EFFECT OF SODIUM SELENITE ON GLUTATHIONE PEROXIDASE AND SUPEROXIDE DISMUTASE ACTIVITY IN THE TISSUES IN HERPETIC KERATITIS

Yu. A. Petrovich, N. A. Terekhina, and K. V. Shmagel'

UDC 617.713-002-022:578.832.1]-07:[617.713-008.931:577.152.1]-02:615.272.4.014.425

KEY WORDS: antioxidative enzymes; selenite; herpetic keratitis.

Intensification of lipid peroxidation (LPO) has been found in herpetic keratitis [2], the commonest virus disease of the human cornea [3]. However, information on the level of activity of the antioxidative enzymes of the cornea in herpetic keratitis is not available. The use of the antioxidant 6-hydroxypyridine in the combined treatment of herpetic keratitis has had a beneficial effect [2].

The aim of this investigation was to study glutathione peroxidase (GP) and superoxide dismutase (SOD) activity in the eye tissues in the course of infection of the rabbit cornea with type I herpes simplex virus (HSV) and also after administration of sodium selenite to these animals.

EXPERIMENTAL METHOD

Experiments were carried out on 50 adult chinchilla rabbits, divided into three groups. The control group (1) consisted of 10 healthy animals. The experimental rabbits were infected with strain I-c of HSV after instillation of a 1% solution of the local anesthetic amethocaine, by application of two drops of virus-containing fluid with a titer of 4 log CPD_{50}/ml to the scarified right cornea.

No selenite was injected into the infected rabbits of group 2 (30 animals). They were used in the experiments 2, 5-7, and 14 days after infection. After 2 days, pinpoint lesions of the corneal epithelium and pericorneal injection of blood vessels were observed in these animals, while after 5-7 days, at the height of the disease, opacity of the cornea, partial erosion of its epithelium, and its invasion by blood vessels were seen. The severity of the pathological changes after 14 days was somewhat reduced.

Starting from the day of infection, the infected rabbits of group 3 (10 animals) received 0.2 mg sodium selenite daily with their food.

TABLE 1. GP and SOD Activity in Tissues of the Anterior Part of the Healthy Rabbit Eye (M ± m)

Eye tissue	GP, μmoles NADPH/min/g tissue		SOD, conventional units/g tissue	
	right eye	left eye	right eye	left eye
Cornea				
epithelium	3.64±0.20	3.58±0.18	1470±46	1460±62
stroma	0	0	150±6	150±3
endothelium	2.8±0.15	2.9±0.23	960±12	930±28
Iris	0.31±0.02	0.32±0.02	190±8	190±11
Lens	1.74±0.22	1.88±0.24	110±6	120±5

Department of Biochemistry, Moscow Medical Stomatologic Institute. Department of Biochemistry, Perm' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Pokrovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 4, pp. 405-407, April, 1987. Original article submitted March 19, 1986.

