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LITERATURE CITED

1. I. D. Volotovskii, V. S. Filin, A. V. Kulikov, et al., Dokl. Akad. Nauk SSSR, 234, 943 (1977).
2. R. I. Salganik, in: Cyclic Nucleotides [in Russian], Krasnoyarsk (1976), pp. 45-46.
3. T. Asano and H. Hidaka, Biochim. Biophys. Acta, 397, 124 (1975).
4. T. Brenner and E. Gorin, Biochem. Med., 20, 160 (1978).
5. L. L. Brunton and S. E. Mayer, J. Biol. Chem., 254, 9714 (1979).
6. P. R. Devoren and E. W. Sutherland, J. Biol. Chem., 238, 3009 (1963).
7. E. Gorin and S. Dickbuch, Horm. Metab. Res. (Stuttgart), 12, 120 (1980).
8. M. Honma, T. Saton, and I. Takezawa, Eur. J. Pharmacol., 47, 1 (1978).
9. C. Klein, J. Biol. Chem., 254, 12573 (1979).
10. F. Okada, M. Honma, and M. Vi, Horm. Metab. Res. (Stuttgart), 12, 80 (1980).
11. J. F. Tine, L. P. Sergent, and G. Rocquet, Med. Armées, 3, 587 (1975).
12. R. H. Williams, J. Barish, and J. Ensinck, Proc. Soc. Exp. Biol. (N. Y.), 139, 447 (1972).

ENZYMIC DETOXICATION OF THE SUPEROXIDE ANION-RADICAL AND OF LIPID PEROXIDES IN THE INTIMA AND MEDIA OF THE ATHEROSCLEROTIC AORTA

V. Z. Lankin, A. M. Vikhert,
V. A. Kosykh, A. K. Tikhaze,
I. E. Galakhov, A. N. Orekhov,
and V. S. Repin

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The hypothesis of the important role of free-radical lipid peroxidation (LPO) in the formation of atherosclerotic lesions of the vascular wall [11, 15] has recently received increasing experimental confirmation [2, 4, 6, 8]. It has been shown, in particular, that aliphatic lipid hydroperoxides [10] and endoperoxides [12] can cause substantial injury to the vascular endothelium. The level of lipid peroxides in the tissues is regulated with the participation of protective enzyme system: superoxide dismutase (SOD) and glutathione peroxidase (GP), which reduce superoxide $O_2^{\cdot}$ anion-radicals and lipid peroxides respectively [3].

For the reasons given above, enzymic utilization of active forms of oxygen and lipid peroxides in the intima and media of the aorta with various types of atherosclerotic lesion was investigated.

EXPERIMENTAL METHOD

Specimens of the thoracic aorta were taken from men aged 40-60 years 1-4 h after sudden death: The principal diagnosis made at autopsy was ischemic heart disease (IHD) with coronary atherosclerosis and cardiosclerosis. Pieces of aorta without visually detectable atherosclerotic changes and also regions of lipid stains and large fibrous plaques were isolated. The intima and media were separated mechanically as described previously [14]. In separate experiments, isolated cells were separated from the samples of intima and media with different types of atherosclerotic lesions by hydrolysis of the connective-tissue matrix with collagenase and elastase [1]. The number of cells isolated was counted in a Goryaev's chamber and the viability of the cells (as shown by vital staining with trypan blue) was not less

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TABLE 1. Activity of "Antioxidant" Enzymes in Different Tissues of Atherosclerotic Lesions in the Human Aorta (M ± m)

