# ANTISTRESS EFFECTS OF A COMBINATION OF SMALL DOSES OF VITAMIN E AND DIMETHYLSULFOXIDE

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UDC 615.356:577.161.3].015.2.615.281.8.547.283.2].015.4

KEY WORDS: stress, vitamin E, dimethylsulfoxide.

Activation of free-radical lipid oxidation (FRLO) is a key mechanism in the injurious action of stress [7, 8]. The marked stress-protective effect of antioxidants has stimulated much research into the adaptation-inducing action of natural and synthetic compounds with antiradical activity. Synthesis of new antioxidants is a promising development [3, 9], but their clinical application is often impeded by their relatively high toxicity. Meanwhile the high antiradical activity and extremely low toxicity of natural antioxidants has drawn the attention of research workers to them. For instance, the study of the properties of vitamin E, an effective antioxidant capable of inserting itself in to biological membranes and exerting a marked membrane-stabilizing action, is continuing [2, 10]. Nevertheless, the experimental and clinical use of vitamin E is often ineffective, for most of a preparation administered fails to reach the principal targets of its biological action, namely membranes. That is why enhancing the bioavailability of vitamin E is a very important problem. Previously the writers studied the adaptation-inducing properties of high doses of dimethylsulfoxide (DMS), a compound with low toxicity and with the unique properties of increasing permeability of biological barriers for various compounds [12].

The aim of this investigation was to study the antistress properties of small doses of vitamin E and DMS when administered together to rats with chronic emotional-painful stress.

## EXPERIMENTAL METHOD

Experiments were carried out on 100 noninbred male albino rats weighing 190-220 g. Chronic emotional-painful stress was produced by a combination of electrodermal stimulation and white noise, applied daily for 3 weeks [1, 6]. Vitamin E (5 mg/kg) and DMS (50 mg/kg) were administered perorally to the animals simultaneously in the form of a suspension in 0.2 ml of vegetable oil 30 min before exposure to stress. Control rats received oil, vitamin E, and DMS together or separately, but were not exposed to stress. Groups of animals receiving oil, DMS, or vitamin E and exposed to stress also were investigated. Vegetative parameters were measured in the different groups of animals as described in [1] and their behavior in the open field test was studied. After the end of exposure and testing the rats were decapitated, the brain was removed, and concentrations of FRLO products [4, 14] and superoxide dismutase activity [13] were determined in cortical homogenates. Lipids were extracted from the remainder of the homogenate by the method in [11] and phospholipids and cholesterol [15] and also the antioxidative activity of the lipids in a co-oxidation system with cumene [5] were determined in the lipid extracts by thin-layer chromatography. Primary molecular products of FRLO [4] and nonenzymic superoxide-scavenging activity were determined in the rats' blood serum.

## EXPERIMENTAL RESULTS

Chronic emotional-painful stress in the rats induced hypertension, which was prevented by a combination of vitamin E and DMS (Fig. 1a). Vitamin E restored the normal values of the blood pressure but did not prevent their elevation in response to immobilization for 2 h, whereas DMS stabilized the blood pressure at a level a little above the control. Vitamin E and DMS, and a combination of the two in particular, restored Hildebrandt's index to

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. S. Rusinov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 9, pp. 274-277, September, 1988. Original article submitted June 12, 1987.

