Effect of the Garlic Preparation Alisat on the Content of Lipid Peroxidation Products, Activity of Some Antioxidant Enzymes, and Blood Lipoprotein Level in Patients with Atherosclerosis

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A 3-month therapy with Alisat (600 mg/day) results in a decrease in the plasma content of lipid peroxidation products (diene conjugates and malonic dialdehyde), activation of the antioxidant enzyme glutathione peroxidase in erythrocytes, increase in the high-density lipoprotein cholesterol, decrease in plasma cholesterol, and decrease in the low-density lipoprotein. This implies that Alisat has an antiatherogenic activity.

Key Words: *garlic; atherosclerosis; lipid peroxides; lipoproteins*

Hypercholesterolemia, disturbances in the plasma lipoprotein spectrum, and activation of lipid peroxidation (LPO) in the blood have been considered as the major factors promoting the development and progression of atherosclerosis [2,3]. Therefore, correction of hyperlipidemia and normalization of LPO in patients with atherosclerosis are fundamental to the prevention of this disease. In contrast to synthetic drugs, preparations based on garlic (Kwai, Garlic) are characterized by minimal side effects [4]. These preparations reduce the oxidizability of human low-density lipoproteins (LDL) [16], suppress and intracellular accumulation of cholesterol (CH) and cellular proliferation in a culture of smooth muscle cells [14], and decrease plasma contents of CH and triglycerides in patients with hyperlipidemia [7]. Alisat (sublimated garlic) is a Russian-manufactured drug. It produces a longer effect than Kwai and Garlic [4]. However, the effects of Alisat on LPO in the blood of atherosclerotic patients and its hypolipidemic activity have not been investigated.

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Our goal was to examine the effect of Alisat on the content of LPO products, activity of the main antioxidant enzymes, and blood lipid levels in patients with atherosclerosis.

MATERIALS AND METHODS

Blood samples from 16 patients with atherosclerosis (ischemic heart disease or stable angina of effort of the II functional class) were studied. The patients received Alisat (600 mg/day) for 3 months against the background of usual antianginal therapy with sustained nitrates. Control group included 10 patients with angina receiving sustained nitrates and placebo. Blood was drawn from the ulnar vein in the morning not earlier than 12 hours after last meal. Blood was sampled before and after 0.5, 1, 2, and 3 months of Alisat therapy and 1 and 2 months after discontinuation of the therapy. The contents of primary (diene conjugates, DC) and secondary (malonic dialdehyde, MDA) LPO products were measured. The content of DC was assayed by optical density at 233 nm [1], the concentration of MDA was measured by the reaction with 2-thiobarbituric acid [13] at 532 nm, and the data were expressed as D_{233} and D_{532} per ml

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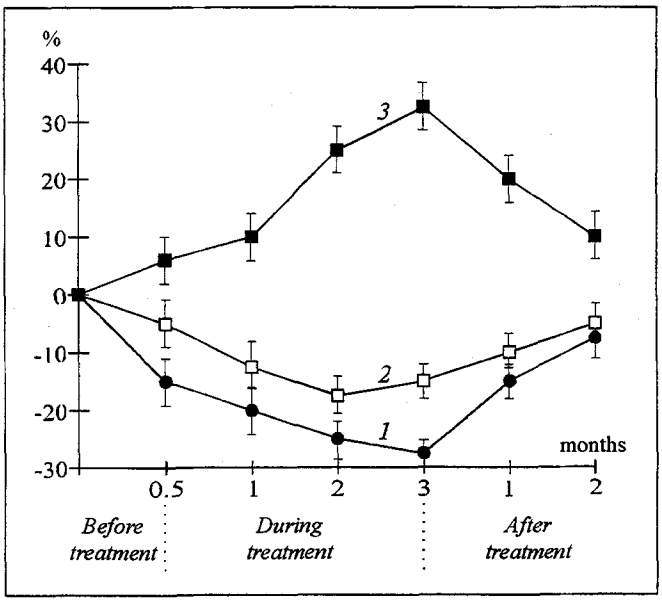

Fig. 1. Changes in the plasma content of diene conjugates (*1*), malonic dialdehyde (*2*), and activity of glutathione peroxidase (*3*) in erythrocytes of patients with atherosclerosis treated with Alisat.

