Enhanced Blood Activity of Selenic Glutathione Peroxidase in Patients with Coronary Heart Disease after Treatment with Selenium-Containing Antioxidant Preparation Adrusen Zinco

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This study analyzed the effects of nutriceutic Adrusen Zinco containing vitamin E and prosthetic groups of antioxidant enzymes (selenium, copper, zinc) on the parameters of free radical oxidation of blood lipids in patients with coronary heart disease and hypercholesterolemia. Adrusen Zinco considerably increased activity of erythrocyte and serum selenic glutathione peroxidase as soon as after 1-month treatment, while erythrocyte SOD activity significantly increased only after 2 months.

Key Words: *antioxidants; glutathione peroxidase; lipoperoxides; hypercholisterinemia; free radical oxidation*

Intensified free radical lipid oxidation plays an important role in the pathogenesis of atherosclerosis [2, 5,14,15]. In tissues of animals with alimentary hypercholesterolemia and in human blood and aorta during atherogenesis, the content of primary (lipohydroperoxides) and secondary (malonic dialdehyde, MDA) products of free radical lipid oxidation increases with simultaneous inhibition of antioxidant enzymes glutathione peroxidase and SOD [1,2,5]. Oxidation of low-density lipoproteins (LDL) in the circulation or in the vascular wall *in situ* increases their atherogeneity and promotes their uptake by aortal monocyte macrophages, which are transformed to foam cells forming a lipidosis zone [14,15]. Therefore, accumulation of lipoperoxides in the blood (lipoperoxidemia) can also be considered as a risk factor for atherosclerosis [2,8]. It seems reasonable to use antioxidants for both primary and secondary prophylaxis of atherosclerosis [5,9], preference to be given to preparations activating antioxidant enzymes, potent natural regulators of lipid oxidation [6,10]. Today, antioxidant preparations containing antioxidant vitamins (provitamin A, vitamins E and C) and trace elements, constituents of SOD (Cu, Zn) and glutathione peroxidase (Se) prosthetic groups are widely used. In this study patients with coronary heart disease (CHD) and hypercholesterolemia were treated for 2 months with the antioxidant preparation Adrusen Zinco (AZ, Societa Industria Farmaceutica Italiana) containing vitamin E (D-α-tocopherol, 10 mg), Cu (1 mg), Zn (22 mg), Se (75 mg), and eicosapentaenoic (68 mg) and docosahexaenoic (45 mg) fatty acids. The parameters of free radical lipid oxidation in blood were measured before and after treatment.

MATERIALS AND METHODS

The study included 15 men aged 51.0±1.7 with CHD treated at the A. L. Myasnikov Institute of Cardiology (Russian Cardiology Research-and-Production Com-

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plex). Total serum cholesterol (CH) was 8.03±1.51 mmol/liter (IIa and IIb hyperlipidemia). The patients took no hypolipidemic preparations for two months before the start of therapy and followed a low-CH diet during the entire examination period. All patients received 2 AZ capsules daily for 2 months. Venous blood was sampled before and after each month of treatment on an empty stomach. The samples were stabilized with sodium citrate and centrifuged. The serum content of lipohydroperoxides was determined colorimetrically by oxidation of F^{2+} to Fe^{3+} by lipoperoxides *in vivo* accumulated in LDL. The content of Fe^{3+} before and after reduction of organic hydroperoxide with triphenylphosphine was determined on a Hitachi 557 spectrophotometer at 560 nm using xylenol orange reaction [12]. The serum content of secondary lipid oxidation products (MDA and other carbonyl compounds) was determined by reaction with thiobarbituric acid (TBA) at 532 nm [3]. To determine activity of antioxidant enzymes in erythrocytes (95% of total activity), 0.1 ml whole blood was mixed 1:9 with a hypotonic 5 mM K, Na-phosphate buffer (pH=7.4), rapidly frozen, and stored at -20°C before measurements (no more than 1 month) [10]. Before the measurement of SOD activity 1 ml hemolysate was shaken on ice with 0.5 ml chloroform:ethanol (3:5) mixture, and sedimented by heme centrifugation. SOD activity was determined at 25°C by inhibition of nitro blue tetrazolium (NBT) reduction by superoxide anion radical generated in the xanthine-xanthine oxidase system at 560 nm [11]. The amount of SOD necessary for 50% inhibition of the reaction under given conditions was taken as an activity unit [11]. Serum and lysate activity of glutathione peroxidase was determined at 25°C by measuring (at 340 nm) the kinetics of glutathione oxidation (by NADPH oxidation in a coupled glutathione reductase reaction) with a Labsystems Oy FP-901 chemical analyzer using tert-butyl hydroperoxide as the substrate (method [13] in our modification [4]). The amount of glutathione peroxidase necessary for oxidation of 1 μmol reduced glutathione for 1 min was taken as an activity unit [4]. The serum content of total CH, LDL CH, and HDL CH was determined enzymatically using Boehringer kits, the hemoglobin content was determined with Biolar kits.

