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EFFECT OF HIGH-DENSITY LIPOPROTEINS ON THROMBIN-INDUCED PLATELET AGGREGATION

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Epidemiologic studies have revealed negative correlation between the plasma high-density lipoprotein (HDL) concentration and clinical manifestations of angina [5]. Since thrombosis is a significant factor in the development of coronary arterial occlusion, it has been suggested that platelets, like vascular endothelial cells, are a target for the action of various classes of lipoproteins, including HDL, possessing antiatherosclerotic properties [6]. Data in the literature on the effect of HDL on platelet aggregation are rather contradictory [2, 4, 6, 9].

In the investigation described below a recently suggested method of recording changes in the radius of aggregates [1] was used to evaluate the effect of HDL on thrombin-induced aggregation of washed human platelets.

EXPERIMENTAL METHOD

HDL were isolated from healthy human blood by successive ultracentrifugation [7]. The homogeneity and purity of the HDL were verified by analytical centrifugation and PAG electrophoresis [10, 11]. The HDL concentration was determined as the protein content, by Lowry's method [8], using bovine serum albumin as the standard. Platelets were obtained from healthy human blood. The determinations were made at room temperature. A sample of 30 ml blood was taken from the cubital vein into a test tube with 6 ml of anticoagulant (2.5 g bihydrated sodium citrate, 1.4 g monohydrated citrate, 2 g dextrose to 100 ml water), indomethacine was added to a concentration of 5 μg/ml, and the sample was centrifuged for 20 min at 200*g*. Next, 5 ml of platelet-enriched plasma was transferred into another tube and the cells were sedimented by centrifugation for 10 min at 1200*g*. The residue was suspended in 5 ml of medium containing 150 mM NaCl, 2.7 mM KCl, 0.37 mM NaH_2PO_4, 1 mM $MgCl_2$, 5 mM glucose, 10 mM HEPES-NaOH, pH 7.4, 50 U/ml heparin, 0.35% bovine serum albumin, and 0.15 mg/ml apyrase (buffer A). Platelets were incubated at 37°C for 30 min and then recentrifuged for 10 min at 1100*g*. The residue was suspended in 5 ml medium of the same composition, except heparin, but containing also 2 mM $CaCl_2$ (buffer B). To study the effect of HDL, the platelets were diluted to 0.25% concentration by volume in buffer B, and immediately before measurement of aggregation, they were incubated with continuous mixing (800 rpm) for 5 min at 37°C in the absence or in the presence of various concentrations of HDL. Platelet aggregation was recorded as the change in dispersion of fluctuations of transmittance [1] of light on an aggregation analyzer ("Gradient," USSR) in PAP-4 microcuvettes ("Bio-Data Corp.," USA), in a volume of 400 μl at 37°C, with a spinning speed of the magnetic mixer of 800 rpm. The results shown were obtained on platelets from three or more donors.

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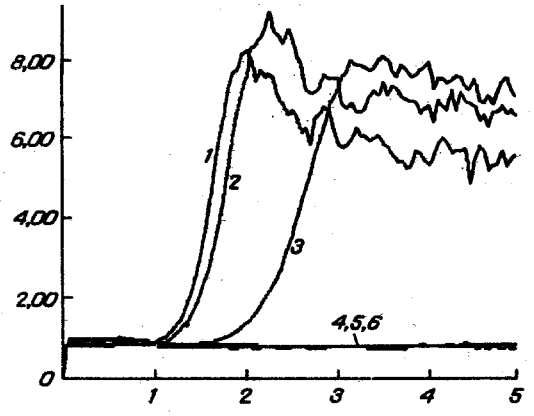

Fig. 1. Changes in radius of aggregates in platelet suspension preincubated with HDL, under the influence of 0.075 U thrombin/ml. Abscissa, time (in min); ordinate, relative dispersion of fluctuations of transmittance (in relative units). Arrow indicates time of addition of 10 μl thrombin solution to 400 μl of platelet suspension. Platelets preincubated: 1) in absence, 2) in presence of 65 μg/ml, 3) 130 μg/ml, 9) 190 μg/ml, 5) 260 μg/ml, and 6) 2100 μg/ml HDL.