Test object	Activity of SOD			Activity of GP		
	homogenate, c.u./mg protein	isolated cells		homogenate, c.u./mg protein	isolated cells	
		c.u./mg protein	c.u/10^6 cells		c.u./mg protein	c.u./10^9 cells
Intima	487±48 (17)	1713±280 (5)	491±64 (5)	0,082±0,011 (18)	0,28±0,03 (5)	93±15 (5)
with no pathological changes						
lipid stains	281±59 (10) P <0,05 P^*<0,01	1264 (2)	291 (2)	0,042±0,014 (11) P <0,05 P^*<0,005	0,18 (2)	27 (2)
fibrous plaques	145±30 (10) P <0,005 P^*<0,005	614±88 (6) P <0,005 P^*<0,05	185±39 (5) P <0,005 P^*<0,05	0,016±0,003 (10) P <0,005 P^*<0,005	0,11±0,02 (6) P <0,005 P^*<0,05	27±3 (5) P <0,005 P^*<0,05
Media						
with no pathological changes	328±38 (18)	2017±199 (7)	431±42 (6)	0,051±0,007 (18)	0,32±0,04 (7)	56±15 (6)
lipid stains	147±17 (11) P <0,005 P^*<0,005	1253±201 (4) P <0,05 P^*<0,05	300±12 (3) P >0,05 P^*<0,05	0,019±0,005 (11) P <0,005 P^*<0,005	0,14±0,01 (4) P <0,05 P^*<0,05	18±3 (3) P >0,05 P^*<0,05
fibrous plaques	145±10 (10) P <0,005 P^*<0,005	495±74 (5) P <0,005 P^*<0,05	124±41 (5) P >0,05 P^*<0,05	0,014±0,002 (10) P <0,005 P^*<0,005	0,10±0,01 (5) P <0,005 P^*<0,05	25±3 (5) P <0,005 P^*<0,05

Legend. Number of experiments given in parentheses. P) significance of differences by Student's t test; P*) by Wilcoxon–Mann–Whitney nonparametric test.

than 90%. Erythrocytes were obtained from blood of clinically healthy men (mean age 43 years) with absence of changes in their coronary arteries, as shown by angiography, and without any of the principal risk factors for IHD development [9]. Hemolysates of erythrocytes were precipitated with a mixture of ethanol and chloroform (3:5) before determination of their enzyme activity [13].

The isolated cells and pieces of tissue were minced in a glass homogenizer with Teflon psetle in 50 mM K, Na-phosphate buffer, pH 7.4, and centrifuged at 800g for 10 min in a refrigerating centrifuge. SOD activity was determined by inhibition of reduction of nitro-BT in a xanthine oxidase system; GP activity was determined by oxidation of NADPH in a coupled glutathione reductase system, using tert-butyl hydroperoxide as substrate at 25°C [5]. The optical density of the specimens was measured on an Aminco DW-2q UV VIS spectrophotometer (USA). The unit of SOD activity (conventional unit, c.u.) was taken to be the quantity of enzyme required to inhibit tetrazolium reduction by 50% under the conditions of determination; the unit of GP activity (c.u.) was taken to be the quantity of enzyme required to oxidize 1 μmole of reduced glutathione in 1 min [5]. The protein concentration in the samples was determined by Lowry's method.

The fact that the level of activity of the test enzymes in the aorta remained unchanged during the 9 h after death was confirmed beforehand by a special experiment [7].

The significance of differences in SOD and GP activity in samples of cells and tissues with different types of atherosclerotic changes was assessed by Student's t test and by the Wilcoxon–Mann–Whitney nonparametric test.

EXPERIMENTAL RESULTS

A very high level of SOD and GP is found in mammalian erythrocytes [13]. The present experiments showed that SOD and GP activity in the erythrocytes was 0.13 ± 0.003 c.u./10^6 cells and 0.25 ± 0.023 c.u./10^9 cells (n = 14) respectively, which is several orders of magnitude below the activities of these enzymes in cells isolated from normal intima and media (Table 1). The presence of abnormally high SOD and GP activity in the aorta (Table 1), which we observed previously during investigations of experimental animals [2], is undoubted evidence of the important role of enzymic protection of the vessel wall against the injurious action of active forms of oxygen and lipid peroxides. SOD activity in a homogenate of the intima of the aorta in the region of lipid stains and of fibrous plaques was reduced by 1.7 and 3.4 times respectively compared with that in the normal intima, whereas in the media of the aorta in these same types of atherosclerotic lesion SOD activity was reduced by 2.2 and 2.3 times respectively (Table 1; Fig. 1).

