Effects of Heparin and Dalteparin on Oxidative Stress During Hemodialysis in Patients With End-Stage Renal Disease

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Introduction. Dialysis-induced oxidative stress is one of the mechanisms of atherosclerotic changes. Heparin, used in hemodialysis, is an anticoagulant drug with anti-inflammatory and antioxidant effects. This study was planned in order to evaluate the antioxidant effects of heparin and dalteparin (low-molecular weight heparin).

Materials and Methods. Twenty-two patients underwent 3 hemodialysis sessions with 48-hour intervals. They underwent hemodialysis with heparin, with a bolus dose of 1000 U followed by 1000 U/h during the procedure. The second hemodialysis was done using hypertonic saline solution instead of heparin, and the third, using dalteparin, 4000 U, infused during hemodialysis. Before and after each dialysis session, we measured serum levels of total blood cholesterol, triglyceride, high- and low-density lipoprotein cholesterols and oxidized low-density lipoprotein cholesterol, in addition to total antioxidant capacity and paraoxonase 1 activity.

Results. Serum concentrations of triglyceride, cholesterol, and oxidized low-density lipoprotein cholesterol, as well as paraoxonase activity and total antioxidant capacity equally increased after the three hemodialysis sessions. Heparin and daltepain increased total antioxidant capacity, but they did not change the ratio of paraoxonase 1 to high-density lipoprotein cholesterol after hemodialysis. No significant differences were found through the study between the two heparin products in their antioxidant activities.

Conclusions. Regarding these findings and considering higher price and less availability of dalteparin in comparison to conventional heparin, we recommend using conventional heparin during hemodialysis as the anticoagulant-antioxidant agent.

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INTRODUCTION

Cardiovascular diseases are the most common cause of death among patients with end-stage renal disease who receive maintenance hemodialysis. Dialysis-induced oxidative stress is one of the mechanisms of atherosclerotic changes which lead to cardiovascular disease. This stress refers to an imbalance resulted from high rate of lipid peroxidation followed by decreased antioxidant levels.[1-7] Many studies hinted that the mentioned

imbalance is started by the interaction between peripheral blood polymorphonuclear cells with dialysis artificial membranes, because of reactive oxygen species generation.[1,3,8-10]

Heparin, an anticoagulant containing a number of additional effects such as anti-inflammatory and antioxidant, is a fundamental substance used during hemodialysis sessions.[1,11,12] This drug is used under 2 forms including conventional and low-molecular weight heparin. The latter is a fragmented form of heparin called *dalteparin* (low-molecular weight heparin) in the market, which has a lower range of side effects than the ordinary drug. Heparin boosts the antioxidant effect of superoxide dismutase by releasing it near the endothelial cells of the vessels. On the other hand, heparin, as a sink of free radicals of oxygen in addition to iron and copper, the well known oxidants in inflamed tissues, plays properly its antioxidant role.[13,14]

Alterations in lipid profile of the patients and the enzymes involved in their metabolism can be used as indicators of antioxidant effects of drugs. Cholesterol and triglyceride are the best known lipids in the process of atherosclerosis. Atherogenic lipoproteins such as oxidized low-density lipoprotein cholesterol (LDLC) resulted from LDLC peroxidation, is the main factor to form foam cells from monocytes beside the endothelium of the vessels and leads to atherosclerotic plaque generation.[15] On the other hand, high-density lipoprotein cholesterol (HDLC) is known as anti-atherogenic lipoprotein inhibiting oxiadation of LDLC through blocking of the LDLC peroxidation. High-density lipoprotein cholesterol has an enzyme in its components called *paraoxonase* (*aryl-di-alkyl-phosphatase*). Paraoxonase is an esterhydrolase provided by the liver. This enzyme is highly dependent upon HDLC to act properly as an anti-atherogenic agent.[16]

Paraoxonase inhibits the gathering of lipid peroxides in the LDLC in an oxidant environment.[17] The absence or decrease of paraoxonase 1 may aggravate oxidative circumstance in macrophages followed by higher atherosclerosis rate. This study was conducted to evaluate antioxidant effects of heparin and dalteparin via measurement of serum lipids, total antioxidant capacity (TAC), and paraoxonase activity. Maintenance hemodialysis as a unique in vivo system was used to evaluate the two drugs and to find the differences in antioxidant effects of heparin and dalteparin through comparing the mentioned indexes of oxidation recorded before and after hemodialysis sessions.

