Acta Biochimica Polonica

Vol. 43 No. 2/1996

403–406

QUARTERLY

Short Communication

The activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in erythrocytes of rats with experimental neoplastic disease

Jolanta Batko, Teresa Warchoł and Halina Karoń

Department of Physiological Chemistry, University of Medical Sciences, Święcickiego 6, 60–781 Poznań, Poland

Received: 13 October, 1995; Accepted: 24 May, 1996

Key words: superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, erythrocytes, Morris hepatoma

In erythrocytes of rats bearing Morris hepatoma 5123 the activities of superoxide dismutase, glutathione peroxidase and glutathione reductase as well as the level of reduced glutathione increased on the 10th day after transplantation of the tumor. In the second phase of the tumor growth (20 days after transplantation), the activities of glutathione peroxidase, glutathione reductase and the level of reduced glutathione in erythrocytes of the experimental animals were lower than in controls, whereas the activity of superoxide dismutase was at that time higher than in controls. On the other hand, the activity of catalase did not significantly differ from that found in healthy rats.

The oxidoreductases: superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase form a system which protects the cell against lesions induced by superoxide and hydroxyl radicals, and against formation of secondary radicals. Reduced glutathione is also involved in these processes.

Erythrocytes are known to be exposed to high concentrations of oxygen and its reduction products [1], superoxide ion being continuously formed on oxidation of oxyhaemoglobin to methaemoglobin. This ion is transformed by dismutation into hydrogen peroxide which is decomposed by glutathione peroxidase and catalase. In the presence of high amounts of H_2O_2 in the reactions of Haber-Weiss and Fenton, may produce the hydroxyl radical, the most toxic among oxygen radicals. Free oxygen radicals and hydrogen peroxide, when present at concentrations exceeding physiological values, can cause lesions of cellular membranes by oxidating polyunsaturated fatty acids. They can also lead to a damage of membrane proteins, mainly by oxidation of their thiol groups.

Intensive studies performed on erythrocytes as a convenient cellular model have demonstrated that in many diseases the activities of enzymes of the cellular antioxidative system were altered [2–4]. The activities of glutathione peroxidase, superoxide dismutase and catalase were altered in patients with diabetes [5], β-thalassemia [6, 7], sickle cell anemia [8], malignant lymphoma [9] and the Down syndrome [10]. In all those patients glutathione metabolism was also altered.

The development of neoplastic disease is accompanied by increased formation of free radicals within the cells, among them erythrocytes, which can lead to their destruction. Therefore it seemed reasonable to study the effect of a neoplastic disease on those erythrocyte enzymes which are responsible for preventing

formation or for scavenging of active forms of oxygen.

MATERIALS AND METHODS

Male white Buffalo rats, about 10 weeks old and weighing about 200 g, were used. A homogenate of Morris 5123 hepatoma tissue was injected bilaterally into thigh muscles as described previously [11]. Activities of the enzymes and the content of reduced glutathione in erythrocytes were determined on the 10th and 20th day after tumor transplantation, and compared with values for erythrocytes from control animals. Blood for the experiments was withdrawn into heparinized tubes by punction of the left heart ventricle and centrifuged at $1000 \times g$ for 10 min at 4°C. Plasma and the upper layer of blood particles containing blood platelets, leukocytes and reticulocytes were removed. Erythrocytes were washed with 3 vol. of NaCl solution (154 mmol/l, pH 7.4) and hemolysed in 10 vol. of distilled water for 15 min. The hemolysate was centrifuged at $3000 \times g$ for 15 min at 4°C, and in the supernatant the activities of the enzymes and the content of reduced glutathione and hemoglobin were determined.

Superoxide dismutase activity was assayed according to Beauchamp & Fridovich [12] and catalase activity was determined according to Beutler [13]. Glutathione peroxidase activity was assayed by the method of Paglia & Valentine [14] and glutathione reductase activity according to Benöhr *et al.* [15]. The content of reduced glutathione was determined by the method of Anderson [16], and haemoglobin concentration according to Drabkin & Austin [17].

For the experiments, groups of 6 animals each, were used. Statistical analysis of the results was performed by the Student's *t*-test.

RESULTS AND DISCUSSION

The results obtained point to differences in the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and reduced glutathione content in erythrocytes of rats bearing Morris 5123 hepatoma, as compared with the values obtained for healthy animals (Table 1).

In the initial phase of the tumor growth (10 days after transplantation) both the activities of all the enzymes studied and the content of reduced glutathione were increased in erythrocytes. On the 20th day of the hepatoma growth, the activities of glutathione peroxidase, glutathione reductase and the level of reduced glutathione were lowered.

