BIOPHYSICS AND BIOCHEMISTRY

Effects of Native and Oxidized Low-Density Lipoproteins on Macrophage Chemiluminescence

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 128, No. 11, pp. 514-517, November, 1999
Original article submitted April 27, 1999

Effects of low-density lipoproteins (LDL) obtained from healthy donors and patients with hypercholesterolemia on spontaneous luminol-dependent and zymosan-induced chemiluminescence of rat macrophages were studied. Unlike LDL from healthy donors, native LDL from patients with hypercholesterolemia inhibited spontaneous chemiluminescence of macrophages. Simultaneous incubation with endotheliocytes from the umbilical vein led to the appearance of inhibitory effect of LDL from healthy donors (incubation for 24 h) and potentiated this effect of LDL from patients with hypercholesterolemia (incubation for 6 and 24 h). The inhibitory effect was more pronounced in LDL incubated with umbilical endotheliocytes under ischemic conditions then after aerobic incubations. This corresponded to higher oxidation of LDL confirmed by accumulation of thiobarbituric acid-reactive substances, increased fluorescence, and high electrophoretic mobility in agarose gel. These data suggest that the model system of spontaneous and zymosan-induced chemiluminescence of macrophages can be used for evaluating the degree of oxidation and potential atherogenicity of LDL.

Key Words: *chemiluminescence of macrophages; low-density lipoproteins; ischemia/reperfusion of endotheliocytes; lipid peroxidation*

Blood phagocytes (monocytes and macrophages) internalizing and degrading low-density lipoproteins (LDL) change into foam cells, which initiate the formation of atherosclerotic plaques. Oxidized LDL (oxLDL) are 3-10-fold more rapidly internalized and degraded by macrophages than in native LDL [5-7] and, therefore, these processes can serve as a measure of LDL oxidation [6,13]. LDL oxidized by incubation with endotheliocytes (EC) enhance adhesion of monocytes to EC [9], reduce their mobility induced by chemotactic factors, including serum-activated zymosan (the most potent activator of the respiratory burst detected by chemiluminescence, CL) [9,12], and produce cytotoxic effects on phagocytes [11].

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Coincubation of LDL and EC leads to LDL oxidation, accumulation of lipid peroxidation (LPO) products, and an increase of electrophoretic mobility in agarose gel [1]. Here we studied the effects of native LDL and LDL oxidized by incubation with EC on spontaneous (sCL) and zymosan-induced (zCL) luminol-dependent CL of macrophages.

MATERIALS AND METHODS

Macrophages were obtained from outbred rats decapitated under ether anesthesia by injection of 20 ml Hank's solution into the peritoneal cavity. The peritoneal exudate was collected into Silica-treated tubes and centrifuged at 200*g* for 10 min. The precipitate was washed with Hank's solution, and cells in the suspension were counted using a Goryaev chamber. Lu-

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minol-dependent CL of macrophages was recorded in a KhLM-3 chemiluminometer (Bikap) at 37°C and constant mixing. The sample contained 10^6 macrophages and 0.1 ml luminol (final concentration 10^{-5} M) in 3 ml Hank's solution (pH 7.4). sCL was recorded for 3-5 min before the plateau phase. Opsonized zymosan (0.1 ml, 1.43 mg/ml) was added to control macrophage samples from each rat, and zCL was measured several times throughout the experiment (Fig. 1). RPMI-1640 medium (250 μl) containing LDL was added into cuvettes with test macrophage samples with luminol. The effect of LDL on sCL in macrophages (coefficient of spontaneous activation) was evaluated by the ratio between sCL amplitude in the test and control samples (taken as the unit). Effect of LDL on zCL in macrophages was estimated by comparing coefficients of induced activation (the ratio between zCL and sCL amplitudes) in the test and control samples. LDL (d=1.019-1.065 g/cm³) were isolated from the plasma of healthy donors (LDLn) and patients with hypercholesterolemia (LDLh) by preparative ultracentrifugation, dialyzed, and added to macrophages immediately (native LDL) or after incubation with EC (oxLDL). EC were obtained from fetal human umbilical vein. Confluent (7-8-day incubation) and subconfluent (4-5-day incubation) cultures of EC (passage 2) were incubated with LDLn or LDLh (100 or 200 μg/ml) in RPMI-1640 medium (Sigma) in the absence of other substrates, embryonal serum, and growth factors (substrate-deficient protein-free medium) for 6 or 24 h under aerobic (A, 95% air and 5% CO_2) or ischemic conditions (I, 95% N_2 and 5% CO_2). Methods for isolation and incubation of LDL and EC were described elsewhere [2]. After incubation, the medium containing modified LDLn or LDLh was sampled and added in a dose of 250 μl (25 or 50 μg LDL protein) to the cuvette with macrophages. Native LDLn and LDLh were studied under similar conditions.