plasma. The activity of the key antioxidant enzyme, glutathione peroxidase, was assayed in erythrocytes by NADPH:H oxidation in a coupled glutathione reductase system [15] using tert-butyl peroxide as a substrate. The superoxide dismutase activity in erythrocytes was assayed as described previously [6] after hemoglobin precipitation [13]. Plasma contents of total CH and triglycerides were measured using Boehringer enzyme kits. Cholesterol of high-density lipoproteins (HDL-CH) was determined after Mn-heparin precipitation of LDL and very-low-density lipoproteins (VLDL) [5]. Cholesterol of LDL (LDL-CH) was calculated from the following formula: CH—HDL-CH—triglycerides/5 [8]. Plasma content of apolipoprotein B (apoB) was measured by enzyme-linked immunosorbent assay in polystyrene multiwell plates using monospecific rabbit antibodies [7] and recorded in a Multiscan-340 or Beckman DU-7 spectrophotometer. The results were analyzed using the Student's differential method.

RESULTS

Plasma contents of DC and MDA increased considerably in patients receiving Alisat and placebo, while the glutathione peroxidase and superoxide dismutase activities were lower than in healthy individuals (Table 1). The contents of DC and MDA

TABLE 1. Effect of Alisat Therapy on LPO Parameters and Blood Lipoproteins in Patients with Atherosclerosis (*M±m*)

Parameter	Group	Duration of therapy, months					Discontinuation of therapy, months	
		initial	0.5	1	2	3	1	2
DC/ml	Alisat	4.26±0.13	3.82±0.14*	3.41±0.15*	3.16±0.18*	3.07±0.21*	3.61±0.16*	4.04±0.17
	Placebo	4.47±0.11	4.67±0.12	4.27±0.15	4.11±0.13	4.59±0.16	4.28±0.17	4.58±0.17
MDA/ml	Alisat	2.71±0.13	2.64±0.13	2.28±0.22*	2.21±0.17*	2.26±0.15*	2.48±0.15	2.59±0.15
	Placebo	2.54±0.11	2.68±0.13	2.41±0.14	2.32±0.13	2.61±0.15	2.39±0.14	2.47±0.16
GPO, act. units/g Hb	Alisat	3.59±0.14	3.79±0.19	4.05±0.18*	4.53±0.27*	4.81±0.24*	4.38±0.35*	3.98±0.28
	Placebo	3.25±0.13	3.31±0.15	3.18±0.18	3.24±0.24	3.58±0.18	3.47±0.17	3.65±0.19
SOD, act. units/g Hb	Alisat	1471±47	1418±61	1396±64	1489±58	1510±71	1487±49	1511±59
	Placebo	1549±53	1528±48	1471±72	1590±44	1611±67	1539±61	1589±54
CH, mg/dl	Alisat	316.4±9.1	307.2±9.0	338.9±10.8	279.3±14.6*	267.3±11.7*	275.9±13.8*	288.0±15.2
	Placebo	330.1±8.3	224.3±7.1	328.2±6.8	337.7±7.3	323.1±9.5	328.1±6.8	336.4±6.6
HDL-CH, mg/dl	Alisat	37.2±0.7	38.6±0.7	40.1±0.8*	42.6±0.9*	44.1±0.8*	39.9±0.7	38.0±0.7
	Placebo	39.8±0.8	40.2±0.7	41.3±0.9	38.7±0.8	37.1±0.8	38.1±0.7	38.9±0.7
LDL-CH, mg/dl	Alisat	238.8±12.2	226.4±16.4	259.2±15.7	297.9±14.2*	189.8±15.8*	198.7±16.2*	210.7±13.7
	Placebo	247.6±14.2	241.6±16.7	241.9±13.8	251.1±15.8	240.5±11.9	246.8±16.7	256.6±19.4
ApoB, mg/dl	Alisat	148.7±8.4	151.6±7.6	158.3±8.1	145.2±8.1	141.3±6.9	157.3±9.7	141.3±9.4
	Placebo	156.3±10.8	161.3±9.1	152.3±7.4	148.3±7.4	155.7±8.5	149.2±8.1	159.4±7.3
ApoB/LDL-CH	Alisat	1.60±0.11	1.53±0.13	1.63±0.12	1.36±0.15*	1.31±0.14*	1.87±0.19	1.44±0.16
	Placebo	1.58±0.14	1.51±0.12	1.59±0.14	1.66±0.14	1.54±0.12	1.65±0.13	1.61±0.11