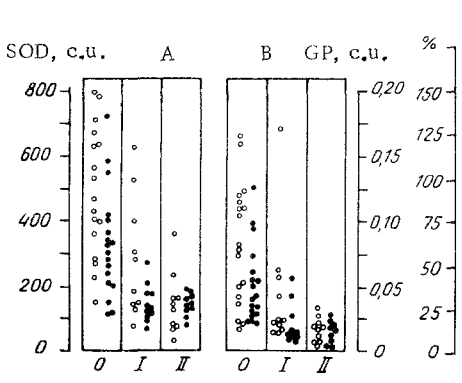

Fig. 1

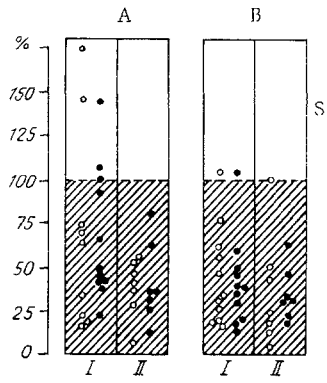

Fig. 2

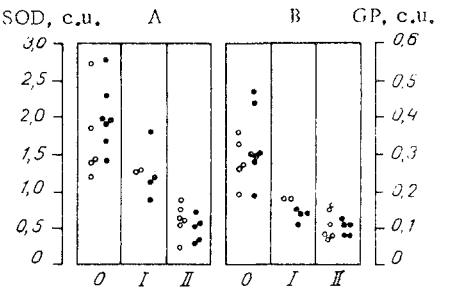

Fig. 3

Fig. 1. Activity of "antioxidant" enzymes in homogenates of intima and media of human aorta with different types of atherosclerotic changes. Here and in Figs. 2 and 3: abscissa, types of atherosclerotic lesion: 0) unaffected region; I) lipid stains; II) fibrous plaques; ordinate: A) c.u. SOD/mg protein, B) c.u. GP/mg protein. Open circles — intima; filled circles — media.

Fig. 2. Relative changes in activity of "antioxidant" enzymes in homogenates of intima and media of the human aorta with different types of atherosclerotic lesions.

Fig. 3. Activity of antioxidant enzymes in cells isolated from intima and media of human aorta with different types of atherosclerotic lesions. (SOD, c.u./mg protein; GP, c.u./mg protein).

GP activity in the homogenate of the intima of the aorta in the region of lipid stains and fibrous plaques was reduced by 2.0 and 5.1 times respectively compared with this parameter in the normal intima, whereas in the media of tha aorta in these same types of atherosclerotic lesion GP activity was reduced by 2.7 and 3.6 times (Table 1; Fig. 1). These results are made even more convincing by the fact that when both types of atherosclerotic lesions were investigated on material from the same aorta, SOD and GP activity was significantly reduced in the affected regions on the overwhelming majority of cases (Fig. 2).

Despite the presence of a large quantity of connective-tissue proteins (collagen and elastin) in the homogenates of the intima and media of the aorta, capable of introducing a definite error into calculations of enzyme activity relative to total protein, similar results were obtained when SOD and GP activity was investigated in isolated aortic cells (Table 1; Fig. 3). SOD activity in cells of the intima, isolated from lipid stains and fibrous plaques, calculated per milligram protein was 1.4 and 2.8 times lower respectively than in cells from the unaffected part of the aorta, whereas activity of this enzyme in cells isolated from the media was reduced by 1.6 and 4.1 times respectively in these same types of atherosclerotic lesion (Table 1; Fig. 3). GP activity in cells of the intima, isolated from the zone of lipid stains and fibrous plaques, calculated per milligram protein, was reduced by 1.6 and 2.5 times respectively, whereas in cells isolated from the media GP activity was reduced by 2.3 and 3.2 times respectively in these same types of atherosclerotic lesion (Table 1; Fig. 3).

The results thus point to a marked fall in enzymic utilization of $\dot{O}_2^-$ and lipid peroxides in the vessel wall affected by atherosclerosis. In turn, this must lead to accumulation of free-radical cytotoxic agents in the vessel wall and it is probably a factor triggering intensification of LPO in atherosclerosis [2].

