Chemiluminescence Assay of the Antioxidant State in Patients with Atherosclerosis

O. A. Azizova, A. P. Piryazev, M. P. Sherstnev, S. V. Drinitsina, and Yu. M. Lopukhin

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We evaluated antioxidant state of 62 patients with coronary heart disease and 47 patients with obliterating atherosclerosis of lower extremities by peroxide-depend chemiluminescence and inhibition of azo-initiated chemiluminescence and hydrogen peroxide-hemoglobin-luminol chemiluminescence system. The flash amplitude of peroxide-dependent chemiluminescence in the plasma from patients was 32% below the control. Antioxidant activity of the plasma from patients was higher than in healthy individuals by 33 and 27% depending on the type of free radical-generating systems. The increase in antioxidant activity was most pronounced in patients with combined pathology: coronary heart disease complicated by obliterating atherosclerosis. These results explain the decrease in peroxide-dependent chemiluminescence of the plasma and whole blood in patients with atherosclerosis compared to that in healthy individuals.

Key Words: *atherosclerosis; chemiluminescence; antioxidant activity; hydrogen peroxide*

Oxidized low-density lipoproteins (LDL) play a key role in the pathogenesis and clinical manifestations of atherosclerosis. Oxidized LDL are responsible for impaired endothelial permeability, appearance of foam cells, migration and proliferation of smooth muscle cells, and formation of fibrous plaques. In light of this, much attention was focused on the resistance of LDL particles to oxidation during the development of atherosclerosis. Previous studies showed that oxidation resistance of LDL decreases in patients with atherosclerosis [1,5]. The degree of LDL oxidation depends on the antioxidant state of tissues and blood. Antioxidant activity (AOA) of the plasma is determined by the content of water-soluble low-molecular-weight compounds (uric and ascorbic acids), sulfur-containing proteins, and lipid-soluble substances (α-tocopherol, bilirubin, and carotenoids).

Chemiluminescence methods for measurements of plasma AOA were recently elaborated. These methods are based on quenching of luminol-dependent chemiluminescence (LDCL) in a free radical-generating system after addition of plasma. Little is known about changes in plasma AOA during atherosclerosis. The method of peroxide-dependent chemiluminescence (PCL) widely used for studying antioxidant state of the plasma or whole blood does not allow us to differentiate free radical generation from AOA of test objects. Here we studied plasma AOA in patients with atherosclerosis by various chemiluminescence methods.

MATERIALS AND METHODS

We examined 62 patients with coronary heart disease (CHD), 47 patients with obliterating atherosclerosis of lower extremities, and 8 healthy donors.

The blood (20 μl) was dissolved in 4 ml isotonic NaCl. Diluted blood (0.5 ml) was placed in a cuvette, and the volume was adjusted to 4 ml with phosphate buffer (100 mM KCl and 34 mM KH_2PO_4, pH 7.4). Erythrocytes were counted in a Goryaev chamber. The flash amplitude of PCL was measured. Erythrocyte

Institute of Physicochemical Medicine, Russian Ministry of Health, Moscow

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TABLE 1. AOA in Patients with Atherosclerosis and CHD (*M*±*m*)

Parameter	Healthy donors	Patients			
		total	Class II CHD	Class III CHD	CHD+atherosclerosis of lower extremities
PCL intensity, 10^6 pulses/sec					
plasma	10±1.9	6.8±1.2*	9.5±1.1^{+o}	6.3±1.0	4.9±0.9
whole blood	16.3±3.2	9±2.2*	17.3±3.4^{+o}	10.0±2.5	7±2.1
AOA_1, arb. units	24±1.6	32±2.8*	26±1.7^{+}	29±2.7^{+}	37±4.8
AOA_2, mM ascorbic acid activity	8.4±1.2	10.7±0.9*	9.3±1.3	11.2±2.4	10.4±0.7

Note. $p<0.05$: *compared to healthy donors; $^{+}$compared to patients with CHD and atherosclerosis of lower extremities; and ^{o}compared to patients with class III CHD.

suspension in the cuvette contained 10^6 cells. PCL flash was recorded after addition of 2 ml 9 mM H_2O_2.

For isolation of the plasma the blood was centrifuged at 3000 rpm for 10 min. The plasma (1 ml) and 3 ml buffer solution (25 mM KH_2PO_4, pH 7.4) were placed in a stirred cuvette and chemiluminescence was induced with 6 ml 0.88 M H_2O_2. Plasma AOA was measured by 2 independent methods: inhibition of LDCL in the presence of azo-initiator 2,2'-azo-bis(2-methyl-propiomidine) dihydrochloride (AOA_1) and inhibition of LDCL in the system containing H_2O_2 and hemoglobin (AOA_2). These methods allow us to estimate AOA of water-soluble compounds in the plasma [2,4].

RESULTS

The flash amplitude of PCL in the plasma and whole blood from patients was 32 and 42% lower than in healthy donors, respectively. AOA_1 in patients was 33% higher than in healthy donors (Table 1). AOA_2 also surpassed the control (Table 1). Thus, AOA of the water-soluble plasma fraction increases in patients with atherosclerosis.

AOA_1 increased with disease progression, while AOA_2 remained unchanged (Table 1). The most pronounced increase in plasma AOA was found in patients with coronary heart disease complicated by obliterating atherosclerosis of lower extremities.

The question arises: what mechanisms underlay this paradoxical increase in plasma AOA observed in our and previous experiments [3,6]? It is known that plasma AOA depends on the presence of low-molecular-weight compounds, including uric and ascorbic acids, carotenoids, and tocopherol [7]. Previous studies showed that the content of vitamin C, tocopherol, and carotenoids decreased during cardiovascular diseases. Taking into account that plasma AOA primarily depends on the presence of uric acid (80%), accumulation of uric acid in the plasma in patients with atherosclerosis can compensate the decrease in the content of other low-molecular-weight antioxidants. It was reported that plasma concentration of uric acid increases during atherosclerosis.

The increased plasma AOA explains the decrease in plasma PCL in patients with atherosclerosis compared to that in healthy individuals, and the dependence of this decrease on the severity of atherosclerotic process.

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