RESULTS

The indications for use of AZ in CHD patients were determined by its possible antioxidant effect due to the presence of several antioxidant components. It should be noted that some patients could respond to AZ treatment with hyperlipoperoxidemia, a prooxidant reaction, which could be provoked by administration of highly unsaturated oxidation substrates (polyenic fatty acids) against the background of antioxidant system deficiency typical of CHD patients [1,2,5]. Therefore, we determined the blood content of lipid oxidation products and antioxidant enzyme activity before the start and after 1 and 2 months of treatment.

As expected, the total blood CH did not significantly change during treatment (Table 1), since the drug contains a relatively small amount of ω3-polyunsaturated fatty acids, while hypolipidemic effect appears after their intake in a daily dose of 2-8 g [7]. Activity of erythrocyte Se-gluthathione peroxidase after 1 and 2 months of AZ treatment increased 1.5-fold and 1.8-fold, respectively. Serum activity of Se-containing glutathione peroxidase also significantly increased (by 10-15%). SOD activity in erythrocytes did not change after 1 month, but increased by 15% after 2 months of AZ therapy. At the same time, no significant changes were observed in the blood content of both primary (lipoperoxides) and secondary (MDA) lipid oxidation products during the entire course of treatment. Thus, despite the presence of readily oxidizable polyenic fatty acids, AZ possesses antioxidant activity which can be realized via acceleration of en-

TABLE 1. Blood Content of Free Radical Lipid Oxidation Products and Activity of Antioxidant Enzymes in Hypercholesterolemic Patients with Coronary Heart Disease (n=15) Treated with Adrusen Zinco Antioxidant Preparation ($M\pm m$)

Parameter	Before treatment	Period of treatment	
		after 1 month	after 2 months
Total CH, mmol/liter	8.03±1.51	8.53±1.97	8.68±1.5
Lipoperoxides, nmol/liter	5.56±0.95	5.21±0.76	5.45±1.08
MDA, nmol/liter	14.7±0.97	14.5±1.11	14.6±0.85
Plasma glutathione peroxidase, U/ml	0.48±0.05	0.55±0.14*	0.53±0.05*
Erythrocyte glutathione peroxidase, U/g Hb	2.9±0.92	4.3±0.68*	5.11±0.59*
Erythrocyte SOD, U/g Hb	579±106	589±96	663±88*

Note. *p<0.05 compared to initial values.

zymatic utilization of lipoperoxides. Therefore, AZ can be recommended as a mild antioxidant in pathological conditions associated with disturbed lipid metabolism and free radical lipid oxidation such as cardiovascular diseases, diabetes mellitus, neoplasms, and others. During the treatment with AZ it is desirable to control the antioxidant state and correct treatment schedule if necessary. It should be noted, that AZ increased glutathione peroxidase activity in all patients, but some of them responded slower than others, and in these patients the dosage could be significantly increased to enhance treatment efficiency. The stimulating effect of AZ on antioxidant enzyme activity gradually increased in the course of treatment, hence more prolonged treatment (for 3-6 months) could produce a more pronounced antioxidant effect.

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