Effect of High-Density Lipoproteins on ADP-Induced Platelet Aggregation in Plasma

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 126, No. 8, pp. 160-163, August, 1998
Original article submitted March 15, 1997

Autooxidized high-density lipoproteins (HDL_2) inhibit ADP-induced platelet aggregation in platelet-rich plasma. Platelet aggregation in the presence of native HDL_2 and HDL_3 and autooxidized HDL_3 does not differ from the control (plasma with buffer). A conclusion is made on the important role of autooxidized HDL_2 as a thrombogenesis-inhibiting factor in atherosclerosis.

Key Words: *platelets; platelet-rich plasma; aggregation; high-density lipoproteins; oxidation*

Modulation of blood cell functions by serum lipoproteins plays an important role in the pathogenesis of atherosclerosis. Special attention is focused on modified, in particular, oxidized lipoproteins, since the principal role of oxidative processes in atherogenesis is beyond doubt [12]. The effect of oxidized low-density lipoproteins on the functional properties of blood cells, in particular, platelets, has been extensively studied [3,11,12]. However, little is known on the effect of oxidized high-density lipoproteins (oxHDL) on platelets [3]; experiments with native HDL yielded contradictory results. The majority of investigators concluded that HDL_3 activate, while HDL_2 inhibit cell aggregation induced by various agents [5,14]. It was shown that the total oxHDL fraction in concentrations below 2 mg/ml activates and in higher concentrations inhibits ADP-induced aggregation of isolated platelets [3]. HDL_3 activate ADP-induced platelet aggregation only in concentrations of 2-4 mg/ml, while the inhibiting effect of HDL_2 was noted at physiological concentrations [5]. It was shown that the total HDL fraction (0.2-2.1 mg/ml) inhibits thrombin-induced aggregation of isolated platelets [2].

These discrepancies probably result not only from different proportions between HDL_2 and HDL_3 in the total HDL fractions [5,14], but also from different degree of peroxidation. Therefore, it seems important to study the effect of native and oxidized HAD subfractions on platelets. It should be noted that the platelet-modulating effect of HDL has been usually studied on washed platelets, although better approximation to *in vivo* conditions can be achieved with platelet-rich plasma. The aim of the present study was to compare the effects of native and oxidized HDL_2 and HDL_3 on ADP-induced platelet aggregation in a platelet-rich plasma (PRP).

MATERIALS AND METHODS

HDL were isolated from donor serum by preparative ultracentrifugation [10] (native HDL were isolated in the presence of 0.01% EDTA) and stored at 4°C for no more than 2 days. Protein content was measured by the biuret microassay (±0.3 mg/ml) [9] in a Beckman spectrophotometer. All reagents were from Sigma.

HDL were dialyzed for 24 h against 100- and 1000-fold volume of 10 mM Tris-HCl (pH 7.4) containing 140 mM NaCl and 5 mM KCl. Autooxidation was effected by incubating HDL in aerobic conditions at 37°C for 10-24 h. The degree of oxidation was assessed by the content of 2-thiobarbituric acid-reactive substances and expressed in nmol malonic dialdehyde (MDA)/mg HDL protein

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0007-4888/98/0008-0783$20.00

(±20%) [14]. The concentration of MDA in native HAD did not exceed 0.3 nmol/mg protein. The concentration of HDL and the degree of oxidation were measured immediately before experiment.

Platelet-rich plasma was prepared from donor blood diluted 1:10 with sodium citrate (130 nM, pH 7.4) by centrifugation at 1000*g* for 15 min and contained 2-4×10^8 platelets/ml. The incubation mixture contained HDL and PRP in a ratio of 1:1, yielding a 2-fold excess of added HDL over donor HDL in PRP [5]. In control samples HDL were replaced by the same volume of the dialysis buffer. Concentrated Tyrode buffer without NaCl and KCl containing (in mM): 200 HEPES, 20 MCl_2, 7.6 $NaH_2PO_4 \times 2H_2O$, and 200 glucose (pH 7.4) was added 20:1 to HDL preparation before incubation. Each experiment was carried out with HDL and PRP isolated from different donors. Three incubation mixtures were processed in parallel in each experiment at 37°C: control, PRP+native HDL, and PRP+oxHDL. The number of mixtures was restricted by the duration of measurements (5 min for each sample). To measure ADP-induced platelet aggregation, aliquots of incubation mixtures were withdrawn at equal time intervals starting from their preparation and transferred to measuring cuvettes. The data obtained with the control mixture and the fact that principal results were obtained for $oxHDL_2$ suggest that platelet aggregation in PRP is modulated by added HDL. We assumed that due to plasma antioxidant activity and low degree of oxidation oxHDL have no effect on oxidative processes in platelets.

Aggregation was recorded in a Biola aggregometer (Moscow) as an increment of light transmission in PRP after addition of 5 μM ADP [6]. The measurements were performed at 37°C with constant stirring (800 rpm) in a volume of 300 μl (3 measurements for each sample). The maximum aggregation rate was determined and used as the aggregation parameter. This parameter is more accurate than the amplitude of the light transmission curve (measurement accuracy for amplitude and aggregation rate is ±25% and ±12%, respectively).