MATERIALS AND METHODS

Patients

In this clinical trial, patients on hemodialysis were recruited to receive heparin, dalteparin, and placebo (hypertonic saline solution). The patients (age range, 18 to 75 years) were those who were on maintenance hemodialysis in Taleghani Hospital in Tehran, Iran. We excluded patients with acute kidney failure, thyroid disorders, acute coronary syndromes, history of cerebrovascular accident, and tertiary hyperparathyroidism, as well as those who were receiving antilipidemic drugs and/or blood transfusion during past month. Overall, 22 patients were included. The eligible patients provided written consent to participate in the study.

Materials

Conventionally used heparin and dalteparin, a low-molecular weight heparin (Fragmin, Pfizer, New York, USA) were used in this study. The hemodialysis machines were Fresenius 4008-B. In order to measure antioxidant effects of heparin and dalteparin, we considered indexes including serum levels of total blood cholesterol, triglyceride, HDLC, LDLC, and oxidized LDLC, in addition to TAC and paraoxonase 1 activity.

Methods

Demographic variables consisting of age and gender along with anthropometric variables, dialysis adequacy, past medical history, and blood pressure were collected. Three sessions of 4-hour hemodialysis were carried out for the patients with 48-hour washout intervals as follows: first, the patients underwent hemodialysis with heparin, which was infused through the catheter that transported the blood into the dialysis machine, with a bolus dose of 1000 U followed by 1000 U/h during the procedure. Then, hemodialysis was done using hypertonic saline solution after a 48-hour washout period. Hypertonic saline, 100 mL/h, was infused and no heparin was used at this stage. Finally, hemodialysis was carried out using dalteparin after another 48-hour washout period. Dalteparin, 4000 U, was infused during hemodialysis.

The participants were requested to keep their nutritional diet and body exercises constant during the study. Fasting venous blood samples (10 mL) were obtained before each session from the brachial vein of the fistula-free hand. The second blood samples were taken after 2 minutes of hemodialysis. The blood samples were centrifuged and restored in -80°C before the tests.

Laboratory Studies

Serum total cholesterol and triglyceride levels were measured using Pars Azmoun kit (Tehran, Iran). Chemical precipitation method by phosphotunistic acid was used for separation of the HDLC. The LDLC was calculated based on the Friedwald formula if serum triglyceride level was less than 400 mg/dL. Oxidized LDLC was determined with enzyme-linked immunosorbent assay using monoclonal antibodies. Paraoxonase 1 was measured by triple specific LaDu method for microtitration 96 microplates using 3 standard human serum including high, middle, and low action as controls. Changes were measured kinetically after 12 minutes, and then, every 20 seconds in the wavelength of 405 nanometers. In order to measure TAC, 20 µmol of serum was added to phosphate-buffered saline (80 µmol/L, pH = 7.4), methemoglubin (6.1 µmol/L), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (640 µmol/L), chromogen, and hydrogen peroxide (250 µmol/L) as substrates. The TAC was measured according to the blue-green color by enzyme-linked immunosorbent assay.

Since paraoxonase 1 is carried by the HDLC, the ratio of paraoxonase 1 to HDLC was calculated instead of paraoxonase 1 per se to eliminate the HDLC concentration effect during oxidative stress. In addition, oxidized LDLC-LDLC ratio and TAC were calculated and corrected based on hemodialysis adequacy (Kt/V).

Statistical Analyses

The data were collected before and after each hemodialysis session and were analyzed using the SPSS software (Statistical Package for the Social Sciences, version 10.0, SPSS Inc, Chicago, Ill, USA). The paired *t* test was used to compare parametric quantitative variables, and the chi-square test was utilized to compare qualitative variables. Mean changes of variables among the three groups were compared by the 1-way analysis of variance test. Nonparametric statistical tests were substituted in case of variables with abnormal distribution (Wilcoxon signed rank test and Kruskal-Wallis test). A P value less than .05 was considered significant.

RESULTS

A total of 22 patients, including 11 men and 11 women, participated in the study. The mean age of the patients was 59.2 ± 10.6 years and the mean duration of dialysis was 5.1 ± 3.6 years (range, 1 to 14 years). As is illustrated in Table 1, serum concentrations of triglyceride and cholesterol significantly increased after the three hemodialysis sessions. Also, paraoxonase activity, arylesterase activity, and TAC, and oxidized LDLC concentration increased significantly in comparison to the time before hemodialysis. There was no significant difference between the three hemodialysis sessions regarding the mean changes of the abovementioned variables (Table 2).