Note. *$p<0.05$ compared with placebo. GPO: glutathione peroxidase, SOD: superoxide dismutase.

decreased after 0.5 and 1 month of Alisat therapy, respectively, both parameters continued to decrease during a 2-month period, after which they remained at a low level. In Alisat-treated patients, a decrease in plasma DC concentration was more rapid and more pronounced than that of MDA ($p<0.05$). The activity of glutathione peroxidase increased by 12.8% during the first month and continued to rise up to termination of the treatment, while the activity of superoxide dismutase remained practically unchanged (Table 1, Fig. 1).

The content of CH dropped starting from the 2nd month of Alisat therapy, the HDL-CH content slightly increased starting from the 1st month, by the end of the 3rd month the increase being equal to 18%. The VLDL-CH and triglyceride contents did not change throughout the observation period. The LDL-CH concentration dropped by 20%; however, the level of apoB (the protein moiety of LDL [2]) did not change. Thus, Alisat had no effect on the content of LDL particles but reduced their CH content, judging from the reduced LDL-CH/apoB ratio. A decrease in the DC concentration correlated positively with its initial level ($r=0.71$) and the initial level of CH ($r= 0.69$). By the 3rd month of Alisat therapy, a negative correlation between the content of DC and HDL-CH ($r=-0.64$) was established. After discontinuation of the therapy, all parameters returned to the original levels. In the control group, no significant changes in the studied parameters were observed.

The mechanisms of the antioxidant activity of Alisat so far remain unclear. The hypocholesterolemic effect of this preparation may be associated with inactivation of hepatic hydroxymethylglutaryl-coenzyme A reductase [10,11]; however, this hypothesis cannot explain the effects of Alisat demonstrated in this study. Nevertheless, our findings suggest that Alisat exhibits a high antiatherogenic activity and should be recommended for the treatment of atherosclerosis.

REFERENCES

1. A. K. Zhuravlev, V. V. Murashko, and P. R. Kamchatov, *Klin. Med.*, **66**, No. 4, 35-37 (1988).
2. A. N. Klimov and N. G. Nikul'chiva, *Lipids, Lipoproteins, and Atherosclerosis* [in Russian], St. Petersburg (1995), pp. 155-175.
3. V. Z. Lankin, A. F. Vikhert, A. K. Tikhadze, and T. I. Bondar', *Vopr. Med. Khimii*, No. 3, 18-24 (1989).
4. A. N. Orekhov and E. M. Pivovarova, *Angiologiya Sosud. Khir.*, No. 1, 158-162 (1995).
5. V. V. Titov, E. D. Brener, and A. A. Zadoya, *Lab. Delo*, No. 1, 36-41 (1979).
6. C. Beauchamp and J. Fridovish, *Anal. Biochem.*, **44**, No. 1, 276-287 (1971).
7. C. Cilady and A. Neil, *J. R. Coll. Physicians Lond.*, **28**, No. 1, 2-8 (1994).
8. E. Engual and P. Parmlan, *Immunochemistry*, **8**, 871-874 (1971).
9. W. T. Friedewald, R. I. Levy, and D. S. Fredrickson, *Clin. Chem.*, **18**, 499-502 (1972).
10. R. Gebhardt, *Arzneimitteforschung Drug Res.*, **41**, 800-804 (1991).
11. R. Gebhardt, Lipids, **28**, 613-619 (1993).
12. J. Maral, K. Puget, and A. Michelson, *J. Biol. Chem.*, **255**, No. 3, 11108-11111 (1980).
13. S. Moncada, R. I. Glyglewski, and S. Bunting, *Arteriosclerosis*, **2**, 193-197 (1982).
14. A. N. Orekhov, V. V. Tertov, I. A. Sobenin, and E. M. Pivovarova, *Ann. Med.*, **27**, 63-65 (1995).
15. D. E. Pagleo and W. N. Valentine, *J. Lab. Clin. Med.*, **70**, No. 1, 158-189 (1967).
16. S. Phelps and W. Harris, *Lipids*, **28**, 475-477 (1993).