LITERATURE CITED

1. A. V. Krushinskii and A. N. Orekhov, Byull. Vses. Kardiol. Nauchn. Tsentr. Akad. Med. Nauk SSSR, No. 2, 36 (1981).
2. V. Z. Lankin, Kardiologiya, No. 8, 42 (1980).
3. V. Z. Lankin, in: Biochemistry of Lipids and Their Role in Metabolism [in Russian], Moscow (1981), pp. 75-95.
4. V. Z. Lankin, E. N. Gerasimova, L. V. Kasatkina, et al., Kardiologiya, No. 6, 71 (1979).

5. V. Z. Lankin and S. M. Gurevich, Dokl. Akad. Nauk SSSR, 226, No. 3, 705 (1976).
6. V. Z. Lankin, A. N. Zakirova, L. V. Kasatkina, et al., Kardiologiya, No. 10, 69 (1979).
7. V. Z. Lankin, A. K. Tikhaze, N. V. Voronina, et al., Byull. Éksp. Biol. Med., No. 3, 327 (1981).
8. V. Z. Lankin, A. K. Tikhaze, and N. V. Kotelevyseva, Kardiologiya, No. 2, 23 (1976).
9. B. G. Khodzhakuliev, T. I. Torkhovskaya, L. G. Artemova, et al., Byull. Éksp. Biol. Med., No. 6, 675 (1980).
10. M. G. Culter and R. Schneider, Atherosclerosis, 20, 383 (1974).
11. J. Glavind, S. Hartmann, J. Clemmensen, et al., Acta Pathol. Microbiol. Scand., 30, 1 (1952).
12. H. A. Kontos, E. P. Wei, J. T. Povlishock, et al., Science, 209, 1242 (1980).
13. H. P. Misra and I. Fridovich, J. Biol. Chem., 247, 3170 (1972).
14. E. B. Smith, P. H. Evans, and M. D. Downham, J. Atheroscler. Res., 7, 171 (1967).
15. A. L. Tappel, Geriatrics, 23, 97 (1968).

ROLE OF BLOOD LIPOPROTEINS IN ADAPTIVE CHANGES IN RAT LIVER MITOCHONDRIA

L. E. Panin, D. I. Kuz'menko, and A. R. Kolpakov

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KEY WORDS: starvation; swelling of mitochondria; lipoproteins; apoproteins; cyclic AMP.

When the body is under intensive functional loading the liver mitochondria undergo complex structural and functional changes, an integral parameter of which is their reversible swelling. Its mechanism has not yet been explained. Adaptive hormones (glucocorticoids, catecholamines) have no direct effect on the mitochondria, and for that reason the action of their mediators is currently under active discussion. Among the latter an important role is played not only by cyclic nucleotides [1] but also, probably, by lipoproteins (LP). Under the influence of glucocorticoids the blood LP level rises [2].

Penetration of lipoprotein particles into hepatocytes takes place through the activity of highly specific receptors located on the outer surface of the plasma membranes [10]. Liver cells, which occupy key positions in metabolism of all classes of LP, can bind LP of all densities to their receptors and ingest them [15]. The study of the intracellular distribution of LP whose protein component was labeled with radioactive iodine, in experiments *in vivo* [7] and *in vitro* [6], showed the label to be present not only in the fraction of secondary lysosomes, where the final stages of degradation of lipoprotein particles take place, but also in fractions of nuclei, mitochondria, and microsomes. These facts provide a solid basis for explaining the effect of serum LP and their apoproteins on the activity of a wide range of biochemical processes taking place in the extracellular space and inside the cell [2, 4, 5, 8, 12-14].

Activation of serum LP metabolism under conditions of stress may have a significant effect on the state of oxidative phosphorylation in the liver mitochondria. It was shown previously [3] that different classes of blood LP, as well as their apoproteins, can activate mitochondrial ATPase in intact and starving rats and rats subjected to intensive physical exertion.

The object of this investigation was to determine the possible role of blood LP and their apoproteins in initiation of swelling of liver mitochondria as one stage in their adaptive changes during functional stress. The comined effect of apoproteins and cAMP, which has a specific effect on individual functions of mitochondria [1], on the swelling process also was investigated.

Laboratory of Biochemistry, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 9, pp. 50-52, September, 1982. Original article submitted April 6, 1982.

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