All parameters are presented as the mean square deviation. The data are processed statistically using the Student *t* test.

RESULTS

Figure 1 shows ADP-induced aggregation curves for platelets incubated in PRP in the presence of dialysis buffer and native and $oxHDL_2$. During a 1.5-h incubation, buffer and HDL_2 added to PRP slightly reduced platelet aggregability, while in $oxHDL_2$-containing samples ADP-induced platelet aggregation decreased 5-fold in amplitude and became reversible. Figure 2, *a* shows aggregation rate in

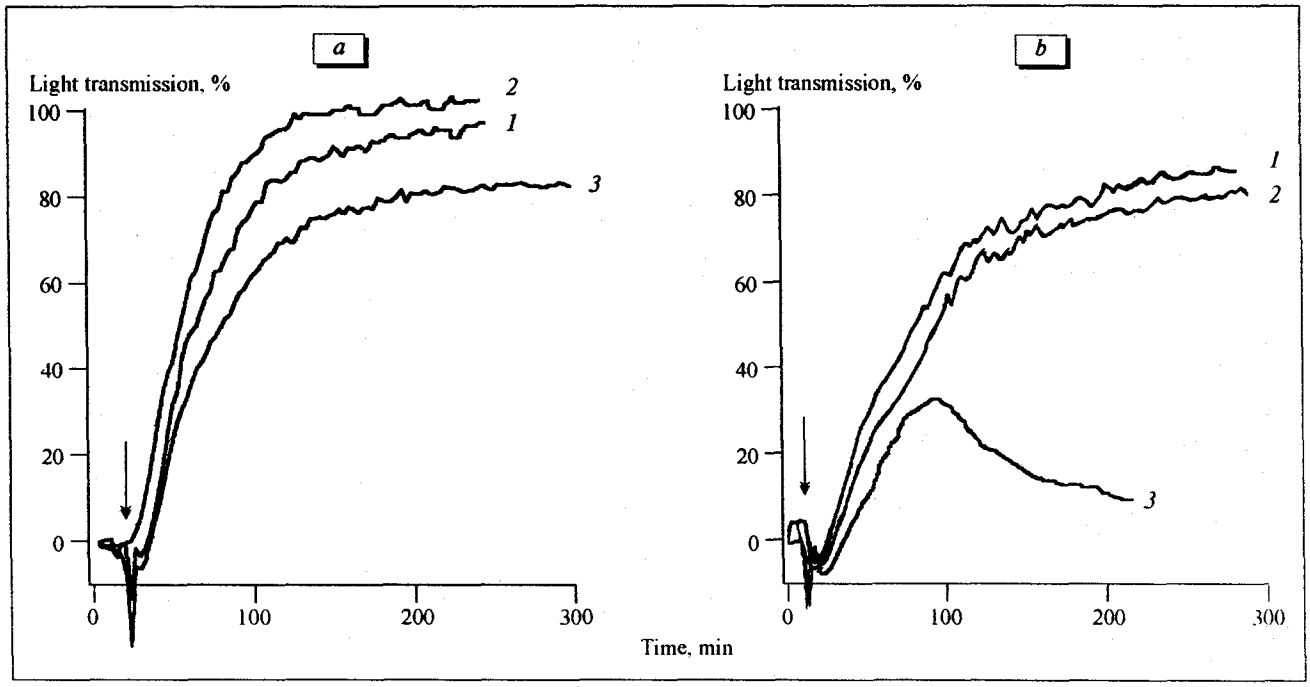

Fig. 1. Time dependence curve of light transmission of platelet-rich plasma after addition of ADP. Plasma was incubated in the presence of buffer (control) or high-density lipoproteins (HDL_2) with varying degree of oxidation for 0 and 1.5 h. *1*) 0 (control), 0.3 (2) and 3.5 nmol MDA/mg protein HDL_2 (3). Arrow indicates the addition of 5 μM ADP.

