid peroxidation, specifically low-density lipoprotein (LDL) oxidation, appears to be a critical step in the pathogenesis of atherosclerotic vascular disease (1). The

oxidation of polyunsaturated fatty acids within LDL is followed by their breakdown and the release of aldehydes and ketones, such as malondialdehyde and 4-hydroxynonenal, which can modify lysine residues on apolipoprotein B (apo B). Modified apo B is no longer recognized by the apo B receptor, but instead is taken up by the macrophage scavenger receptor. This leads to unregulated uptake of LDL cholesterol and the formation of foam cells, and eventually results in a fatty streak, the first phase of an atherosclerotic lesion (3,7). High levels of oxidized LDL have been reported in HD patients (8,9).

There are some conflicting reports on oxidative modifications of the lipids in hemodialysis patients (8,9). The relative heterogeneity of data published is probably due to different factors including intra- and inter-patient variability, selection of patients and different dialysis methods, particularly those using various membranes with different compositions.

Recently, the use of vitamin E-coated multilayer dialysis membranes has been proposed to prevent ROS (reactive oxygen species) production and improve antioxidant host defense mechanisms (12). Markers of oxidative stress such as lipid peroxidation product MDA (10), advanced glycation end-products (AGE) (11), advanced oxidation protein products (AOPP) (12) and enhanced susceptibility to LDL oxidation (13) are elevated in hemodialysis patients.

Several serum components act as antioxidants and neutralize free radicals. Ascorbic acid, vitamin E, beta carotene, and serum proteins are all antioxidants. Since the antioxidant system has many components, a deficiency of any component can cause a reduction in the total antioxidant status of an individual. Thus, it is more meaningful to assess the total antioxidant capacity of serum using a single assay in a clinical laboratory rather than measuring the individual components of the total antioxidant system in serum. Automation of the antioxidant assay has become available recently, and is used in routine clinical laboratories due to the simplicity and cost effectiveness of the automated technique.

The objective of the presented investigation was to determine TAO activity in the sera of hemodialysis patients by using an automated technique and to evaluate the relation between LDL oxidation and hemodialysis with respect to membrane types. Another aim was to evaluate whether vitamin E-modified multilayer hemodialysis membranes have an in vivo potential effect on protection from LDL oxidation.

Materials and Methods

Twenty-two nonsmoking patients receiving chronic hemodialysis treatment for at least 12 months were evaluated. The patients (n=22) were selected from those who had been clinically stable for the previous 3 months and were included in this study after receipt of informed consent. End-stage renal disease developed secondary to chronic tubulointerstitial nephritis (5 patients), chronic glomerulonephritis (6 patients), polycystic kidney disease (2 patients), and amyloidosis (1 patient). The other cases (8 patients) were of unknown origin. All patients were dialyzed three times a week for four hours in each session, using a bicarbonate bath.

Our study was performed in two steps. In the first step, TAO status together with the uric acid levels of the sera obtained from the 22 hemodialysis patients were determined. The differences between TAO and uric acid values of pre- and post-dialysis session were evaluated and compared with those of the control group. Because TAO and uric acid levels decreased with the dialysis, these two parameters were assessed in various membranes. Eleven patients (first group) were selected from those who used a polysulfone membrane (mean age= 39.2 ± 7.3, and 7 male, 4 female) and the other group of eleven patients (second group) was selected from those who used hemophan (mean age= 41.5 ± 8.8, and 6 male and 5 female) for hemodialysis. Both groups had similar age and sex composition and had the same duration of hemodialysis. Two weeks later, a vitamin E-bonded membrane was used in the patients to whom the polysulfone membrane was applied and the results were compared with those of the polysulfone membrane group.

To investigate whether decreased serum TAO activity is related to LDL oxidation, the resistance to oxidation and AOP of the LDL fraction with respect to membrane types were assessed. Plasma samples were obtained from each patient before and after hemodialysis for isolations of LDL fractions.

All three kinds of dialyzer (low-flux dialyzer) membranes were hollow fiber. The manufacturer of the hemophan type membrane was Kawasumi Lab. Inc. Tokyo, Japan; the manufacturer of the polysulfone type membrane was Fresenius Medical Care, Hamburg, Germany; and the manufacturer of the vitamin E-bonded filters was Excebrane CL*E15-18L, Terumo, Japan. The composition of these membranes was as follows:

Hemophan: Diethyleaminoethylcellulose, potting compound: polyurethane; Polysulfone: polycarbonate, potting material: polyurethane; Excebrane: multilayer structure of cellulose, acrylic/fluororesin copolymer, oleyl alcohol and alpha-tocopherol.