 0007-4888/88/0009-1228$12.50 

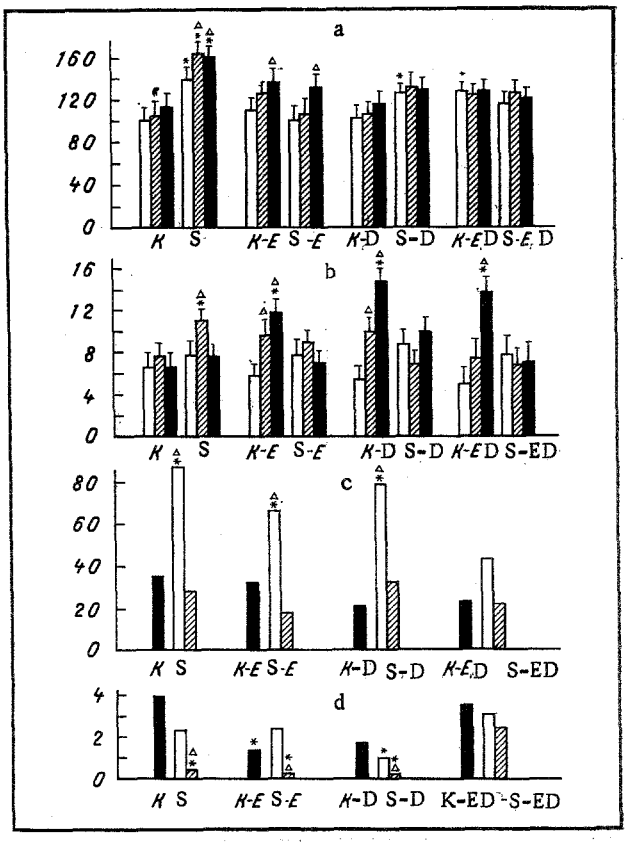


Fig. 1. Changes in physiological parameters in rats receiving vitamin E and DMS and exposed to chronic emotional-painful stress. a) Arterial pressure (mm Hg), b) Hildebrandt's index: unshaded columns – background, obliquely shaded – after 1 h of immobilization. Black columns – after 2 h of immobilization. *) $p < 0.001$ compared with corresponding control, triangle) the same, compared with background; c) number of squares crossed during 5 min of observation; d) number of visits to center during 5 min of observation; black columns – control, unshaded columns – hyperactive animals from stressed groups (50% of animals), obliquely shaded columns – hypoactive animals. *) $p < 0.01$ compared with control, triangle) the same for comparison of hyper- and hypoactive animals. K) Control animals receiving oil; K-E) vitamin E, K-D) DMS, K-ED) vitamin E + DMS, S) stressed animals receiving oil; S-E) vitamin E; S-D) DMSO, S-ED) vitamin E + DMS.

normal (its value is regarded as an indicator of interaction between the respiratory and cardiovascular systems; see Fig. 1b). In the control animals not subjected to stress, administration of the preparations caused changes in Hildebrandt's index indicating a disturbance of coordination between these systems, i.e., normally small doses of DMS or vitamin E can be regarded as a stress-inducing factor, as reflected in parameters of vegetative functions.

Comparison of parameters characterizing behavior of the rats in an open field shows that only a combination of vitamin E + DMS had an antistress effect (Fig. 1c, d). Reducing the degree of manifested hyperactivity and preventing stress-induced separation of the rats within the group into animals with normal activity and with hyperactivity. Other characteristic features of the action of a combination of vitamin E and DMS were a decrease in the number of defecations and normalization of the number of visits to the center of the field. Vitamin E and DMS, given together or separately, shortened the latent period.

Concentrations of primary molecular products of FRLO (conjugated dienes and ketodienes) were somewhat reduced in the brain and increased in the blood serum of rabbits exposed to stress. In conjunction with the results of investigation of the internal organs, this is evidence that the state of the animals corresponded to Selye's stage of adaptation, as a result of 3 weeks of stress. Superoxide dismutase was activated in the animal's brain and nonenzymic superoxide-scavenging activity in the blood serum; concentrations of phospholipids and cholesterol and the cholesterol:phospholipids ratio in the lipid extracts were