Conventional heparin and dalteparin significantly decreased paraoxonase 1-HDLC ratio. However, adjustment by Kt/V showed that heparin and daltepain increased TAC, but they did not change

Table 1. Laboratory Serum Variables Before and After Hemodialysis with Heparin, Dalteparin, and Hypertonic Saline Solution*

	Heparin			Dalteparin			Hypertonic Saline Solution		
Variable	Before	After	*P*	Before	After	*P*	Before	After	*P*
Cholesterol, mg/dL	143 ± 44	168 ± 57	< .001	141 ± 42	163 ± 51	< .001	140 ± 44	162 ± 50	< .001
Triglyceride, mg/dL	153 ± 54	175 ± 77	< .05	154 ± 60	176 ± 70	< .01	157 ± 78	191 ± 115	< .001
HDLC, mg/dL	46 ± 10	57 ± 14	< .001	46 ± 12	55 ± 12	< .001	45 ± 9	55 ± 11	< .001
LDLC, mg/dL	67 ± 36	75 ± 45	< .05	64 ± 30	73 ± 40	< .01	65 ± 30	70 ± 32	< .01
Oxidized LDLC, mg/dL	34 ± 14	40 ± 15	< .001	34 ± 17	39 ± 18	< .001	38 ± 15	44 ± 17	< .001
TAC, mU/L	1.1 ± 0.2	1.4 ± 0.2	< .001	1.0 ± 0.2	1.3 ± 0.2	< .001	1.2 ± 0.2	1.4 ± 0.2	< .001
Paraoxonase 1, U/L	57 ± 30	68 ± 40	< .001	57 ± 33	66 ± 37	< .001	58 ± 32	66 ± 36	< .001
Arylesterase, U/L	67 ± 30	83 ± 37	< .001	66 ± 28	76 ± 37	< .001	72 ± 32	84 ± 37	< .01

*HDLC indicates high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; and TAC, total antioxidant capacity.

Table 2. Comparison of Mean Changes in Variables in Hemodialysis With Heparin, Dalteparin, and Hypertonic Saline Solution*

Variables	Changes After Hemodialysis			*P*
	Heparin	Dalteparin	Saline	
Cholesterol, mg/dL	22.0 ± 21.1	21.1 ± 14.5	22.1 ± 13.1	.93
Triglyceride, mg/dL	27.3 ± 44.1	23.4 ± 26.3	36.4 ± 49.1	.45
HDLC, mg/dL	10.3 ± 9.0	8.5 ± 5.7	10.5 ± 7.9	.71
LDLC, mg/dL	7.2 ± 13.9	7.9 ± 11.6	5.7 ± 6.9	.76
Oxidized LDLC, mg/dL	5.5 ± 4.6	4.2 ± 4.4	6.3 ± 5.8	.41
TAC, mU/L	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	.45
Paraoxonase 1, U/L	7.5 ± 19.2	8.0 ± 8.8	8.4 ± 7.9	.97
Arylesterase, U/L	15.9 ±19.2	14.7 ± 15.1	12.0 ± 21.6	.82

*HDLC indicates high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; and TAC, total antioxidant capacity.

Table 3. Adjusted Laboratory Variables for HDLC and Hemodialysis Adequacy in Hemodialysis With Heparin, Dalteparin, and Hypertonic Saline Solution*

Variables	Hemodialysis with Heparin			Hemodialysis with Dalteparin			Hemodialysis with Hypertonic Saline Solution		
	Before	After	*P*	Before	After	*P*	Before	After	*P*
Oxidized LDLC/LDLC	0.6 ± 0.2	0.6 ± 0.2	NS	0.6 ± 0.2	0.6 ± 0.3	NS	0.6 ± 0.3	0.7 ± 0.3	NS
Paraoxonase 1/HDLC	1.3 ± 0.7	1.2 ± 0.7	< .05	1.3 ± 0.9	1.1 ± 0.8	< .05	1.4 ± 0.9	1.3 ± 0.8	NS
Paraoxonase 1/HDLC/Kt/V	2.7 ± 6.6	2.5 ± 6.3	NS	2.9 ± 7.7	3.1 ± 8.1	NS	2.9 ± 7.1	2.8 ± 7.1	< .05
TAC/Kt/V	1.8 ± 3.3	2.1 ± 3.5	< .001	1.7 ± 3.2	2.2 ± 3.7	< .001	1.9 ± 2.4	2.3 ± 4.2	NS
Oxidized LDLC/LDLC/Kt/V	0.8 ± 1.4	1.0 ± 1.9	NS	0.9 ± 1.3	0.8 ± 1.0	NS	1.1 ± 1.9	1.1 ± 1.8	NS

*LDLC indicates low-density lipoprotein cholesterol; HDLC, high-density lipoprotein cholesterol; and TAC, total antioxidant capacity.

paraoxonase 1-HDLC ratio after hemodialysis (Table 3). Adjustment by Kt/V showed that saline solution significantly reduced the ratio of paraoxonase 1 to HDLC/Kt/V and made no significant changes in TAC/Kt/V (Table 3).

