EFFECT OF GLUTATHIONE ON CHOLEROGENIC DIARRHEA AND ON DISTRUBANCES OF THE ANTIOXIDATIVE SYSTEM OF RAT INTESTINAL AND LIVER CELLS

V. A. Yurkiv, N. I. Popkova, and A. M. Gerasimov

UDC 616.34-008.311.4-022.7:579.843.1]-092.9-07:[616.34+616.36]-008.939.626-074

KEY WORDS: glutathione; cholerogenic diarrhea; glutathione peroxidase; glutathione reductase; glutathione transferase; superoxide dismutase

It was shown previously that cholera toxin (CT) causes marked disturbances of the glutathione (GL) redox system of enzymes in intestinal and liver cells [6]. Probably cAMP-dependent reactions of protein phosphorylation, triggered by the toxin, directly or indirectly involve GL-dependent enzymes. Considering the important role of GL in the regulation of metabolic processes [1], including those mediated by cyclic nucleotides [11], it can be tentatively suggested that correction of disturbances of the GL system may be one way of modifying the cell response to CT.

In the investigation described below the effect of GL was studied on the severity of the diarrhea syndrome and the course of changes in activity of the GL redox system and of superoxide dismutase (SOD) in the intestine and liver or rats exposed to CT.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 120-140 g. The animals were deprived of food for 24 h before the experiments. The rats were anesthetized (2% hexobarbital solution, intraperitioneally), two ligatures were tied around the jejunum at a distance of 18-20 cm apart, and 0.5 ml of a solution of CT (7.5 μg/kg) and 2 ml of a solution of polyethylene-glycol 4000 (PEG, 100 μg PEG/1 ml), pH 4, were injected into the ligated segment, after which 0.3 ml of the contents of the loop was withdrawn in order to determine the initial PEG concentration. Instead of CT, the control animals received an injection of 0.5 ml of physiological saline.

The effect of GL on the development of diarrhea was studied in three series of experiments: preincubation of GL with CT, injection of GL into the intestine before CT, and injection of GL (intraperitioneally) after injection of CT into the intestine. In series I, 1 ml of 12 μM or 2.3 mM solution of GL was incubated with 0.5 ml of a 2.4 μM solution of CT (pH 7.4) at 37°C for 30 min, and the mixture was injected into the intestine. In series II, 1 ml of one of the above GL solutions was injected into the intestine, and 0.5 ml of the same solution of CT was injected 15 min later. In series III, GL solution (pH 7.4) was injected intraperitioneally in a dose of 1 g/kg body weight 15 min after injection of 0.5 ml of CT and PEG into the intestine. When the effect of GL on enzyme activity was studied in normal animals it was injected intraperitoneally in the same dose without ligation of the jejunum. The rats were decapitated under superficial ether anesthesia 4 h after closure of the abdomen and the contents of the ligated loop of small intestine were withdrawn in order to determine the final EEG concentration. The ratio of the initial to the final concentration of PEG served as the criterion of the degree of development of the diarrhea syndrome. The PEG concentration was estimated by the method in [15]. The liver was perfused and the ligated loop of small intestine was irrigated with 0.15 M Tris-HCl buffer, pH 8.0, containing 0.3 mM phenylmethylsulfonyl fluoride. Cytosol fractions were isolated from the liver and jejunal musosa as described previously [8]. The fractions were stored if necessary at −40°C for 7-10 days. Glutathione transferase (GT) activity was determined at 37°C with 1-chloro-2,4-dinitrobenzene by the method in [10], glutathione peroxidase (GP) activity was determined against hydrogen peroxide

Laboratory of Molecular Bases of the Pathogenesis of Infectious Diseases, Central Research Institute of Epidemiology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Pokrovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 10, pp. 435-438, October, 1985. Original article submitted November 2, 1984.