Excluded from this study were subjects with diabetes mellitus, chronic liver disease, malignancy, and acute infections and those receiving multivitamin preparations or vitamin E during the study. Duration of hemodialysis treatment of the patients was (mean ± SD) 52.45 ± 34.6 months. Twenty nonsmoking healthy persons comprised the control group (11 male, 9 female: mean age ± SD, 39 ± 28.6). Blood samples of healthy subjects and of HD patients just before and after the dialysis were used in accordance with the policies and regulations of local ethical committees.

Blood Routine Parameters: Uric acid, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride were assayed in a Synchron CX7 (Beckman-Coulter CA, USA) by routine enzymatic methods. Apolipoprotein and lipoprotein-a examinations were performed by the immunonephelometric assay (Beckman-Coulter, CA, USA).

Measurement of Total Antioxidant Status: The reagents of Total Antioxidant Status were supplied from Randox (Antrim, UK). The assay principle wasbased on the suppression of ABTS+ radical cation (2,2'-Azino-di-[3-ethylbenzthiazoline sulfonate]) by the antioxidant content of the sample. The assay procedure of the reactive was adapted to an RA-XT (Tarrytown, NY, USA) autoanalyzer. TAO statuses were expressed as mmol/L.

AOP and Resistance to Oxidation of LDL Fraction: Determinations were performed before and after the dialysis session in all groups.

LDL Preparation: LDL isolated from 1 ml EDTA plasma within 6 ml 64 mM citrate buffer containing 50,000 IU/L sodium heparin was precipitated by centrifugation as described previously (14). The precipitated pellet was resuspended in 1 ml PBS (phosphate buffered saline, pH 7.3) and then used for antioxidant defense potential (AOP) and resistance to oxidation of the LDL fraction.

Measurement of RO: Resistance to oxidation was assessed as described by Dasgupta et al. (15). This assay is based on the determination of malondialdehyde (MDA) with thiobarbituric acid assay levels before and after 24 h incubation with copper-II sulfate by incubation with metal-catalyzed peroxidation, and then the peroxidation products of the LDL fraction were measured. Levels of MDA (nmol/ml sample/24h) are inversely proportional to resistance to oxidation. To obtain a positive proportion, the results were converted to 1/nmol/ml.h.

Assessment of Antioxidant Potential: AOP was assessed as described by Durak et al. (16), which is based on the determination of TBARS levels in fish oil (0.2 μl per ml) enriched sample medium before and after exposure to superoxide radicals (O2-.) produced by xanthine (10 mM)/xanthine oxidase (0.012 U) system. For this purpose, TBARS levels in the reaction mixture were measured before and after the radical attack. The difference between the values was inversely proportional to the AOP of the samples. The AOP was assessed from the difference between TBARS levels of a blank and a sample studied and the results were expressed as nmol/ml.h.

Statistical analysis: Student's t and paired t-tests were used to compare the results and Spearman correlation analysis was used for the correlation calculation.

Results

The biochemical characteristics of the patients during the prehemodialysis period are shown in Table 1. Our results showed that the serum TAO status of the patient group was significantly lower (1.355 ± 6.7, n= 22) than that of the control group (1.60 ± 0.12, n= 20) (p< 0.001) and also showed a significant decrease after dialysis (p< 0.001). Similarly, uric acid values decreased significantly after dialysis (Table 2). When we investigated the difference in activity in two types of conventional membranes, there was no significant difference in activity of TAO (Table 3).

In the patients to whom the polysulfone type dialyzer was applied, the AOP of LDL showed a significant decrease (p< 0.01). In this group, although not statistically significant, the resistance to oxidation (RO) of LDL decreased after hemodialysis (Table 4). In contrast to these findings, when the vitamin E-bonded membrane was applied to the same group, while there was no difference in the results of AOP, a significant increase with the dialysis session was detected in the results of the resistance to oxidation of LDL (Table 5). As a result, a

Table 1.   Biochemical characteristics of the patients.

Patients (n=22)	
	MEAN ± SD
Age	43.3 ± 12.7
Duration of HD (months)	52.45 ± 34.6
Total cholesterol (mg/dl)	173.2 ± 39.5
HDL-Cholesterol (mg/dl)	38.6 ± 7.8
LDL-Cholesterol (mg/dl)	99.6 ± 25.2
Apo-A (g/L)	0.98 ± 0.21
Apo-B (g/L)	0.77 ± 0.23
Lipo-a (g/L)	0.32 ± 0.19
Triglyceride (mg/dl)	151.5 ± 70.6

Table 2.   TAO and UA levels of sera in patients (n= 22) before and after HD.