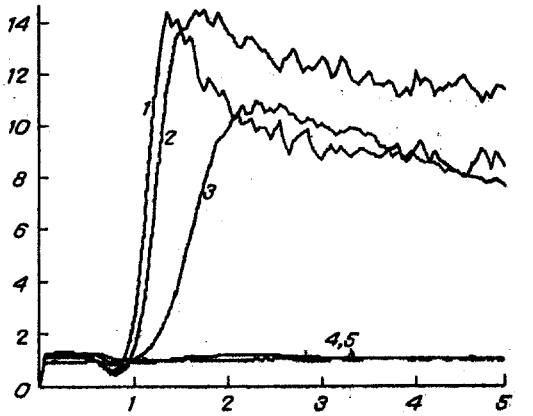

Fig. 2. Changes in radius of aggregates in platelet suspension preincubated with HDL, under the influence of 0.225 U thrombin/ml. Platelets preincubated: 1) in the absence, 2) in presence of 420 μg/ml, 3) 1050 μg/ml, 4) 1320 μg/ml, and 5) 2100 μg/ml HDL. Remainder of legend as to Fig. 1.

EXPERIMENTAL RESULTS

Figure 1 gives data on the effect of preincubation of the washed cells for 5 min with HDL on thrombin (0.075 U/ml)-induced platelet aggregation. Clearly HDL in a concentration of 65 μg/ml had virtually no effect on the rate of development of aggregation, whereas in a concentration of 130 μg/ml it considerably shifted the aggregation versus time curve to the right, thereby altering the gradient of the curve. Complete inhibition of platelet aggregation, induced by 0.075 U/ml thrombin, was observed within the range of concentrations of HDL from 190 to 2100 μg/ml.

A threefold increase in concentration of the inducer changed the quantitative characteristics of inhibition of platelet aggregation by HDL. A typical trace of changes in the radius of aggregates obtained by investigation of the action of HDL on platelet aggregation induced by 0.225 U thrombin/ml is illustrated in Fig. 2. Under these conditions HDL in a concentration of 420 μg/ml had virtually no effect on the temporal parameters of development of thrombin-induced aggregation, and only if HDL was present in a concentration of 1050 μg/ml was this process delayed, and within the concentration range of 1320-2100 μg/ml aggregation was completely inhibited.

HDL itself, incidentally, did not change the mean radius of the aggregates in the platelet suspension tested, but as will be clear from Figs. 1 and 2, they have a marked antiaggregation action.

Preliminary experiments showed that HDL in a concentration up to 2100 μg/ml caused no changes in the radius of aggregates in platelet-enriched plasma, and likewise did not affect ADP- or thrombin-induced platelet aggregation after different periods of preincubation (from 5 to 45 min; data not given). Thus the use of the procedure of double reprecipitation of platelets with intermediate incubation for 30 min was carried out in order to remove contaminating lipoproteins, which are present in comparatively high concentrations in plasma (1-2 mg/ml) [3].

A similar conclusion regarding the need to remove contaminating lipoproteins from platelets in order to discover the inhibitory action of HDL was reached in a study using a platelet suspension obtained by gel-filtration [4]. The authors cited also showed that after preincubation of gel-filtered platelets for 1 h with 1000 μg g/ml of HDL, aggregation was reduced by 53%, and the time of development of the process, induced by 0.5 U thrombin/ml, was delayed. Under our own conditions, incubation of a suspension of washed platelets for 5 min with 130 μg HDL/ml was sufficient to exhibit their inhibitory effects on platelet aggregation induced by 0.075 U thrombin/ml. As might be expected, an increase in the dose of the inducer made inhibition of platelet aggregation by HDL less effective. Since whole blood, besides HDL and other endogenous inhibitors of platelet aggregation, contains components with proaggregative activity and, in particular, low-density lipoproteins [4, 6], it is perfectly logical to suggest that a disturbance of the balance between antiaggregants and proaggregants may lie at the basis of changes in the aggregating activity of platelets in hereditary or acquired disturbances of lipid metabolism.

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