0007-4888/87/0004-0455$12.50

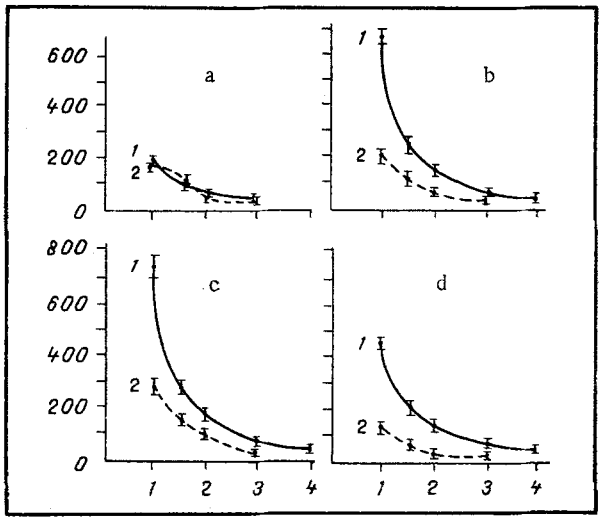

Fig. 1

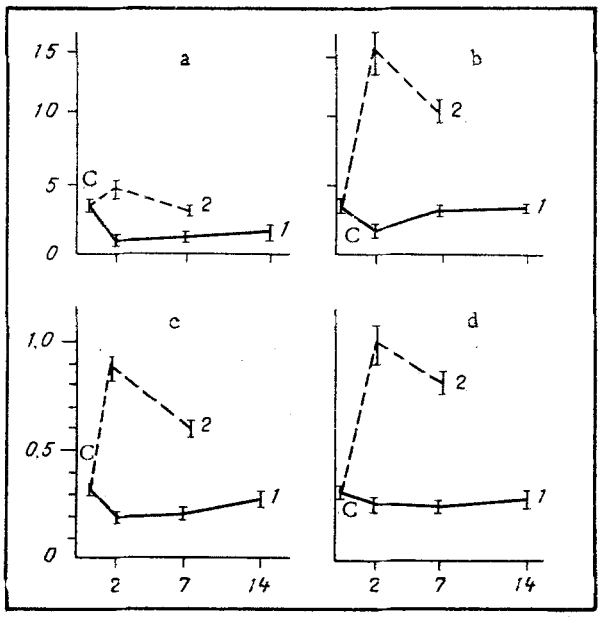

Fig. 2

Fig. 1. Intensity of corneal chemiluminescence after infection of right eye with HSV. Abscissa, time after beginning of contact of corneal homogenate with H_2O_2 and $FeSO_4$ (in min); ordinate, intensity of chemiluminescence (in counts/10 sec). 1) Right eye, 2) left eye. a) Healthy rabbit; b, c, d) 2, 5-7, and 14 days after infection.

Fig. 2. Effect of sodium selenite on GP activity in tissues of right (a, c) and left (b, d) eyes after infection of rabbit's right eye with HSV. Abscissa, time after infection (in days); ordinate, enzyme activity (in μmoles NADPH/min/g tissue). C) Control (healthy rabbit); 1) herpetic keratitis; 2) sodium selenite + herpetic keratitis. a, b) Corneal epithelium; c, d) iris.

Activity of GP [1] and SOD [10] in the epithelium, stroma, and endothelium of the cornea, in the iris, and lens of the animals was measured on an SF-16 spectrophotometer.

Chemiluminescence analysis was undertaken on corneal homogenates from five healthy and 15 infected rabbits. The corneas were excised around the limbus from enucleated eyes frozen in liquid nitrogen, quickly ground and transferred to scintillation flasks with 8 ml of 0.2 M phosphate buffer (pH 7.4), and kept for 30 min at 37°C. Next 1 ml of 1% H_2O_2 and 1 ml of 25 mM $FeSO_4$ were added. The number of counts in 10 sec was recorded on an SBS-2 scintillation counter (with one photoelectronic multiplier).

The numerical data were subjected to statistical analysis by Student's t test, with comparison of the various parameters determined for the right (infected) and left (contralateral) eyes of the same animal, and also comparing them with parameters for the eyes of normal rabbits.

EXPERIMENTAL RESULTS

The intensity of chemiluminescence of the corneas was increased by 2.5-3 times, 2, 5-7, and 14 days after infection with HSV (Fig. 1). GP activity was higher in the epithelium and endothelium of the cornea than in the other tissues of the eye (Table 1). GP activity in the lens was about 50% lower, and in the iris it was lower by an order of magnitude. In the corneal stroma no GP activity could be found. SOD activity in the epithelium and endothelium of the cornea was several times less than in the iris and an order of magnitude higher than in the lens.

With respect to the distribution of levels of GP and SOD activity, the tissues of the rabbit eye were basically similar to those of the human's, cow's, rat's, and dog's eyes [9, 12, 14].