	Before HD Mean ± SD	After HD Mean ± SD	p value
TAO (mmol/L)	1.355 ± 6.7	1.238 ± 0.13	p < 0.001
UA (mmol/L)	0.305 ± 0.05	0.128 ± 0.07	p < 0.001

Table 3.   Comparison of serum TAO (mmol/L) with respect to membrane types.

Membrane types	Before HD	After HD
Polysulfone (n= 11)	1.35 ± 8.6	1.24 ± 8.3
Hemophan (n= 11)	1.36 ± 4.3	1.23 ± 0.16
	Paired t-test	
p	p > 0.05	p > 0.05

Table 4.   AOP and RO of LDL fractions in HD patients (n= 11) during HD procedure using Polysulfone type dialyzer.

	AOP (/nmol/ ml.h)	RO (/ nmol/ ml.h)
Before HD	42.5 ± 7.51	1.45 ± 0.83
After HD	32.96 ± 4.71	1.19 ± 0.48
	Paired t-test	
p	p<0.01	p >0.05

Table 5.   AOP and RO of LDL fraction in the same group of HD patients (n=11) before & after HD using vitamin E-bonded membrane.

	AOP (/ nmol/ ml.h)	RO (/ nmol/ml.h)
Before HD	27.22 ± 6.95	1.58 ± 0.11
After HD	29.91 ± 5.5	2.22 ± 0.14
	Paired t-test	
p	p > 0.05	p < 0.01

vitamin E-bonded membrane may be considered a more suitable medium with respect to oxidant stress because of the increase in the resistance to oxidation in LDL.

The correlation of the decrease in uric acid levels with the TAO was found to be significant before hemodialysis (r= 0.535, p=0.01) and moderate after hemodialysis (r= 0.405, p= 0.06). A significant inverse correlation was also observed between serum LDL and TAO level (r= -0.429, p= 0.046).

Discussion

Oxidative stress has recently been implicated in the progression of renal diseases (17,18) and there is increasing evidence that the oxygen radical scavenger system is severely impaired in patients on chronic hemodialysis therapy (19-22) with a chronic deficiency in the major antioxidant systems (23,24). Increased oxidative metabolism in blood granulocytes has been described both in end-stage renal failure (ESRF) and hemodialysis patients (25). The generation of oxidized low-density lipoprotein (ox-LDL) is a common feature of oxidative stress, and high levels of ox-LDL have been reported in hemodialysis patients (26,27).

It has been reported in several studies that serum concentrations of superoxide dismutase, catalase and glutation peroxidase, which are antioxidant enzymes, decreased and lipid peroxidation by products such as malondialdehyde MDA increased in hemodialysis patients (14,25,28). Our findings may contribute to evaluations of the decreased efficiency of the cellular defense mechanism against ROS.

Hemodialysis was considered to aggravate the uremia-related pro-oxidant/antioxidant imbalance. This may result from increased ROS levels generated by

circulating neutrophils when they enter into contact with dialysis membranes. The dialysis membrane is subjected to immunologic response by low molecular weight plasma constituents such as IgG and complement components to make the membrane biologically active for granulocytes. Activation of blood granulocytes can increase reactive oxygen species (29). The second possible reason for the continuing decrease antioxidant response after hemodialysis may be the removal of an uremic inhibitor by dialysis. This suggests that an uremic dialyzable inhibitor could be partly responsible for the impaired TAO activity observed. However, it is also possible that post-translational modification of the constituents of TAO and AOP could contribute to impaired activity. A number of modifications are possible, including glycation, oxidation and carbamylation. A loss through the dialysis membrane of hydrosoluble antioxidants such as ascorbic acid, together with their possible increased consumption by oxidation, and other factors such as the absence of a complete correction of the uremic toxicity, malnutrition and the progressive worsening of the clinical condition due to aging and comorbidity may contribute to a decreased antioxidant power of plasma during dialysis.

Up to now it has been demonstrated that enhanced susceptibility to LDL oxidation is found (13) and small-dense LDL subtractions, recognized for their enhanced susceptibility to oxidation, have been shown in hemodialysis patients (30).