 0007-4888/85/0010-1362$09.50

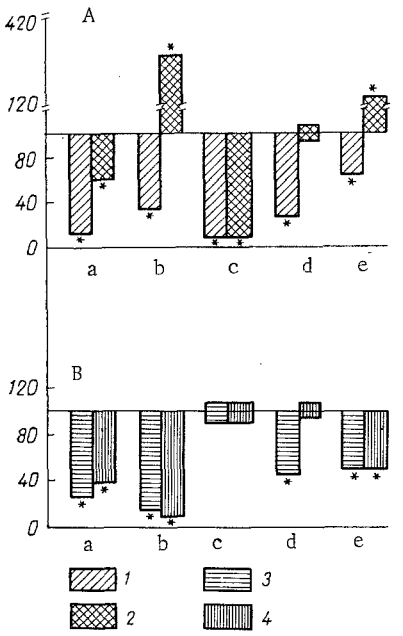

Fig. 1. Effect of GL on activity of enzyme of antioxidative system in rat jejunum under the influence of CT. Ordinate, activity of enzyme (in % of control). A) Control, B) animals undergoing operation. a) GT, b) GP-TBH, c) GP-H_2O_2, d) GR, e) SOD. 1) GL, 2) control for operation, 3) CT, 4) CT, 4) CT + GL. *P < 0.05.

and tert-butyl hydroperoxide (GP-H_2O_2 and GP-TBH) at 30°C [13], glutathione reductase (GR) activity was determined at 37°C as described in [2], and SOD activity was determined at 30°C by the method in [7]. Protein was estimated by Lowry's method [12]. The significance of differences between experimental and control values was established by Student's test [3].

EXPERIMENTAL RESULTS

During diarrhea the PEG concentration in the lumen of the intestine falls and the ratio of the original concentration to the final concentration (diarrhea coefficient) ought to be greater than 1. Under the experimental conditions described above, after the action of CT this coefficient was 1.9 ± 0.1. Intraperitoneal injection of GL significantly alleviated the diarrhea syndrome and the ratio between concentrations was 0.8 ± 0.08 (P < 0.05).

Since CT contains disulfide bonds, the possibility could not be ruled out that the protective action of GL was due to its penetration into the intestinal lumen, and the modification of the toxin, as was observed when unithiol was used [5]. However, after preincubation of 2.4 μM CT with GL in the ratios of 1:5 and 1:1000, and injection of the mixture into the intestinal lumen, the diarrhea coefficient was 1.8 ± 0.14 and 2.3 ± 0.2 respectively. If GL was injected into the intestinal lumen before CT in the same proportions, the diarrhea coefficient likewise did not fall (2.0 ± 0.24 and 2.0 ± 0.3). Hence it follows that blocking of the diarrhea syndrome by GL was due neither to its direct action on CT nor to extracellular modification of the receptor on the side of the muscosa. The possibility of a change in sensitivity of the intestinal cells to CT on account of GL-induced reorganization of metabolism cannot be ruled out.

The protective effect of exogenous GL against toxic agents, including poisoning with bacterial toxins, is well known [14], and is in agreement with an increase in the reserves of exogenous thiols, when depressed as a result of toxic action [9]. It was accordingly interesting to study whether the action of GL on cholerogenic diarrhea is connected with a change in activity of those enzymes which control the reduced GL level in the cell.

The level of GR activity in the intestinal mucosa of rats receiving GL after CT was much higher than in animals with diarrhea (Fig. 1C). A similar tendency also was observed in relation to GT activity in the mucosa and GR activity in the liver (Fig. 2). GR activity in the mucosa and liver was higher in this case than during diarrhea by 210 and 190% respectively (P < 0.05). GT activity in the small intestine was 150% higher (P < 0.05) than during diarrhea.

