Effects of Low-Density Lipoproteins on Blood Coagulation and Fibrinolytic Activity

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In vitro experiments showed that copper-oxidized low-density lipoproteins activate factors of the prothrombin complex in the whole blood and inhibit fibrin generation in both blood and plasma. Moreover, oxidized low-density lipoproteins inhibit fibrinolysis and impair the structure of fibrin clot, which results in hypercoagulation.

Key Words: *lipid peroxidation; low-density lipoproteins; blood coagulation; fibrinolysis*

The role of lipid peroxidation of plasma lipoproteins in atherogenesis is now well established [5]. On the other hand, atherosclerosis is characterized by activation of the blood coagulation system and inhibition of fibrinolysis [7]. Enhanced blood coagulation can result from activation of factor VIII against the background of high concentration of plasma lipids [14], activation of platelet secretory function [4], changes in the vessel wall [9] and blood rheology [6], release of tissue plasminogen activator inhibitor from endothelial cells [11], and the presence of apolipoprotein A [13].

We previously demonstrated that oxidized serum added to whole blood or plasma induced hypercoagulation due to activation of the prothrombin complex and inhibition of fibrinolysis [1]. A question arises of which blood components are responsible for this effect? It should be noted that serum albumin, low- (LDL), and high-density lipoproteins (HDL) are characterized by the highest propensity for free radical oxidation in the plasma [10]. The observed phenomena can result from the effects of all these components.

Our aim was to study the effects of oxidized LDL on coagulation and fibrinolysis in the blood and plasma.

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MATERIALS AND METHODS

LDL (d=1.006-1.065 g/cm^3) were isolated by ultracentrifugation (120,000*g*) from blood plasma of healthy donors starved for 12 h [12]. LDL were dialyzed against 1000 volumes of phosphate buffer at 4°C for 24 h and oxidized in the presence of copper ions in a final concentration of 2.5 μmol [8]. The oxidation was evaluated by the concentration of MDA reacting with thiobarbituric acid [15].

In *in vitro* experiments, LDL were added to the blood stabilized with sodium citrate (9:1) or blood serum in a volume proportion of 1:9, and coagulation and fibrinolytic activities were measured after 5- and 60-min incubation with LDL at 37°C [2,3]. Blood or plasma samples diluted with physiological saline (1:9) served as the control.

The data were analyzed statistically by ANOVA and Student's *t* test at $p<0.05$.

RESULTS

The effects of oxidized LDL on blood coagulation and fibrinolysis were evaluated by the following parameters: prothrombin time (PTT) characterizing the external mechanism of blood coagulation; thrombin time (TT) reflecting the effect of blood anticoagulants on fibrinogenesis; maximum amplitude of thromboelastogram (TEG) reflecting the functional state of the

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TABLE 1. LDL Oxidation and Parameters of Blood Coagulation and Fibrinolytic Activity ($M \pm m$)

Parameters		Incubation period, min	Control	Non-oxidized LDL	LDL_L	LDL_H
PTT, sec	whole blood	5	25.1±1.1	26.3±0.8	27.4±1.2	22.3±0.9*
		60	29.1±1.2	30.9±1.7	30.9±1.3	26.0±0.7*
	plasma	5	22.7±0.9	23.3±1.4	24.8±1.2	33.7±1.9*
		60	26.4±1.6	27.7±1.4	28.2±1.4	38.1±2.1*
TT, sec	whole blood	5	23.4±1.3	24.4±1.1	24.0±1.1	23.1±1.4
		60	26.8±1.4	27.2±1.2	25.4±1.6	28.0±1.8
	plasma	5	19.8±0.7	18.6±1.5	16.6±1.3	26.0±1.6*
		60	25.0±1.1	25.7±1.9	24.5±0.9	38.0±3.1*
TEG maximum amplitude, mm		5	77.0±3.6	84.0±3.9	75.0±3.0	68.0±3.6*
		60	66.0±2.9	69.0±2.7	58.0±2.4*	43.0±2.8*
Fibrinolytic activity, %		5	43.0±2.7	45.0±2.3	40.5±2.6	42.0±2.7
		60	42.0±2.1	44.0±2.5	42.0±2.2	35.0±2.0*

Note: *$p<0.05$ compared to non-oxidized LDL.

clot; and activity of the fibrinolysis system (percent of clot lysis for 1 h).

Preliminary experiments showed that residual non-oxidized LDL (0.2-0.5 nmol MDA/mg protein) and Cu^{2+} (2.5 μmol) produced no significant effect on coagulation and fibrinolysis.

Low oxidized LDL (LDL_L, MDA 1-3 nmol/mg protein) introduced into whole blood did not change PTT (Table 1), but highly oxidized LDL (LD_H, MDA 6-8 nmol/mg protein) reduced PTT by 15.2 and 15.9% after 5 and 60 min of incubation, respectively, compared with non-oxidized LDL.

PT also remained unchanged in the presence of LDL_L but increased after 5- and 60-min incubation with LDL_H by 30.9 and 27.2%, respectively (Table 1).

Thus, only LDL_H affected coagulation of the whole blood *in vitro*. The observed changes were oppositely directed: the prothrombin complex was activated and fibrinolysis was inhibited.

LDL did not change PTT in the plasma irrespectively of the degree of oxidation (Table 1).

TT increased after 5- and 60-min incubation with LDL_H by 38.5 and 32.4%, respectively, compared with non-oxidized LDL. Thus, the formation of fibrin clot in the plasma was inhibited similarly to in whole blood. However, the effects of oxidized LDL on coagulation parameters differed from those in the whole blood. Since no changes in PTT were observed in experiments with the plasma, it was hypothesized that erythrocytes can play a role in activation of the prothrombin system.

The observed inhibition of clotting can be attested to abnormalities in the clot structure. Incubation of plasma samples with LDL_L for 60 min reduced the maximum amplitude of TEG by 16% compared to incubation with non-oxidized LDL. The decrease in TEG induced by LDL_H was even more profound: 19.1 and 37.7% after 5- and 60-min incubation (Table 1). These findings suggest that oxidized LDL directly affect fibrin and fibrinogen molecules, which disturbs the structural and/or functional properties of fibrin clot.

Incubation with LDL_L or LDL_H for 5 min did not change fibrinolytic activity (Table 1). However, 60-min incubation with LDL_H reduced fibrinolysis by 19.5%. Thus, oxidized LDL modify fibrin molecules. Moreover, prolonged incubation with highly oxidized LDL also inhibits fibrinolysis and promotes hypercoagulation.

The effects of LDL preparations on blood coagulation and fibrinolysis were similar to the effects of oxidized blood plasma observed in our previous experiments [1].

Thus, oxidized LDL activate external pathway of blood coagulation *in vitro*, inhibit fibrin production and fibrinolysis, and modify clot structure. LDL_H produce the most pronounced effect on blood coagulation and fibrinolysis. These results indicate that oxidation of LDL is a potential factor responsible for disturbances in the blood coagulation system during atherosclerosis.

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