BIOPHYSICS AND BIOCHEMISTRY

Effect of Oxidized LDL on Hemolytic Resistance of Erythrocyte

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 8, pp. 160-162, August, 2002
Original article submitted March 15, 2002

Using the method of peroxide-induced chemiluminescence we showed that incubation of the whole blood with oxidized LDL or oxidized blood plasma increased plasma hemoglobin concentration, which linearly depended on the degree of LDL oxidation. Similar effects were observed in erythrocyte suspension. Hemolytic activity of oxidized plasma 3-4-fold surpassed that of LDL isolated by ultracentrifugation. LDL capacity to oxidation in the presence of Cu^{2+} increased by 50% and osmotic hemolysis of erythrocytes increased by 53% in coronary patients in comparison with healthy donors. These results indicate that oxidized LDL induce erythrocyte hemolysis.

Key Words: *hemolysis; erythrocytes; chemiluminescence*

It is now acknowledged that oxidized LDL play an important role in atherosclerotic injury to blood vessel walls. However under these conditions not only vascular walls, but also blood cells are affected. The mechanism of this damage is not quite clear. At the same time, free-radical processes impair the structure and function blood cell [1,2]. However, it remains unclear whether oxidized LDL can damage erythrocyte membranes.

We therefore tried to elucidate whether oxidized LDL can impair the structure of erythrocyte membranes.

MATERIALS AND METHODS

Blood (10 ml) was collected from the ulnar vein into tubes with 3.8% sodium citrate (9:1 blood-sodium citrate ratio). LDL or blood plasma (BP) were oxidized in the presence of Cu^{2+}. The degree of oxidation was evaluated by the content of malonic dialdehyde (MDA) in the reaction with thiobarbituric acid [6]. LDL were isolated by ultracentrifugation [3,5].

The degree of erythrocyte hemolysis was evaluated by changes in hemoglobin concentration in the extracellular medium [4]. H_2O_2 (3%, 0.5 ml) was added to BP (0.5 ml) and the maximum flash of peroxide-induced chemiluminescence (PCL) was recorded. In some cases hemoglobin concentrations were measured by colorimetry using Hemoglobin-Novo kit, in parallel with PCL. The degree of erythrocyte osmotic hemolysis was evaluated by turbidimetry.

RESULTS

Oxidized LDL increased the intensity of plasma PCL after 1-hour incubation with the whole blood at 37°C compared to nonoxidized LDL (Fig. 1). Plasma PCL is determined by the reaction of H_2O_2 with hemoglobin. This suggests that oxidized LDL promote hemolysis of blood erythrocytes. Then we studied the relationship between the intensity of plasma PCL and degree of LDL oxidation. The results indicate that the higher the degree of LDL oxidation, the lower the

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0007-4888/02/1342-0137$27.00

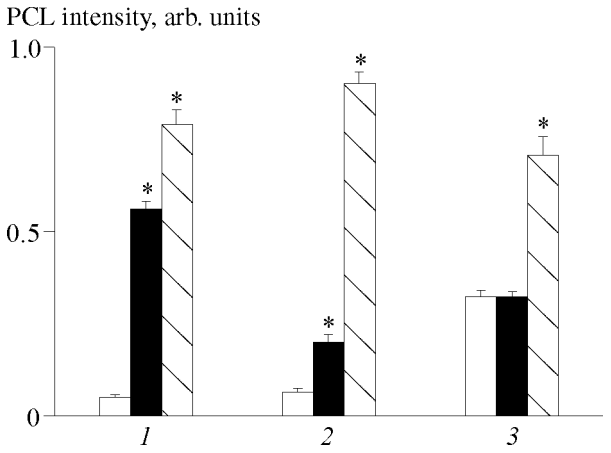

Fig. 1. Intensity of peroxide-induced chemiluminescence (PCL) of blood plasma (*1*, *2*) and erythrocyte suspension supernatant (*3*). Light bars: control (incubation with 0.85% NaCl); dark bars: incubation with nonoxidized LDL (*1*) or nonoxidized plasma (*2*, *3*); cross-hatched bars: incubation with oxidized LDL (*1*) or oxidized plasma (*2*, *3*). *$p<0.05$ compared to the control.

