GLUTATHIONE AND SUPEROXIDE DISMUTASE REDOX ENZYME SYSTEM DURING DEVELOPMENT OF TOXICOINFECTIOUS SHOCK CAUSED BY MURINE PLAGUE TOXIN

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Data on changes in concentrations of cyclic nucleotides and prostaglandins E_1 and $F_{2\alpha}$ in the blood and various organs and on disturbance of carbohydrate metabolism under the influence of toxins of *Yersinia pestis*, obtained in the writers' laboratory [8-10], are evidence of a disturbance of intracellular homeostasis and of the mechanisms controlling it.

The activating effect of cAMP on glutathione-dependent enzymes, especially glutathione transferase (GT) and glutathione peroxidase (GP) from various tissues [5, 6], suggested the possibility of a combined change in the cAMP level and activity of glutathione-dependent enzymes under the influence of plague toxin. The time course of the cGMP concentration [8], in view of the effect of the redox status relative to guanylate cyclase activity [7], also is invariably accompanied by changes in peroxidation processes in the cell, and ought correspondingly to be reflected in the function of its antioxidative (AO) system.

Accordingly, bearing in mind the connection between glutathione metabolism and functioning of the adenylate- and guanylate-cyclase and prostaglandin-synthetase systems, when switched to the pathological level of regulation associated with the action of plague toxin, there can be no disputing involvement of the redox enzyme system of glutathione and superoxide dismutase (SOD) in the pathological process.

To broaden our ideas on the molecular mechanisms of toxicoinfectious shock in plague and to assess the state of the protective forces of the body, we studied the time course of activity of the AO system in tissue homogenates of rat lung and liver exposed to the action of murine plague toxin (PT).

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 150-170 g. Toxicoinfectious shock was induced by intraperitoneal injection of 1 ml of physiological saline containing 0.8 mg (LD_{100}) of an original preparation of toxin (Becker's fraction II). Control animals received an injection of the same volume of physiological saline. The rats were killed after 0.5, 1, 2, and 5 h with 3% hexobarbital solution (1.5 ml), the apex of the heart was excised, and after the blood had drained, the lungs were removed. The liver was perfused with 0.05 M K-phosphate buffer, containing 0.3 mM phenylmethylsulfonyl fluoride, pH 7.4. The organs were weighed and transferred into cold buffer (the same as mentioned above, 4°C) and 20% homogenates of liver and lung were prepared on a Braun homogenizer. They were then frozen in liquid nitrogen. The homogenates were kept when necessary at −20°C for 14-21 days.

SOD activity was determined by the method in [11] at 30°C, GT at 37°C, and GP activity (relative to hydrogen peroxide – GP-H_2O_2, and to *tert*-butyl hydroperoxide – GP-TBHP) at 30°C, by the method in [12]; glutathione reductase (GR) activity was determined at 30°C by the method in [1]. The protein content in the test material was determined by Lowry's method. The results were subjected to statistical analysis by Student's test [4].

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TABLE 1. Activity of Glutathione-Dependent Enzymes (in nmoles/mg protein) and of SOD (in units of activity/mg protein) in Organs of Healthy Animals ($M \pm m$; $n = 10$)

Test object	GT	GP-H_2O_2	GP-TBHP	GR	SOD
Liver	469±81,7	57,5±7,2	52,6±6,0	45,6±4,0	92,3±17,8
Lungs	190,8±17,4	108±8,9	95,5±10,5	60,3±6,3	65,1±11,4

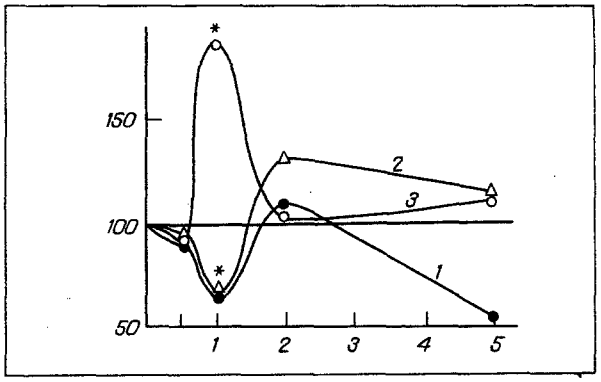

Fig. 1. Time course of activity of antioxidative enzymes in lung tissue after injection of PT. Here and in Figs. 2 and 3: abscissa, duration of action of toxin (in h); ordinate, differences from control, in %. 1) SOD, 2) GP-TBHP, 3) GR. Here and in Figs. 2 and 3, asterisk indicates significance of differences between parameters compared ($p < 0.05$).

EXPERIMENTAL RESULTS

As Table 1 shows, GT and SOD activity in the liver of intact (healthy) animals was higher than in the lungs by 2.5 and 1.5 times respectively ($p < 0.05$). Activity of the other enzymes studied was 1.5-2 times higher ($p < 0.05$) in the lung tissue, indicating the presence of a large reserve of AO activity of the lung.