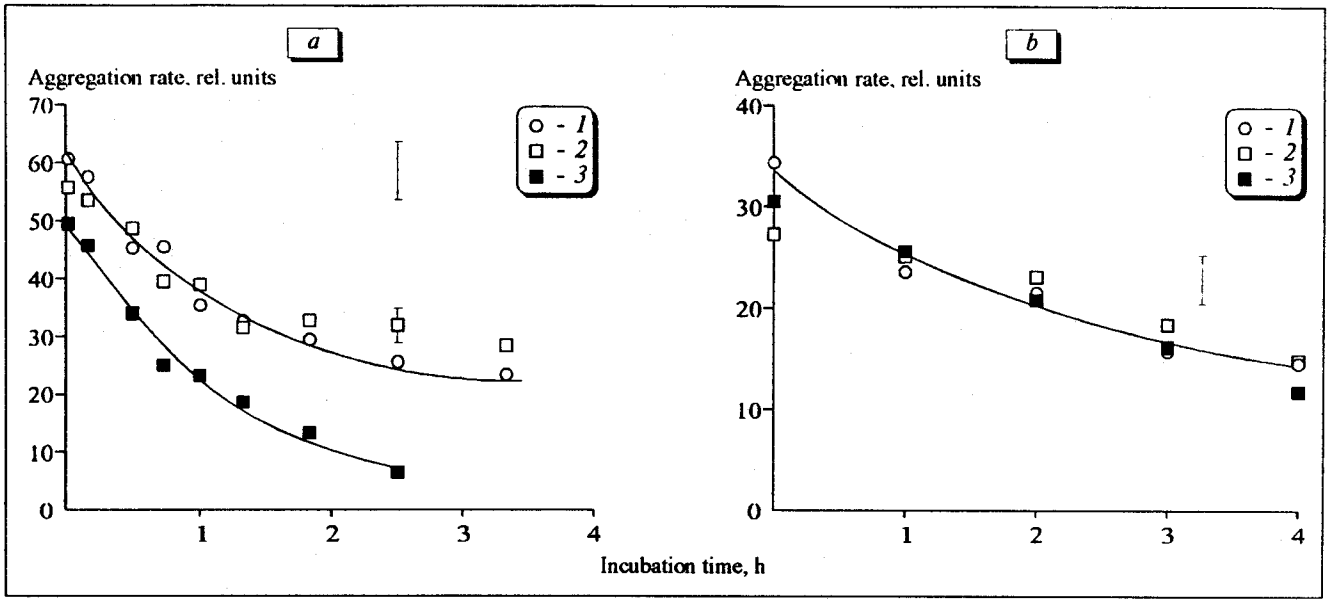

Fig. 2. Platelet aggregation rate in platelet-rich plasma incubated with buffer or high-density lipoproteins (HDL) as a function of incubation time. *a*) HDL_2 (in mixture): 1.5 mg protein/ml (*1*), 0.15 (*2*) and 4.4 nmol MDA/mg protein (*3*). *b*) HDL_3 (in mixture): 1.4 mg protein/ml (*1*), 0.1 (*2*) and 5.6 nmol MDA/mg protein (*3*).

PRP in the presence of HDL_2 as a function of incubation time. The inhibiting effect of $oxHDL_2$ on ADP-aggregation became evident 1 h after addition and increased during subsequent incubation. The incubation usually lasted no more than 4 h, since cell aggregation in all mixtures markedly decreased during this time period. Similar results were obtained in 3 independent experiments. The effect did not depend on the concentration of added HDL_2 (0.5, 0.7, and 1.5 mg/ml, degree of oxidation 1.3, 4.4, and 4.5 nmol MDA/mg protein HDL_2).

In three independent experiments (1.2, 2.7, and 1.4 mg HDL_3/ml, degree of oxidation 2.2, 0.8, and 5.6 nmol MDA/mg protein HDL_2) $oxHDL_3$ had no effect on ADP-induced platelet aggregation within the 4-h incubation (Fig. 2, *b*). It should be noted that addition of HDL to PRP caused a minor transient decrease in cell aggregability in comparison with control samples (Fig. 2): 1% significance level for native and $oxHDL_3$ and 5% for $oxHDL_2$, the decrease caused by native HDL_2 was insignificant. Similar effect was reported for low-density lipoproteins; it did not depend on the degree of lipoprotein oxidation [1]. The aggregation rate in the presence of low-density lipoproteins decreased by 15-20% in comparison with the control, while for HDL_3 this decrease did not exceed 12±7%. We have previously concluded that changes in cell membrane caused by low-density lipoproteins inhibit ADP-induced aggregation [1]. In light of this conclusion and the data of Curtiss *et al.* [4], the effect of HDL on platelet aggregation can be explained as follows: HDL interact with low-density lipoprotein binding sites on platelets, thus modulating the parameters of ADP-induced platelet aggregation. This competition between HDL and low-density lipoproteins for the common binding sites on platelets is responsible for the inhibiting effect of HDL on platelet aggregation induced by oxidized low-density lipoproteins [3,11].

Thus, our experiments showed that:

1. Native HDL_2 and HDL_3 and $oxHDL_3$ have no effect of ADP-induced platelet aggregation;
2. Oxidized HDL_2 inhibit ADP-induced platelet aggregation.

It was previously shown that HDL_2 inhibit ADP-induced aggregation of isolated platelets [5]. It was proved that this effect is determined by apolipoprotein E-rich HDL_2 fraction. It can be hypothesized that oxidation-induced structural changes in lipoprotein particles [7] promote their binding to cells. The principal result in our experiments was obtained with HDL_2; hence, it does not contradict to published data [13], where total HDL fraction had no effect on ADP-induced platelet aggregation in PRP.

Since mild oxidation of HDL particles occurs *in vivo*, modulation of ADP-induced cell aggregation with $oxHDL_2$ can be directly involved into pathological processes [3]. The inhibiting effect of oxidized HDL_2 on platelet aggregation contributes to the antiatherosclerotic effect of HDL.

The study was supported by the Russian Foundation for Basic Research (grant No. 96-04-49449).

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