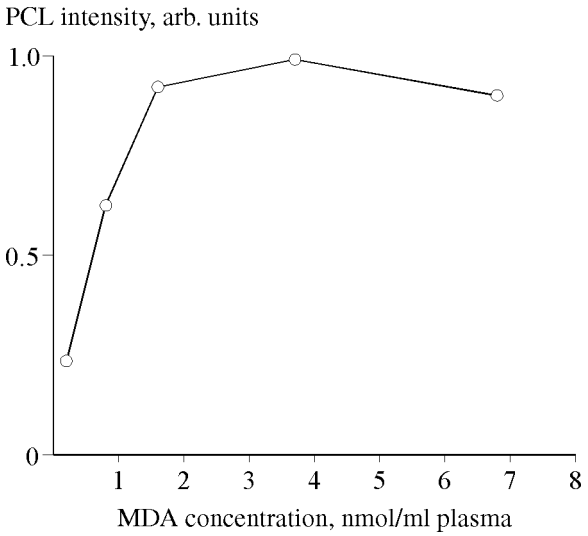

Fig. 2. Relationship between the intensity of plasma PCL and degree of oxidation of LDL.

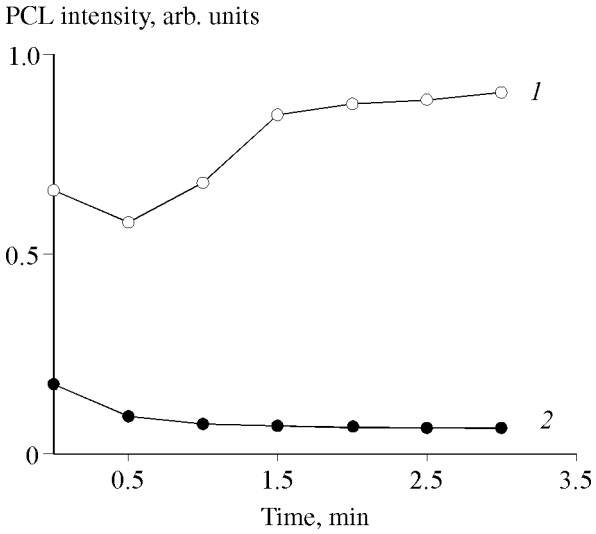

Fig. 3. Typical PCL curves for erythrocyte supernatant with oxidized (*1*, hemoglobin 15.43 g/liter) and nonoxidized (*2*, hemoglobin 0 g/liter) plasma.

hemolytic resistance of erythrocytes incubated with these LDL. The amplitude of plasma PCL increased with increasing the degree of LDL oxidation (Fig. 2).

The incubation of the whole blood with oxidized blood plasma markedly increased the intensity of PCL and hence, erythrocyte hemolysis (Fig. 1). Oxidized LDL caused less intensive hemolysis than oxidized plasma. This indicates that not only PCL, but also other components of oxidized plasma possess hemolytic activity. The next question is whether PCL generation results from the direct effect of oxidized LDL and oxidized plasma on erythrocytes or it is mediated by other blood cells. In our experiments the effect of oxidized plasma on suspension of washed erythrocytes was similar to the effect of oxidized plasma on the whole blood (Fig. 1). Moreover, PCL of erythrocyte supernatant containing hemoglobin was characterized by a higher amplitude compared to hemoglobin-free supernatant (Fig. 3). This suggests that PCL intensity depends on the content of hemoglobin.

These data suggest that oxidized LDL can damage erythrocytes and reduce their resistance to hemolysis. The next step was to elucidate whether hemolytic resistance of erythrocytes was reduced in atherosclerosis patients, specifically in coronary patients with increased content of oxidized LDL in the blood. Sixty-seven coronary patients and 30 donors were examined. LDL capacity to oxidation in the presence of Cu^{2+} and the degree of osmotic hemolysis of erythrocytes were evaluated. Both parameters increased in patients (by 50 and 53%, respectively). These data indirectly confirm our hypothesis on hemolytic activity of oxidized LDL.

Oxidized LDL induce erythrocyte hemolysis. In coronary patients oxidation of blood plasma inversely correlated with osmotic resistance of erythrocytes.

The study was supported by the Russian Foundation for Basic Research (grant No. 99.04-48255).

REFERENCES

1. Yu. A. Vladimirov, O. A. Azizova, A. I. Deev, *et al.*, *Itogi Nauki Tekhn.*, **29**, Moscow (1991).
2. A. N. Klimov and N. G. Nikul'cheva, *Lipid and Lipoprotein Metabolism and Its Disorders* [in Russian], St. Petersburg (1999).
3. A. N. Klimov and N. G. Nikul'cheva, *Dyslipoproteinemias and Coronary Disease* [in Russian], Moscow (1980).
4. A. V. Kozlov, A. N. Osipov, and Yu. A. Vladimirov, *Biofizika*, **35**, No. 2, 347-349 (1990).
5. A. I. Shcherbakova, N. V. Petrova, I. M. Karmanskii, *et al.*, *Vopr. Med. Khim.*, No. 1, 102-108 (1981).
6. R. C. McKnight and F. E. Hunter, *J. Biol. Chem.*, **241**, 2757-2762 (1966).
7. J. R. O'Braen, *Nature*, **207**, 306-307 (1965).