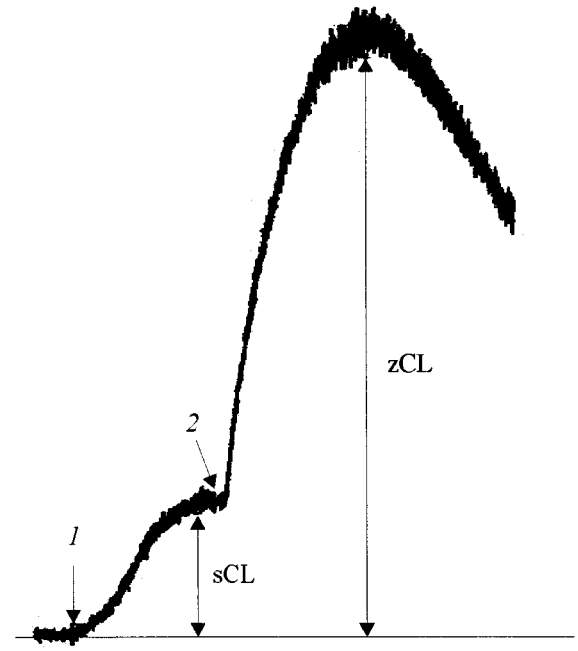

Fig. 1. Luminol-dependent chemiluminescence (CL) in macrophages. sCL: amplitude of spontaneous CL; zCL: amplitude of CL after addition of zymosan. 1) start of recording sCL; 2) addition of zymosan and srart of recording zCL.

TABLE 1. Effects of LDLn and LDLh on sCL in Peritoneal Macrophages (*M±m*)

Time and conditions of LDL incubation with EC	Coefficient of spontaneous activation, rel. units	
	LDLn	LDLh
Without incubation (initial)	0.98±0/07 (12)	0.73±0.02⁺ (14)
Aerobic ischemia		
6 h	0.69±0.28 (2)	0.61±0.18 (4)
	1.05±0.21 (2)	0.52±0.05* (4)
24 h	0.71±0.06* (6)	0.66±0.02* (8)
	0.70±0.05* (6)	0.53±0.02*⁺ (8)

Note. *$p<0.01$ compared with native LDL; ⁺$p<0.01$ compared with LDLn (Student's t test). The number of observations is shown in brackets.

RESULTS

In the control, the suspension of rat peritoneal macrophages in the presence of luminol demonstrated a sCL response, whose intensity was different in various rats and reflected individual functional activity of macrophages.

Native LDLn did not affect the amplitude of sCL in macrophage suspension (in comparison with the control), while native LDLh produced an inhibitory effect confirmed by the mean ratio between sCL amplitudes in test and control samples (Table 1) and the number of samples, where the amplitude of sCL decreased or increased compared with the control (12 and 2, respectively).

LDLn modified by incubation with EC for 6 h under aerobic and ischemic conditions did not change the amplitude of sCL in macrophages, while the incubation of LDLh with EC under ischemic conditions considerably increased their inhibitory influence on sCL. After prolonged 24-h incubation with EC, the inhibitory effect of LDLn on sCL in macrophage suspension appeared, while the effects of LDLh remained unchanged or even potentiated (in some observations) (Table 1, Fig. 2). After 24-h incubation with EC, inhibition of sCL in macrophages by LDLn and LDLh was observed in 26 of 28 observations. Inhibitory