The time course of activity of enzymes of the redox system of glutathione and SOD in the lung tissue reflects the early effect of the action of the murine PT: a 35% ($p < 0.05$) fall of the SOD and GP-TBHP levels coupled with an 87% ($p < 0.05$) rise for GR, 1 h after injection (Fig. 1). In the late stages of observation all the parameters studied returned to their control values. GP-H_2O_2 and GT activity did not differ statistically significantly throughout the experiment from the control level (data not shown). The opposite direction of the changes in the glutathione-reducing enzyme (GR) and enzymes oxidizing it (GP-TBHP, GP-H_2O_2, GT) will be noted, and is evidence of an inappropriate accumulation of reduced glutathione, and correspondingly, it reflects the inadequacy of function of the factors controlling glutathione-dependent enzymes. As a result the conditions were created for disturbance of the balance of the highly organized multicomponent AO system. An argument in support of the suggestion is the sharp increase (by 1.8-2 times, $p < 0.05$) in the ratios between activities of the enzymes GR/GT, GR/GP-TBHP, GR/GP-H_2O_2, 1 h after injection of the toxin (Fig. 2). By the end of the experiment the ratios between the pairs of components of the AO system did not differ from the control.

However, the pathological changes in the metabolic reserves of the OA system of the lung tissue, assessed by the parameter GR/GT · GP-H_2O_2 · GP-TBHP, arising in the early stages of toxicoinfectious shock, continued until the 5th hour of exposure to PT. The increase of 3.7 times in this potential ($p < 0.05$) after 1 h reflects accumulation of reduced glutathione relative to the oxidized fraction, and in conjunction with inhibition of its oxidation, this may be the result of depression of mitochondrial respiration and of oxidative phosphorylation and inhibition of activity of the pentose phosphate cycle [13]. The 5-h effect of PT consisted of lowering the potential as a result of exhaustion of the reserve capacity of the AO system and, correspondingly, the appearance of a deficiency of reduced glutathione, threatening destruction of the cell.

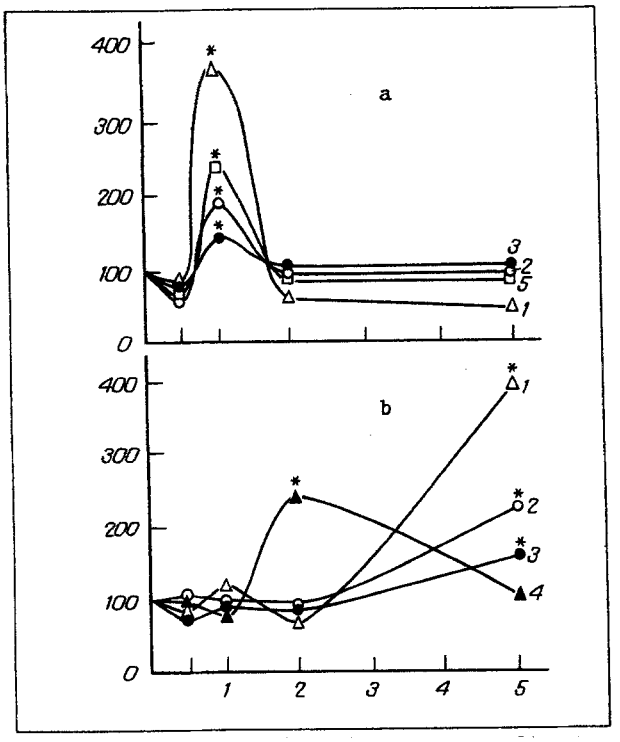

Fig. 2. Ratios of enzyme activities of detoxication system. a) In lung tissue, b) in liver tissue. 1) GR/GT · GP-TBHP · GP-H_2O_2; 2) GR/GP-TBHP; 3) GR/GT; 4) SOD/GP-H_2O_2; 5) GR/GP-H_2O_2.

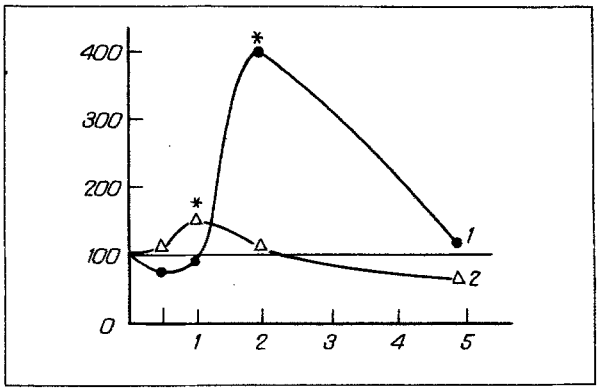

Fig. 3. Activity of AO enzymes of liver tissue during development of toxicoinfectious shock due to plague.