Both GP and SOD activity was reduced in the infected iris and epithelium and endothelium of the infected cornea (Fig. 2). GP activity in the corneal epithelium after 2 and 5-7 days was several times lower than in the control. GP activity in the corneal epithelium and iris was reduced at these times by 1.5 times. SOD activity in the epithelium of the infected corn-

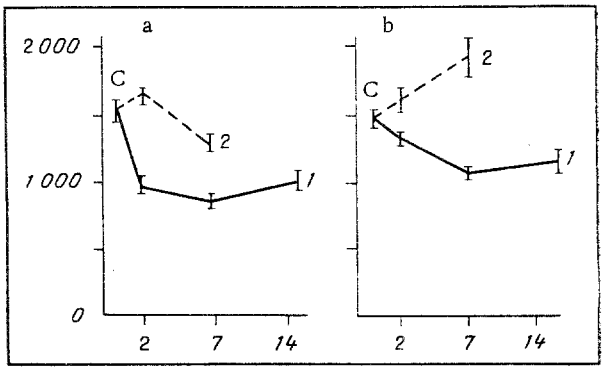

Fig. 3. Effect of sodium selenite on SOD activity in corneal epithelium after infection of right eye of a rabbit with HSV. Abscissa, time after infection (in days); ordinate, enzyme activity (in conventional units/g tissue). C) Control (healthy rabbit). 1) Herpetic keratitis; 2) sodium selenite + herpetic keratitis. a, b) Corneal epithelium of right and left eye, respectively.

ea was reduced by one-third (Fig. 3). No changes in GP or SOD activity were found in the lens of the infected eye. Activity of these enzymes in the tissues studied 14 days after infection was closely similar to that in healthy animals. Only SOD activity at this time remained rather lower in the cornea and iris.

The decrease in activity of both enzymes was less marked in the cornea and iris of the contralateral eye 2 and 5-7 days after infection than in the infected eye.

GP activity in the corneal epithelium of the right (infected) eye was increased several times under the influence of sodium selenite after 2 and 5-7 days compared with its level in rabbits not receiving selenite. An increase in activity of this enzyme also was observed in the corneal epithelium of the left (contralateral) eye (Fig. 2). In the iris GP activity after 2 and 5-7 days was higher in rabbits receiving selenite than in infected rabbits not receiving selenite. The increase in GP activity under the influence of sodium selenite is understandable, because GP is a selenium-dependent enzyme [6].

As regards the effect of sodium selenite on SOD activity, it can be postulated that the mechanism of its action is indirect. We know, for instance, that an important role in the effect of HSV on intracellular enzymes is played by the action of the virus on biomembranes with activation of LPO [2, 5]. This is confirmed by the results of chemiluminescence analysis (Fig. 1). The decrease in SOD and GP activity may to some extent be the result of hydrolysis of the enzymes by lysosomal proteinases, whose free activity rises when the eye is infected with HSV [7]. Sodium selenite, by preventing damage to the membranes, reduces the outflow of lysosomal proteinases, and reduces proteolysis of the cytoplasmic proteins, including SOD and GP. The antioxidative effect of sodium selenite is also determined by its inhibitory effect on xanthine oxidase [11], a "supplier" of the superoxide anion [10].

To attempt to explain the reaction of the contralateral eye, it must be noted that it was infected latently by HSV [4]. The HSV genome in the latent form is located in the corresponding ganglion of the trigeminal nerve [6, 13] and one of the principal pathways of spread of HSV in the body is the neuronal path.

Activation of LPO in infection of the eye with HSV is thus due, besides other factors, to a certain extent to inhibition of GP and SOD, and administration of sodium selenite prevents the reduction of activity of the antioxidant system under these circumstances.

LITERATURE CITED

1. D. V. Gutkin and Yu. A. Petrovich, Byull. Éksp. Biol. Med., No. 1, 33 (1982).
2. B. S. Kasavina, Yu. F. Maichuk, L. D. Dalgat, et al., Byull. Éksp. Biol. Med., No. 7, 27 (1982).
3. A. A. Kasparov, Abstracts of Proceedings of the 6th All-Union Congress of Ophthalmologists [in Russian], Vol. 6, Moscow (1985), pp. 3-14.
4. V. I. Loktina, Abstracts of Proceedings of the 3rd All-Russian Congress of Ophthalmologists [in Russian] Vol. 2, Moscow (1975), pp. 323-326.