Because of the great interest in the evaluation of the role of ox-LDL in the atherosclerosis process, the TAO of plasma, AOP and the resistance to oxidation of LDL have to be investigated.

Several researchers suggested that plasma TAO would be impaired in patients with ESRD (10); nevertheless, other investigators found that plasma TAO activities were higher in hemodialysis patients than in controls, and decreased by the end of dialysis session. These observations were attributed to uric acid, which was present at a high concentration in plasma before dialysis; thereby, it was termed the uric acid paradox (31). In contrast to these observations, the activity was lower than that of the controls before dialysis by automated TAO technique. However, in our study cases, plasma uric acid concentrations were within the normal limits before dialysis and decreased significantly after dialysis together with the decrease in plasma TAO. In our opinion, before

making a decision, the types of assay method should be evaluated in detail.

Some authors showed that a cellulose membrane was responsible for an acute stimulation of the oxidative metabolism of leucocytes while a synthetic membrane was able to reduce it (32).

According to our results, after dialyses plasma TAO activity showed no difference with respect to standard polysulfone and hemophan type membranes. Although Cu^{+2} –induced oxidation of LDL fractions showed no difference, the AOP of LDL decreased after the dialysis session. This might have been caused by the high content of advanced oxidation protein products in the LDL fraction, which are more avid than native proteins for trapping radicals or enrichment of LDL with triglycerides (33,34). However, to clarify these hypotheses, further research is needed.

In recent years, new approaches to antioxidant therapy such as hemolipodialysis, infusion of antioxidants by dialyzate, and vitamin E-bonded membranes in hemodialysis have been introduced (34,37). The newest scientific work in this field is based on the detailed characterization of the multilayer structure and composition of the vitamin E-bonded membrane (35-38). Several investigations have shown that these membranes can improve some antioxidant parameters (12), and lower lipid peroxidation both in vitro and in vivo (10,38), and decrease advanced glycation end-product (AGE) accumulation (31).

The cellular functions of vitamin E have recently been demonstrated in blood cells including a decreased production of superoxide anion by activated monocytes and polymorphonuclear leukocytes (39). Since ROS production is enhanced during the dialysis session, this cell-mediated action of vitamin E should be taken into account in the beneficial action of vitamin E supplementation in hemodialysis patients. Raggi et al. observed in an in vitro study that a vitamin E-modified membrane may offer some advantages compared to a polysulfone membrane in inducing less oxidative stress in granulocytes, resulting in a smaller production of oxygen free radicals (40).

The present study confirms earlier reports describing profound alterations in the circulating antioxidant levels of patients with ESRD undergoing chronic hemodialysis therapy. The aggravation of the antioxidant deficit as a

result of a single dialysis session indicates that part of the stress occurs during the dialysis procedure. The decrease in both TAO and AOP suggests that the defense system against the oxidant stress is insufficient and decreased resistance to the oxidation of LDL may contribute to this condition in hemodialysis with conventional membranes. However, the AOP of LDL did not change in dialysis with the vitamin E-bonded membrane, whereas a significant decrease occurred with the standard polysulfone membrane in the same patient group. This finding indicates that the use of vitamin E-bonded membrane at least in part may be beneficial by compensating for the decrease in antioxidant potential and the increase in the resistance to the oxidation of the LDL fraction during dialysis. On the other hand, we postulate to assess the total antioxidant status instead of assaying each constituent separately to evaluate an individual's defense mechanism against the reactive oxygen species. This test procedure is automated, convenient and available for use in most analyzers.

In light of our results, improving LDL resistance to oxidation by using a vitamin E-bonded membrane appears to be an attractive alternative to prevent accelerated oxidative processes in hemodialysis patients.

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Abbreviations

ABTS+	Radical cation (2,2'-Azino-di- [3-ethylbenzthiazoline sulfonate])
AGE	Advanced glycation end-products
AOP	Antioxidant defence potential
AOPP	Advanced oxidation protein products
apo B	Apolipoprotein B
Apo-A	Apolipoprotein A
CRF	Chronic renal failure
ESRD	End-stage renal failure
ESRF	End-stage renal failure
GSH-Px	Glutathione peroxidase
HD	Hemodialysis
LDL	Low-density lipoprotein
MDA	Malondialdehyde
ox-LDL	oxidized low-density lipoprotein
PBS	Phosphate buffered saline
RO	Resistance to oxidation
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TAO	Plasma total antioxidant
TBARS	Thiobarbituric acid reactive substance
UA	Uric acid

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