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NEPHROLOGY – ORIGINAL PAPER

Low-density lipoprotein (LDL) apheresis reduces atherogenic and oxidative markers in uremic patients with hyperlipidemia

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Abstract Uremic patients with hyperlipidemia are classified at high atherogenic risk due to oxidative stress induced by regular hemodialysis process (hemoincompatibility) and a high level of oxidized low-density lipoprotein (ox-LDL). This study aimed to investigate whether LDL apheresis was capable of reducing oxidative and atherogenic markers in uremic patients with hyperlipidemia. We found that oxidative metabolites (methylquanidine, dityrosine, and ox-LDL) and atherogenic markers (lipoprotein (a), LDL, and LDL/HDL ratio) were significantly reduced ($P < 0.05$) after LDL apheresis. On the other hand, plasma total antioxidant status (TAS) was not influenced after LDL apheresis. Our results suggest that LDL apheresis reduces oxidative and atherogenic markers and do not influence plasma TAS in uremic patients with hyperlipidemia. This may lead to a decreased risk of atherosclerosis in these patients. However, supplementation of dietary proteins may be necessary because of the removal of some "useful" proteins (e.g., albumin and globulin) after LDL apheresis.

Keywords Atherosclerosis · Hemodialysis · Hyperlipidemia · LDL apheresis · Oxidative stress · Uremic patients

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Introduction

Hemodialysis is the main therapeutic strategy to remove metabolic wastes (uremic solutes) in patients with end-stage renal failure. During the hemodialysis process, neutrophils are activated due to the interaction between blood and dialysis membranes and fluids (hemoincompatibility) [1–3]. The activated neutrophils then release a large amount of reactive oxygen species (ROS) into the bloodstream leading to an increase in oxidative stress, a phenomenon known as "neutrofil burst" [4, 5]. In its turn, oxidative stress promotes atherosclerosis.

In addition to oxidative stress, the high plasma level of low-density lipoproteins (LDL) is another risk factor for atherosclerosis. The LDL are transformed into oxidized LDL (ox-LDL) which lead to a series of pathologic processes resulting in atherosclerosis [6].

The aim of this study was to evaluate whether LDL apheresis is capable of reducing atherogenic markers and oxidative metabolites in uremic patients with hyperlipidemia. In addition, the plasma total antioxidant status (TAS) was investigated in prior to and after LDL apheresis.

Materials and methods

Chemicals and reagents

All chemicals and reagents used in this study were purchased from Sigma–Aldrich Inc. (3050 Spruce Street, St. Louis, MO, USA). The analytical kit in plasma ox-LDL was purchased from Mercodia AB (Sylveniusgatan 8A, ST2-75450 Uppsala, Sweden). The other plasma biochemical tests were performed by IMMULITE instrument (DPC Cirrus Inc., Los Angeles, CA, USA).

Experimental subjects and design

Twenty-three uremic patients with hyperlipidemia (plasma LDL $>$ 120 mg/dl) were included in this study. The LDL apheresis therapy for these patients was performed according to the standard operating procedures (SOP) of the Formosan Blood Purification Foundation (Taipei, Taiwan). Briefly, the LDL apheresis was performed by KM-8800 (Kuraray Co., Ltd, Osaka, Japan) with a double-filtration plasmapheresis procedure (Plasmacure PS-06 and Evaflux 4A, Kuraray Medical Inc., Okayama, Japan). Total blood volumes of 10.05 $\pm$ 0.27 l were processed in the 2-h plasmapheresis procedure. The dosage of heparin used in the 2-h apheresis procedure was 4,000 IU. Twenty healthy volunteers were included in this study and served as controls. The general data of the subjects are listed in Table 1.

Plasma sample analysis

Plasma samples were collected from subjects prior to (DF1) and after (DF2) LDL apheresis. Plasma biochemical tests, including lipoprotein a (Lp(a)), LDL, high density lipoprotein (HDL), methylguanidine (MG), oxidized LDL (ox-LDL), dityrosine, and plasma total antioxidant status (TAS), were performed in all plasma samples.

Data analysis

All data were expressed as the mean $\pm$ standard deviation (SD). Data analysis was carried out using ANOVA. The significant difference was considered at $p < 0.05$.

Results

Plasma MG [7] and dityrosine [8] are metabolic products of protein oxidation, whereas ox-LDL is a

Table 1 General data of subjects

	Gender	Age	Hemodialysis vintage (months)	LDL (mg/dl)
Uremic patients	Male = 9	48.6 $\pm$ 14.6	89 $\pm$ 35	127.2 $\pm$ 7.2
	Female = 14	59.2 $\pm$ 9.6	78 $\pm$ 43	143 $\pm$ 19.3
Healthy volunteers	Male = 11	51.0 $\pm$ 13.9	–	81.5 $\pm$ 7.2
	Female = 9	57.4 $\pm$ 11.0	–	81.4 $\pm$ 11.5

Table 2 Analysis of plasma oxidative markers for experimental subjects

	MG (fluorescence)	Dityrosine	Ox-LDL (U/l)
Uremic patients ($n = 23$)			
DF1	309.2 ± 114.5	5,954.8 ± 1,875.2	40.3 ± 4.2
DF2	264.9 ± 108.0	4,499.4 ± 1,726.8	32.2 ± 4.8
Decrease %	23.1 ± 11.5	22.3 ± 20.3	19.7 ± 11.0
P value	<0.05	<0.05	<0.05
Healthy volunteers ($n = 20$)	202.9 ± 64.2	1,611.8 ± 379.7	37.4 ± 5.5

DF1 and DF2 represented the plasma samples collected prior to and after performing LDL apheresis, respectively. The decrease % represented the comparison between DF1 and DF2. The *P* value showed the significance of the decrease %

product of LDL oxidation. Therefore, the plasma levels of the above-mentioned metabolites are a marker of oxidative stress. Table 2 shows the concentration of these oxidative metabolites in plasma samples collected from subjects. We showed that the plasma concentration of MG, dityrosine, and ox-LDL significantly decreased by 21.3 ± 11.5, 22.3 ± 20.3, and 19.7 ± 11.0% ($P < 0.05$), respectively, in hyperlipidemic uremic patients after LDL apheresis.