DISCUSSION

Cardiovascular diseases make up a great proportion of mortality among patients on maintenance hemodialysis, 10 to 20 times more than that in the general population.[18] Renal dyslipidemia is one of the major cardiovascular risk factors characterized by an impaired catabolism of triglyceride-rich lipoproteins through accumulation of atherogenic remnant particles.[18,19] Lipoprotein lipase (LPL) system is a contributing factor which is the major lipase in the catabolism of triglyceride-rich lipoproteins. Lipoprotein lipase functional pool is situated at the vascular surface. It facilitates consumption of lipoproteins by cells and consequently decreases the serum level of those lipids. Heparin injection during hemodialysis is the major factor for releasing LPL into the circulating blood and extracting and degrading it by the liver. Plasma LPL activity is usually lower after using low-molecular weight heparin because of less releasing LPL followed by less disturbance of lipoprotein metabolism than that after injection of conventional heparin.[18,20] However, animal studies revealed that both types of heparin have the same efficacy in releasing LPL, while low-molecular weight heparin is less efficient in retarding hepatic uptake.[18,20,21] Therefore, serum lipoprotein level would be higher after dialysis using low-molecular weight heparin.

In chronic kidney disease per se, dyslipidemia and increased oxidation rate is seen.[22,23] Dyslipidemia includes higher serum concentrations of triglyceride and very low-density lipoprotein cholesterol; prolonged persistence of postprandial chylomicron remnants; accumulation of small dense LDLC; modification of apolipoproteins by glycation, oxidation, and carbamoylation; increased lipoprotein(a); and accumulation of noncardioprotective acute-phase HDLC.[22] In addition, dialysis, may affect the lipoprotein metabolism mediated by some factors such as dialysis modality (hemodialysis versus peritoneal dialysis), type of the liquid used, and type of dialysis filters (synthetic or cellulose).

Oxidative stress is a nontraditional risk factor of cardiovascular disease in patients on hemodialysis. Increased free radicals of oxygen as well as antioxidant insufficiency could cause oxidative stress followed by lipoprotein oxidation.[15,23] For instance,

LDLC will be oxidized to produce oxidized LDLC that is the main reason of foam cells generation near the endothelium and then atherosclerosis. Hemodialysis accelerates the mentioned process due to its artificial membranes which are in closed contact with blood. This procedure pushes the setting to create atherosclerotic changes leading to cardiovascular diseases.

Obviously, increased serum lipids during hemodialysis refers to high oxidant injury, which also plays a major role in many disorders including atherosclerosis as well as diabetes mellitus, hypertension, chronic inflammatory process, and cancer, because of tissue damage by reactive oxygen species. Maher and colleagues[24] were the pioneers of research on oxidative effects of uremia and hemodialysis, who concluded in 1987 that oxidative stress rose in hemodialysis. This was certificated by the pursuant. Dasgupta and associates[25] found that cellulose membranes of dialysis filters could stimulate complement immune system to release cytokines and oxidants. Tayeb and colleagues[26] explained that maintenance hemodialysis, even by biocompatible dialysis filters, increased the rate of cardiovascular death, infectious consequents, and serum C-reactive protein concentration.

The current study showed that increased serum lipids and oxidative stress occurred during hemodialysis regardless of the types of anticoagulants used. Alterations in paraoxonase 1 and arylesterase were corrected by adjusting them for HDLC and hemodialysis adequacy (Kt/V). This adjustment shifted the results to accept more strongly the antioxidant character of conventional and low-molecular weight heparin compared to hypertonic saline solution (Table 2). Although the sample size in current study was calculated and considered in order to obtain a standard power, multicenter studies on greater numbers of participants is required in order to extrapolate the results. In addition, designing a controlled trial with a long period of follow-up could appoint the incidence of cardiovascular problems in patients on hemodialysis. We did not evaluate paraoxonase 2 and paraoxonase 3 because of the inefficiency of the laboratory techniques. Hence, these should also be studied through a more technical research.

CONCLUSIONS

According to the findings of this study, which are in agreement with the results of other studies, and considering the high price and unavailability of dalteparin, we recommend conventional heparin through hemodialysis as the anticoagulant-antioxidant agent which yields acceptable results compared to low-molecular weight heparin. This idea, however, is regardless of other effects of heparin products and is only about antioxidative and anticoagulant effects irrespective of their complications.

CONFLICT OF INTEREST

None declared.

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