5. Yu. A. Petrovich, N. G. Gol'dfel'd, and N. A. Terekhina, Oftal'mol. Zh., No. 1, 52 (1986).
6. Yu. A. Petrovich and R. P. Podorozhnaya, Usp. Sovrem. Biol., 91, No. 1, 127 (1981).
7. Yu. A. Petrovich, N. A. Terekhina, V. Yu. Maichuk, et al., Abstracts of Proceedings of the 3rd All-Union Symposium on Medical Enzymology [in Russian], Astrakhan' (1979), p. 11.
8. Yu. A. Petrovich and N. A. Terekhina, Vopr. Virusol., No. 6, 643 (1977).
9. K. C. Bhuyan and D. K. Bhuyan, Biochim. Biophys. Acta, 542, 28 (1978).
10. I. Fridovich, Annu. Rev. Biochem., 44, 147 (1975).
11. U. Kela and R. Vijayavaeguja, Curr. Sci. (India), 49, 392 (1980).
12. T. M. Redmond, E. J. Duke, W. H. Coles, et al., Exp. Eye Res., 38, 369 (1984).
13. K. G. Warren, R. G. Marusyk, M. E. Lewis, et al., Arch. Virol., 73, 85 (1982).
14. D. R. Whinehart and K. Garner, Ophthalm. Res., 10, 189 (1978).

EFFECT OF ETHANOL AND THE CATALASE INHIBITOR AMINOTRIAZOLE ON LIPID PEROXIDATION IN THE RAT MYOCARDIUM

L. F. Panchenko, S. V. Pirozhkov, S. V. Popova, and V. D. Antonenkov

UDC 616.127-008.939.15-39-02:615.917:547.262]-092.9

KEY WORDS: ethanol; catalase; microperoxisomes; lipid peroxidation; antioxidants; myocardium.

The peroxisomal enzyme catalase, which takes part in the detoxication of hydrogen peroxide, is one component of the antioxidative protective system of the cell [3, 5]. It was shown previously that during long-term combined administration of 3-amino-1,2,4-triazole (aminotriazole) and ethanol ultrastructural changes characteristic of the morphological picture of alcoholic cardiomyopathy in man are observed in the cardiomyocytes of rats [9]. In the case of separate administration of the two substances the pathological process does not develop. The necessity for inactivation of catalase when the experimental model of alcoholic cardiomyopathy is created may indicate a probable role of peroxide processes in the pathogenesis of this disease.

The aim of this investigation was to study the effect of chronic administration of ethanol and aminotriazole on the level of lipid peroxidation (LPO) in the rat myocardium. The action of natural (vitamin E, reduced glutathione) and artificial (dibunol*) antioxidants on alcohol-induced lipid peroxidation also was studied.

EXPERIMENTAL METHOD

Male Wistar rats weighing initially 160-180 g were used. The animals were kept on a semisolid diet, balanced with respect to the principal components (proteins, lipids, carbohydrates), which included vitamins and mineral salts [1]. Parallel experiments were carried out on rats of four groups: 1) control, 2) ethanol, 3) aminotriazole, 4) ethanol + aminotriazole. The animals of groups 2 and 4 received ethanol (34-36% of the total calorific value of the diet, 10-12 g/kg body weight/day) as part of their diet. Aminotriazole, in the form of a 10% aqueous solution, was injected intraperitoneally in a dose of 1 g/kg 3 times a week. The total duration of the experiments was 12 weeks. The rats were deprived of food and the last injections of aminotriazole were given 18-20 h before sacrifice. Immediately after decapitation blood was collected from the animals, and the ethanol concentration in it was determined by gas chromatography. The ethanol concentration in the blood at the time of sacrifice of the animals did not exceed 0.5-0.7 mM. After thoracotomy the heart was perfused with cold isolation medium (0.15 M KCl, 20 mM Tris-HCl buffer, pH 7.4). The organ was removed and quickly frozen in dry ice, after which pieces of tissue were kept at −80°C for not more than 2-3 weeks. The heart tissue was homogenized in isolation medium in a Dounce homogenizer

*4-Methyl-2,6-di-tert-butylphenol.

All-Union Research Center for Medico-Biological Problems in the Prevention of Drunkenness and Alcoholism, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 4, pp. 407-410, April, 1987. Original article submitted July 9, 1986.

 0007-4888/87/0004-0458$12.50