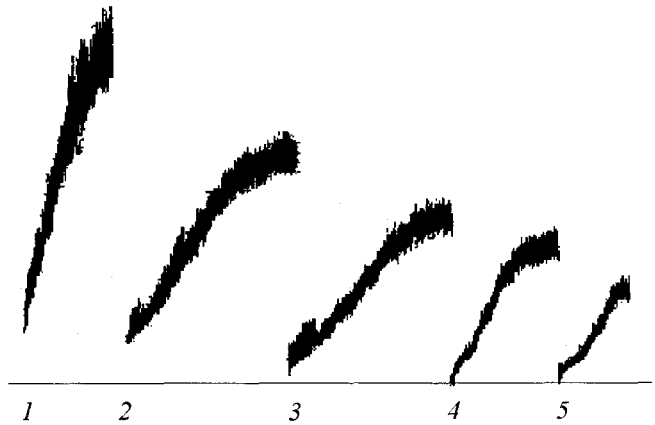

Fig. 2. Effects of native and oxidized LDL from patients with hypercholesterolemia (LDLh) incubated with endothelial cells (EC) under aerobic and ischemic conditions for 24 h: control macrophages (*1*), native LDLh (*2*), LDL incubated with EC under aerobic (*3-5*) or ischemic conditions for 24 h (*4, 5*). LDLh were added into chemiluminometer cell in doses of 25 (*2-4*) and 50 μg protein (*5*).

capacity of LDL was more pronounced after incubation with EC under ischemic than under aerobic conditions. Incubation of LDL with a highly sensitive confluent culture of EC (compared with subconfluent culture) and increasing the dose of LDL from 25 to 50 μg/ml also potentiated their inhibitory effect on sCL in macrophages (Fig. 2). It should be noted that incubation medium initially increased the amplitude of sCL of macrophages, but after 24-h incubation with EC slightly decreased this parameter (data not shown). Therefore, the inhibition of sCL observed in our experiments was due to the effects of the incubation medium and LDL modified after incubation with EC.

In 14 of 66 observations, the effects of native LDL and LDL modified by incubation with EC on zCL of macrophages were studied. Short-term (3-5 min) preincubation of macrophages with LDLn or LDLh (during sCL recording) was accompanied by a decrease in the amplitude of their response to zymosan. The coefficients of induced activation under effects of native and modified (by 24-h incubation with EC) LDLn and LDLh on macrophages were 3.0±0.85 and 2.4±1.36, respectively, compared with 4.2±2.6 in the control.

Differences in the effects of native LDLn and LDLh on sCL in macrophages corresponded to 2-fold differences in plasma cholesterol content (4.0±0.04 and 7.9±1.0 μmol/liter, respectively, $p<0.01$) and higher electrophoretic mobility of LDLh in agarose gel (1.88± 0.04 *vs.* 1.27±0.04 rel. units for LDLn, $p<0.01$), which indicated a greater negative charge of LDLh compared with that of LDLn and characterized oxidative modification of LDL. These differences between LDLn and LDLh are responsible for apoB-protein recognition by scavenger receptors on macrophages and accelerate uptake and degradation of LDLh by macrophages (compared to LDLn) [8,10].

The induced or augmented inhibitory effects of LDLn or LDLh (respectively) on sCL of macrophages after 6- and 24-h incubation with EC correlated with the higher degree of oxidation confirmed by a 6-10-fold increase in the content of thiobarbituric acid-reactive substances [1] and higher electrophoretic mobility in agarose gel. Oxidation of phospholipid components and apoB-protein in LDLn or LDLh was more pronounced after incubation with EC under ischemic than under aerobic conditions [1]. Therefore, the inhibitory effects of LDL on sCL in macrophages were obviously due to oxidation of various LDL components.

The incubation medium inhibited sCL in macrophages due to the presence of biologically active mediators, whose nature requires detailed analyses.

The inhibitory effects of oxLDL on sCL and zCL in peritoneal macrophages and oxLDL-induced inhibition of the basal mobility of macrophages and their response to chemotactic factors (including serum-activated zymosan [9,12]) suggest the interrelation between the mobility and activity of macrophages. Decreased mobility of macrophages (chemotaxis) and inhibition of respiratory burst in these cells are probably associated with their transition to other metabolic pathways and represent an obligate stage before LDL uptake and degradation [9,12]. The interrelation between the cell mobility and CL intensity was found also in blood monocytes. oxLDL stimulate the mobility of monocytes and their adhesion to EC [12] and enhance CL [4].

Initially modified LDL (LDLh) and LDL oxidized by incubation with EC (LDLn and LDLh) inhibited not only sCL, but also zCL in macrophages. The preserved (though weakened) response of macrophages to zymosan indicates maintenance of their functional activity. On the other hand, it was shown that incubation of polymorphonuclear leukocytes with oxidized yolk phospholipid liposomes enhances their response to zymosan [3].

Inhibitory effect of LDL on sCL and zCL in macrophages reflects the degree of LDL modification. Therefore, these models can be used for evaluation of oxidation and potential atherogenicity of LDL.

This study was supported by the Russian Foundation for Basic Research (grant No. 97-04-48472).

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