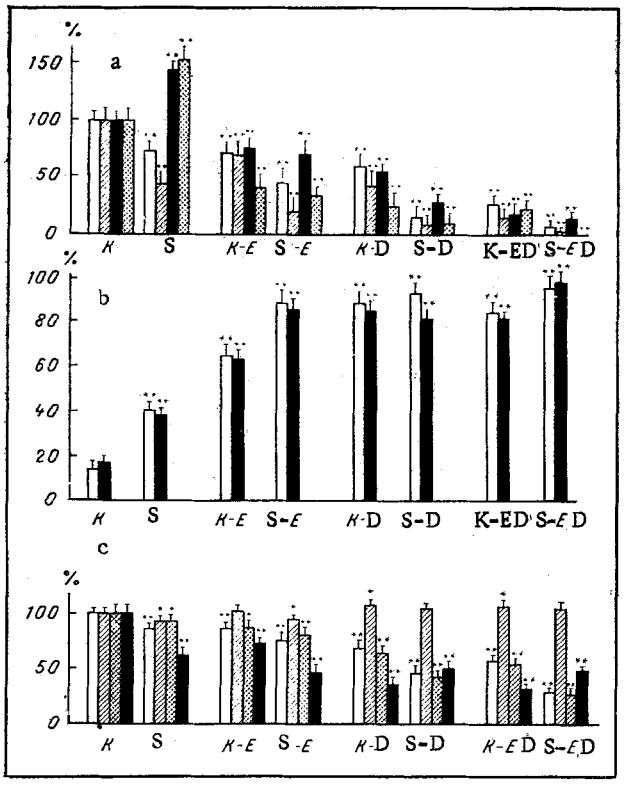


Fig. 2. Changes in FRLO parameters and antiradical protection in brain and blood serum of rats. a) Concentrations of FRLO products in brain homogenates and blood serum: unshaded columns — conjugated dienes in brain, black columns — in blood serum; oblique shading — ketodienes in brain, cross-hatching — in blood serum. Values of parameters for K group taken as 100%; b) unshaded columns — superoxide dismutase activity of brain columns — nonenzymic superoxide-scavenging activity of blood serum. Ordinate, inhibition of reduction of nitro-BT in sample containing supernatant of homogenate of 50 mg brain tissue or 200 µl of protein-free supernatant of blood serum; c) unshaded columns — cholesterol concentration, oblique shading — phospholipid concentration, cross hatching — cholesterol:phospholipids ratio, black columns — antioxidative activity of lipid extracts of brain. Values of parameters for K group taken as 100%. *$p < 0.01$, **$p < 0.001$ compared with K group. Remainder of legand as to Fig. 1.

reduced (Fig. 2a-c). Administration of vitamin E and DMS and, in particular, of a combination of both reduced the concentrations of FRLO products in the brain and blood serum and the cholesterol concentration and cholesterol:phospholipids ratio in lipid extracts of the brain, and sharply increased superoxide dismutase and superoxide-scavenging activity. The effects of the antioxidants were more marked in rats exposed to stress (Fig. 2a-c). DMS and vitamin E reduced the antioxidative activity of the lipid extracts, evidently on account of an increase in the proportion of readily oxidized fractions in the composition of the lipids (Fig. 2c).

The results indicate that a combination of vitamin E + DMS possesses the positive properties of each component, but also exhibits various qualitatively different antistress properties, probably due to interaction between vitamin E and DMS by mechanisms which are not yet fully clear. The most likely mechanism of the effective antistress action of a combination of vitamin E, scavenging of hydroxyl radicals by DMS, facilitation of access of vitamin E to membranes on account of the superconducting properties of DMS, protection of vitamin E against oxidation by free-radical compounds during delivery to the membranes on account of the presence of an excess of DMS, the indirect antiradical action of vitamin E and DMS on account of activation of enzymic and nonenzymic scavenging of superoxide radicals, and lowering of the cholesterol concentration and cholesterol:phospholipids ratio in biomembranes.

Their effective antistress activity coupled with their low toxicity is evidence of the promising outcome of research into the action of small doses of vitamin E together with DMS, with the aim of promoting the clinical use of this combined preparation in the treatment of diseases accompanied by activation of FRLO.

## LITERATURE CITED


1. M. G. Airapetyants, I. P. Levshina, and N. V. Gulyaeva, Zh. Vyssh. Nerv. Deyat., 36, No. 3, 554 (1986).
2. A. N. Erin, V. A. Tyurin, N. V. Gorbunov, et al., Dokl. Akad. Nauk SSR, 281, No. 2, 447 (1985).
3. V. V. Ershov, L. G. Plekhanova, and I. S. Belostotskaya, Bioantioxidants [in Russian], Vol. 1, Chernogolovka (1986), pp. 6-7.
4. V. A. Kostyuk, A. I. Potapovich, and E. F. Lunets, Vopr. Med. Khimii, 30, No. 4, 125 (1983).
5. A. N. Kudrin, V. S. Smolenskii, V. M. Khusainov, et al., Author's Certificate 127801 (USSR).
6. I. P. Levshina, O. L. Levina, and N. V. Gulyaeva, Zh. Vyssh. Nerv. Deyat., 35, No. 2, 330 (1985).
7. F. Z. Meerson, Physiology of Adaptive Processes [in Russian], Moscow (1986), pp. 521-631.
8. L. L. Prilipko, O. M. Orlov, S. M. Ivanova, et al., Dokl. Akad. Nauk SSSR, 265, No. 4, 1010 (1982).
9. L. D. Smirnov and K. M. Dyumaev, Vest. Akad. Med. Nauk SSSR, No. 11, 89 (1984).
10. N. G. Khrapova, Bioantioxidatns in the Regulation of Metabolism under Normal and Pathological Conditions [in Russian], Moscow (1982), pp. 59-73.
11. J. Folch, M. Lees, and G. H. Sloane-Stanley, J. Biol. Chem., 226, No. 3, 497 (1957).
12. N. Kharasch and B. S. Thyagarajan, Ann. N.Y. Acad. Sci., 411, 391 (1983).
13. M. Nishikimi, N. A. Rao, and K. Yagi, Biochem. Biophys. Res. Commun., 46, No. 2, 849 (1972).
14. H. Ohkawa, N. Ohishi, and K. Yagi, Anal. Biochem., 95, No. 2, 351 (1979).
15. N. N. Osborn, Microchemical Analysis of Nervous Tissue, New York (1974), pp. 122-152.