Peculiarities of Oxidative Stress in the Plasma of Patients with Familial Hypercholesterolemia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 2, pp. 213-216, February, 1998
Original article submitted December 31, 1996

Plasma level of lipid-standardized vitamin E is decreased, while the concentration of inflammatory transmitters C3a(desArg) and C5a(desArg) is elevated in heterozygous familial hypercholesterolemia caused by apoB,E-receptor deficiency. Leukocytes isolated from these patients exhibit enhanced spontaneous aggregation of superoxide anion generation and are resistant to activation with exogenous platelet activation factor (10^{-6} M).

Key Words: *familial hypercholesterolemia; oxidative stress; vitamin E; anaphylatoxins; platelet activation factor*

Familial hypercholesterolemia (FH) caused by defective functioning of apoB,E-receptor manifests itself in elevated concentration of low-density lipoproteins (LDL) and early atherosclerosis development. This pathology is characterized by a high cholesterol content in atherogenic apolipoprotein B (apoB)-containing particles and increased concentration and prolonged circulation of these particles in the blood. It is now accepted that contact of endothelial cells with cholesterol-enriched native LDL rapidly reduces fluidity of their membranes, stimulates endocytosis, induces formation of stress filaments, and modulates arachidonic acid metabolism and NO-synthase activity, i.e., leads to formation of oxidative stress in endotheliocytes [12]. Activated endothelial cells produce a number of inflammatory transmitters, for instance, platelet activation factor (PAF); PAF is a biological stimulator not only of platelets but also of monocytes and leukocytes. On the other hand, high plasma LDL cholesterol induces accumulation of cholesterol-free liposome-like particles and cholesterol crystals in the subendothelial space leading to complement activation through, first, the classical and than alternative pathways [7]. Generation of C3a and C5a anaphylatoxins leads to the formation of inflammatory foci in the subendothelial space due to broad biological activity of these substances, in particular, chemotactic activity of C5a for mononuclear phagocytes and neutrophils, and their ability to stimulate neutrophil adhesiveness and oxidative metabolism in leukocytes.

Oxidation of lipid and protein components in apoB-containing lipoprotein particles depends on the intensity of oxidative stress generated by endotheliocytes, resident phagocytes, and blood cells, and on the resistance of these particles to oxidative stress which is determined by hydrophobic antioxidants. The present study was undertaken to evaluate the state of blood leukocytes from FH patients and their ability to respond to exogenous stimuli, and to assess plasma level of anaphylatoxins as inflammatory markers. It was interesting to measure plasma level of vitamin E, since this parameter reflects the resistance of lipoprotein particles to oxidative stress.

MATERIALS AND METHODS

The study comprised 16 healthy donors and 16 patients with FH caused by apoB,E receptor deficiency.

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0007-4888/98/0002-0187$20.00

TABLE 1. Plasma Content of Lipids (mM) and Vitamin E (μM)in Healthy Donors and Patients with FH (*M±m*)

Parameter	Control (*n*=16)	FH (*n*=16)
Age (years)	40.18±2.0	41.7±2.15
Total cholesterol, mM	4.82±0.46	10.20±1.61*
LDL cholesterol	3.07±0.36	8.46±0.99*
HDL cholesterol	1.19±0.27	0.92±0.19
Triglycerides, μM	1.24±0.19	1.81±0.28*
Vitamin E, mM	23.14±2.81	43.89±4.75*
Vitamin E/total cholesterol×10^{-3}	4.80	4.32
Vitamin E/(total cholesterol+ triglycerides)×10^{-3}	3.82	3.65

Note. Here and in Table 2: *p<0.001 compared with the control.

Blood was drawn from the ulnary vein after a 12-h fasting. Total and LDL cholesterol and triglycerides in blood serum were measured on a biochemical analyzer (KONE). The content of vitamin E was determined in an organic extract from Na_2EDTA-stabilized plasma (1 mg/ml) as described previously [13]. Total concentrations of anaphylatoxins and their desArg-derivatives C3a(desArg) and C5a(desArg) were measured using Amersham radioimmunoassay kits. Blood plasma containing 1 mg/ml Na_2EDTA was collected after centrifugation at 2500 rpm and 4°C for 30 min, immediately frozen at -70°C, and stored no longer than 30 days.

For isolation of cells, sodium citrate (2%) and glucose were added to the blood. Leukocytes were isolated by centrifugation in a Ficoll gradient after preliminary erythrocyte precipitation with dextran T-500 [10]. Generation of superoxide radicals by leukocytes was assessed spectrophotometrically by measuring absorbance of reduced cytochrome *c* at 550 nm [1].

The following reagents were used: cytochrome *c* and DL-α-tocopherol (Sigma), dextran T-500 (Pharmacia), superoxide dismutase from human erythrocytes (Rosepidkompleks, Rostov-on-Don), and Hank's solution (without phenol red, Moscow Plant of Bacterial Preparations). 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine (phospholipid PAF) was synthesized by hydration, alkaline deacylation of phosphatidal cholines from bovine heart followed by acetylation with acetic anhydride as described elsewhere [3].