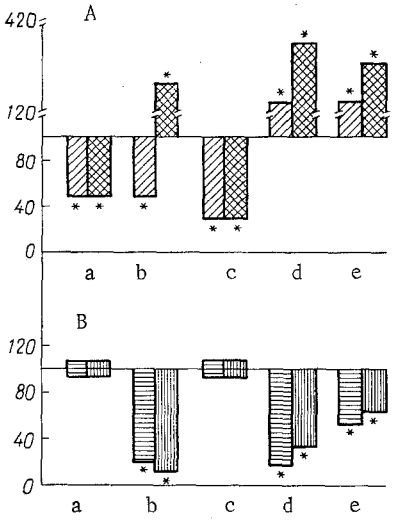

Fig. 2. Effect of GL on activity of enzymes of antioxidative system in rat liver under the influence of CT. Legend as to Fig. 1.

Meanwhile the trend of changes in enzyme activity of the control animals (operation alone or treatment with GL without operation) shows that the operation of ligation of the intestine itself, and injection of GL without the operation, are procedures that affect the activity of the enzymes studied both in the intestine and in the liver significantly, and sometimes in opposite directions (Figs. 1A and 2A). In particular, the effect of GL on GR and SOD activity in the liver (increase) and also on GT, GP-H_2O_2, and GP-TBH activity in both tissues (decrease) will be noted. The operation without injection of CT caused a decrease in GT and GP-H_2O_2 activity in the liver and mucosa, but an increase in GP-TBH, SOD, and GR activity in the liver and GP-TBH and SOD activity in the intestine. This means that changes in the enzyme systems of the liver and intestine observed in the ligated jejunum after injection of CT reflect, at least partially, the special nature of the experimental model rather than the specific features of the action of cholera toxin, and this makes interpretation of the mechanisms of pharmacologic action more difficult.

So far as the effect of GL on diarrhea is concerned, the most marked of the changes in enzyme activity discovered was stimulation or preservation of GR activity in the intestine under the influence of GL, when reduced by injection of CT (Fig. 1). Considering that activation of this lone enzyme of GL reduction takes place against the background of depressed levels of the other enzymes studied, which catalyze its oxidation, theoretically this ought to assist preservation of GL in the reduced state in the cell. The poly-functional character of intracellular GL [1] and, in particular, connection of its metabolism with prostaglandin synthetase and adenylate cyclase systems (key stages in the cascade of pathochemical and funcational manifestations of the action of cholera toxin) suggests that the antidiarrheal action of GL discovered in this investigation must be regarded as an element of strictly pathogenetic therapy.

LITERATURE CITED

1. A. M. Gerasimov, "Antioxidative enzyme system of the animal cytosol," Author's Abstract of Dissertation for the Degree of Doctor of Medical Sciences, Moscow (1981).
2. A. M. Gerasimov, L. A. Koroleva, O. S. Brusov, et al., Vopr. Med. Khim., No. 1, 89 (1976).
3. G. F. Lakin, Biometrics [in Russian], Moscow (1980).
4. V. I. Pokrovskii and V. A. Yurkiv, Ter. Arkh., No. 4, 102 (1981).
5. V. A. Yurkiv and V. I. Melikhov, Byull. Éksp. Biol. Med., No. 10, 432 (1981).
6. V. A. Yurkiv and N. I. Popkova, Byull. Éksp. Biol. Med., No. 8, 190 (1984).
7. C. Beauchamp and I. Fridovich, Anal. Biochem., 44, 276 (1971).
8. G. Clifton and H. Kaplowitz, Cancer Res., 37, 788 (1977).
9. C. D. Jeffries, J. Bact., 86, 1358 (1963).
10. W. H. Habig, M. J. Pabst, and W. B. Jakoby, J. Biol. Chem., 249, 7130 (1974).
11. J. Isaacs and F. Binkley, Biochim. Biophys. Acta, 498, 29 (1977).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).
13. D. E. Paglia and W. N. Valentine, J. Lab. Clin. Med., 70, 158 (1957).

14. D. C. Szymanski and C. D. Jeffries, Proc. Soc. Exp. Biol. (N.Y.), 129, 966 (1968).
15. A. Ty, Microbiol. J., 24, 287 (1979).