Table 3 shows the clinical markers associated with atherosclerosis. Lp(a), LDL, and the LDL/HDL ratio significantly decreased by 56.3 ± 17.5, 66.4 ± 11.6, and 42.2 ± 17.1%, respectively, in hyperlipidemic uremic patients after LDL apheresis ($P < 0.05$).

As shown in Fig. 1, plasma TAS did not change significantly after LDL apheresis.

As shown in Fig. 2, we observed that plasma albumin and globulin decreased by 19.3% (albumin: DF1 = 3.88 ± 0.40 g/dl, and DF2 = 3.13 ± 0.37 g/dl) and by 28.3% (globulin: DF1 = 2.79 ± 0.41 g/dl, and DF2 = 2.00 ± 0.45 g/dl), respectively.

Discussion

The principle of LDL apheresis is to remove macro-biomolecules in plasma through two membranes (double-filtration) [9]. The first membrane of LDL apheresis separates whole blood into blood cells and plasma. The second membrane separates LDL from plasma filtered by the first membrane. The LDL-separated plasma is then infused back to the patient. Thus, LDL apheresis removes from blood macromolecules like protein-bound MG, dityrosine, ox-LDL, Lp(a), and LDL [10, 11]. On the other hand, the removal of macro-biomolecules by LDL apheresis is non-selective, and therefore, some "good" proteins will be partially removed by LDL apheresis as well. For instance, albumin (% removal = 19.3%) and globulin (% removal = 28.3%) will be removed after LDL apheresis (data shown in Fig. 2).

Figure 1 showed that the plasma TAS was not significantly altered in hyperlipidemic uremic patients before (DF1) and after (DF2) LDL apheresis. These data indicated that LDL apheresis did not

Table 3 Analysis of plasma atherogenic markers for experimental subjects

	Lp(a) (mg/dl)	LDL (mg/dl)	LDL/HDL ratio
Uremic patients ($n = 23$)			
DF1	11.7 ± 8.3	136.9 ± 17.4	3.4 ± 1.1
DF2	4.3 ± 2.7	45.8 ± 15.9	1.9 ± 1.0
Decrease %	56.3 ± 17.5	66.4 ± 11.6	42.2 ± 17.1
P value	<0.05	<0.05	<0.05
Healthy volunteers ($n = 20$)	13.2 ± 12.5	81.5 ± 9.2	1.5 ± 0.4

DF1 and DF2 represented the plasma samples collected prior to and after performing LDL apheresis, respectively. The decrease % represented the comparison between DF1 and DF2. The *P* value showed the significance of the decrease %

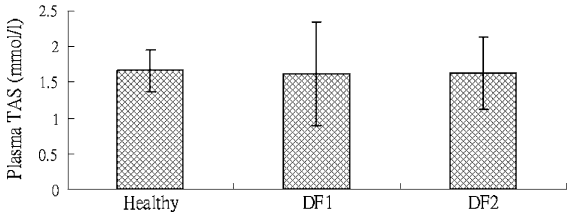

Fig. 1 Analysis of plasma antioxidant status (TAS) for experimental subjects. DF1 and DF2 represented the plasma samples collected prior to and after performing LDL apheresis, respectively

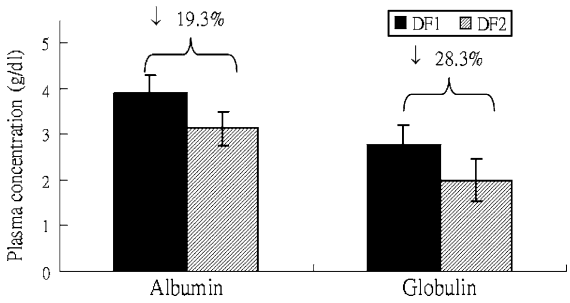

Fig. 2 Analysis of plasma proteins in hyperlipidemic uremic patients prior to and after LDL apheresis

influence plasma TAS. We know that plasma antioxidant defenses are including enzymatic (e.g., SOD, catalase, and GSH peroxidase) and non-enzymatic (e.g., vitamins, uric acid, and polyphenols) substances, and these antioxidant substances are not removed by LDL apheresis due to a relatively low molecular weight (LDL apheresis mainly removes substances of macro-molecular weight, such as LDL). This may be the reason why LDL apheresis will not influence plasma TAS significantly. These findings are consistent with previous studies [12, 13].

Conclusion

As described earlier, LDL apheresis can reduce not only plasma LDL, but oxidative metabolites and atherogenic indices in uremic patients with hyperlipidemia. In addition, plasma TAS will not be influenced in patients prior to and after LDL apheresis. These data suggest that LDL apheresis may decrease the risk of complications associated with oxidative stress (especially atherosclerosis) in uremic patients with hyperlipidemia. Nevertheless, supplementation of proteins (e.g., albumin) is necessary due to protein depletion by LDL apheresis.

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