Considering the development of inflammatory changes, or even necrosis of the tissue, under the influence of PT [3], an increase in cellular permeability and outflow of enzymes from the cell can be postulated, and evidently determines the fall of GP-TBHP and SOD activity (Fig. 1). On the other hand, the pathomorphological changes in the cell lead to disturbance of the spatial micro-organization of the AO system and bring about the imbalance of its function described above, for close structural-functional correlation is known to exist between the components of this system [7]. The possibility of inactivation of enzyme biosynthesis and of a direct effect of the toxin on the enzymes likewise cannot be ruled out.

A fourfold ($p < 0.05$) increase in SOD activity 2 h after injection of PT, and a 1.5-fold increase for GP-TBHP 1 h after its injection were found in the liver (Fig. 3), evidently in response to intensification of free-radical and peroxidation processes in that organ. The sharp rise in the level of SOD, the most general cellular generator of hydrogen peroxide, against a background of unchanged activity of the enzyme of its utilization, namely GP-H_2O_2 (data not shown), are evidence of damage to the mechan-

isms of regulation of the glutathione and SOD redox system. This fact is illustrated by the increase of 150% ($p < 0.05$) in the SOD/GP-H_2O_2 ratio (Fig. 2b). The increase in GP-TBHP activity without any accompanying changes in GR (GR activity did not differ from the control at any of the points investigated) paves the way for imbalancing of the AO system in this organ also. The increase in the GR/GT ratio by 61% above the control values ($p < 0.05$) and of the GR/GP · TBHP ratio by 114% ($p < 0.05$) 5 h after injection of PT is evidence of a lasting imbalance of the AO system, characterizing profound disturbances of homeostasis of the liver cells in plague toxicosis. Accordingly the fourfold ($p < 0.05$) increase in the GR/GP-TBHP · GT · GP-H_2O_2 potential toward the end of the experiment can be regarded as an emergency measure taken by the cell to protect it against destruction on account of the reserves of reduced glutathione, through intensification of glutathione-reducing processes. However, this measure is of no essential importance because the accumulation of reduced products is accompanied by inhibition of their oxidation through inhibition of respiration [13] and accordingly, it prevents death of the cell.

Thus the imbalance of the AO system of lung tissue combined with activation of glutathione-reducing processes arising in the early periods of plague toxemia is replaced by exhaustion of its reserve capacity. This may be one of the main causes of the appearance of severe metabolic disturbances in this organ, leading to death of the animals. So far as the liver is concerned, these two factors do not develop in its cells until the 5th hour of the experimental model of this pathological process due to the possibly greater resistance of the liver to plague toxin.

LITERATURE CITED

1. A. M. Gerasimova, L. A. Koroleva, O. S. Bruso, et al., Vopr. Med. Khim., No. 1, 89 (1976).
2. A. M. Gerasimov, "The antioxidative enzyme system of the animal cytosol," Doctoral Dissertation, Medical Sciences, Moscow (1981).
3. T. I. Domaradskaya, "Antigens of the plague microbe isolated into the culture medium during its growth," Candidate's Dissertation, Medical Sciences, Saratov (1980).
4. G. N. Zaitsev, Mathematical Statistics in Experimental Botany [in Russian], Moscow (1984).
5. L. S. Kolesnichenko and V. I. Kulinskii, Role of Cyclic Nucleotides and Secondary Messengers in the Regulation of Enzyme reactions [in Russian], Petrozavodsk (1988), p. 126.
6. N. S. Manterova and L. S. Kolesnichenko, Role of Cyclic Nucleotides and Secondary Messengers in the Regulation of Enzyme Reactions [in Russian], Petrozavodsk (1988), p. 135.
7. A. S. Sobolev, Cyclic Nucleotides [in Russian], Minsk (1982), p. 141.
8. T. D. Cherkasova, P. R. Vengrov, V. I. Melikhov, et al., Zh. Mikrobiol., No. 7, 70 (1987).
9. T. D. Cherkasova, P. R. Vengrov, V. I. Melikhov, et al., Byull. Éksp. Biol. Med., No. 3, 313 (1988).
10. T. D. Cherkasova, P. R. Vengrov, V. A. Yurkiv, and V. P. Avrovov, Byull. Éksp. Biol. Med., No. 10, 428 (1988).
11. C. Beauchamp and I. Fridovich, Analyt. Biochem., 44, 276 (1976).
12. W. H. Habig, M. J. Pabst, and W. B. Jacoby, J. Biol. Chem., 249, 7130 (1974).
13. S. Kadis and S. J. Ajl, Microbial Toxins, T. Montie and S. Kadis (eds.), New York (1970), pp. 42-45.