RESULTS

Plasma content of vitamin E in patients with FH was considerably higher than in healthy individuals (Table 1). It was shown that 65-75% vitamin E in the blood is transported by apoB-containing lipoprotein particles and cleared from the circulation through the apoB,E-receptor [6]. This accounts for the elevation of the plasma vitamin E content in heterozygous FH patients, in whom the number of apoB,E receptors constitutes 30-70% of normal. Vitamin E protects lipids from oxidative stress, being an important hydrophobic trap of free radicals. Therefore, its adequacy for blood lipid protection can best be assessed by measuring the level of lipid-standardized vitamin E. This method clearly demonstrated that despite high blood concentration of vitamin E in FH patients, their lipids are worse protected than in healthy donors (Table 1).

Plasma level of anaphylatoxins in FH patients far surpassed that in healthy individuals (Table 2). It has been previously shown [11] that cholesterol-mediated activation yields about 10 C5a and 100 C3a molecules per one C5b-9 complex formed in the subendothelium. C3a and C5a are small peptides readily diffusing in aqueous phase and possessing high affinity for blood cell receptors. Free anaphylatoxins are rapidly cleaved by carboxypeptidase N, which removes C-terminal arginine yielding C3a(desArg) and C5a(desArg). Unlike C5a, C5a(desArg) exhibits no spasmogenic activity, but its neutrophil-stimulating effect is preserved, though decreased by an order of magnitude. Chemotactic properties of C5a and C5a(desArg) for monocytes are similar; both agents stimulate degranulation and activation of oxidative metabolism in leukocytes. Free C5a(desArg) can be detected in the plasma only if its concentration exceeds binding capacity of monocyte and neutrophil receptors. C5a and C5a(desArg) are internalized by neutrophils and cleaved by their hydrolytic enzymes to inactive fragments and amino acids [4]. Excessive generation of anaphylatoxins can up-regulate the synthesis of interleukin-1 tightly involved in the mechanisms of damage to the endothelium and impaired blood coagulation.

Production of superoxide anion radicals by leukocytes isolated from healthy donors and incubated in Hank's medium in the absence of inductors was 3.0±0.5 nmol/10^6 cells/h. Leukocytes from FH patients were characterized by higher level of spontaneous activation (Table 3), which is probably due

TABLE 2. Plasma Contents of C3a(desArg) and C5a(desArg) (*M±m*)

Experimental conditions	C3a(desArg)	C5a(desArg)
	ng/ml	
Control (*n*=16)	288±65.3	3.6±2.7*
FH (*n*=16)	783±94.7	15.5±2.1*

to stimuli produced by activated endothelial cells and platelets. The elevated content of C5a(desArg) in FH patients is probably responsible for enhanced endogenous generation of superoxide anion radicals by blood leukocytes [9]. However, involvement of other inflammatory transmitters such as kallikrein, arachidonic acid metabolites, monokines, and PAF cannot be excluded. PAF can be synthesized by various activated cells, including platelets, endothelial cells, monocytes, basophils, and neutrophils. In concentrations below the threshold concentration of platelet activation, PAF stimulates neutrophils and induces their chemotaxis, aggregation, generation of superoxide anion radicals, and degranulation [2]. Addition of 10^{-6} M PAF to the incubation medium revealed a principal difference in the reaction of leukocytes from FH patients and healthy individuals to exogenous PAF: PAF enhanced production of superoxide anion radicals in leukocytes from donors (which agrees with published data [3]) and had no effect on leukocytes from FH patients (Table 3). The mechanisms of this phenomenon remain unclear. It can be hypothesized that in hypercholesterolemic individuals different inflammatory stimuli induce accumulation of PAF or its analogs in leukocytes. The assumption on the increased content of PAF in leukocytes of FH patients is in accordance with a higher plasma level of the PAF precursor lysoPAF and more intense PAF production by leukocytes from these patients in response to ionophore stimulation [5]. These facts and findings that PAF produced by stimulated leukocytes mainly remains in the cell [8] suggest the existence of elevated concentration of PAF in leukocytes in FH.

Thus, our experiments showed that FH is characterized by 1) decreased plasma content of lipid-standardized vitamin E; 2) increased concentration of inflammatory transmitters C3a(desArg) and C5a(desArg) anaphylatoxins; 3) enhanced spontaneous generation of superoxide anion radicals in blood leukocytes and their resistance to activation with exogenous PAF.

TABLE 3. Spontaneous and PAF-Induced (1 μM) Reduction Cytochrome c by Leukocytes of Donors and FH Patients (*M±m*)

Experimental conditions	Activation, nmol/10^6 cells/h	
	spontaneous	PAF-induced
Control (*n*=16)	3.0±0.2	3.9±0.3
FH (*n*=16)	4.6±0.3	3.4±0.4

Authors are grateful to Prof. V. N. Titov and the staff of Laboratory of Clinical Biochemistry (Institute of Experimental Cardiology) for their help in vitamin E assay.

The study was supported by the Russian Foundation for Basic Research (grant No. 95-04-11937)

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