The concentration of reduced glutathione in tissues is dependent on both its synthesis and regeneration [18]. Oxidized glutathione is reduced by glutathione reductase which utilizes NADPH formed in the pentosephosphate cycle, mainly in the reaction catalysed by glu-

Table 1

Activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-R) and the content of reduced glutathione in erythrocytes of rats with Morris hepatoma 5123

	SOD (U/g Hb)	CAT (IU/mg Hb)	GSH-Px (IU/g Hb)	GSH-R (IU/g Hb)	GSH (μmol GSH/g Hb)
Control animals	580.2 ± 30.7	51.4 ± 3.2	15.4 ± 0.8	5.8 ± 0.5	4.2 ± 0.4
10 days after transplantation	664.4 ± 42.4[b]	68.4 ± 7.1[b]	18.0 ± 1.4[b]	7.3 ± 0.8[b]	6.3 ± 0.7[b]
20 days after transplantation	680.8 ± 48.2[b]	53.7 ± 4.8	10.4 ± 1.7[a]	4.0 ± 0.4[a]	3.2 ± 0.4[a]

Values are expressed as means ± SD.

[a]Values significantly lower ($P \leq 0.001$) than those for control rats.

[b]Values significantly higher ($P \leq 0.001$) than those for control rats.

The unit of superoxide dismutase (U) was defined as the amount of the enzyme causing 50% inhibition of the rate of reduction of nitro blue tetrazolium [12]. The activities of other enzymes were expressed in international units (IU) defined as the amount of the enzyme catalyzing the reaction of 1 μmole of the substrate per minute at 25°C. All values were expressed per g of haemoglobin.

cose-6-phosphate dehydrogenase. On the 20th day after transplantation, glutathione reductase activity in erythrocytes was decreased, that of superoxide dismutase increased, whereas catalase activity did not differ from that in the control animals. Similar results were obtained by Gonzales *et al.* [19] who studied the activities of these enzymes in erythrocytes of patients with neoplastic diseases.

Our earlier studies on the effect of experimental tumor on the metabolism and structure of erythrocytes demonstrated changes in the rate of glycolysis and of enzymes of the pentose-phosphate cycle [20] as well as changes in the lipid and carbohydrate composition of cell membranes [21, 22]. In simultaneous studies on the number of reticulocytes and erythrocytes and the hemoglobin content in the blood of Morris 5123 hepatoma bearing rats and of healthy animals, it was found that development of the tumor was accompanied by anemia. The latter, expressed by lowered erythrocyte content and decreased level of hemoglobin appeared after more than 20 days after tumor transplantation. On the other hand, increased amounts of reticulocytes were observed beginning with the 10th day of tumor growth. It seems that the observed alterations in the activity of the redox system enzymes and in the content of reduced glutathione were due both to the neoplasmatic process and the accelerated erythropoesis in the rat organism.

Our results support the view that the neoplastic disease is not limited to tissues directly attacked by the tumor but evokes a number of changes in other organs and tissues, as exemplified by erythrocytes.

REFERENCES

1. Uyesaka, N., Hasegawa, S., Ishioka, N., Ishioka, R., Shio, H. & Schechter, A.N. (1992) *Biorheology* **29**, 217–229.
2. Olivieri, O., Girelli, D., Trevisan, M.T., Bassi, A., Zorzan, P., Bambara, L.M. & Corocher, R. (1991) *J. Rheumatol.* **18**, 1263–1264.
3. Descamps-Latscha, B., Nguyen, A.T., Golub, R., Baruchel, A., Debray-Sachs, M. & Assan, R. (1984) in *Oxygen Radicals in Chemistry and Biology* (Bors, W., Saran, M. & Tait, D., eds.) pp. 965–968, Walter de Gruyter, Berlin.
4. Bhuyan, D. & Bhuyan, K. (1983) in *Oxy Radicals and Their Scavenger Systems Cellular and Medical Aspects* (Greenwald, R.A. & Cohen, G., eds.) pp. 343–348, Elsevier Biomedical Press, Amsterdam.
5. Murakami, K., Kondo, T., Ohtsuka, Y., Fujiwara, Y., Shimada, M. & Kawakami, Y. (1989) *Metabolism* **38**, 753–758.
6. Concetti, A., Massei, P., Rotilio, G., Brunori, M. & Rachmilewitz, E.A. (1976) *J. Lab. Clin. Med.* **87**, 1057–1054.
7. Gerli, G.C., Beretta, L., Pellegata, A. & Agostini, A. (1980) *Scand. J. Haematol.* **25**, 87–92.
8. Das, S.K. & Nair, R.C. (1980) *Br. J. Haematol.* **44**, 87–92.
9. Bewick, M., Coutie, W. & Tudhope, G.R. (1987) *Br. J. Haematol.* **65**, 347–350.
10. Percy, E.M., Dalton, A.J., Marcovic, V.D., Crapper Mc Lachlan, D.R., Hummel, J.T., Rusk, A.C.M. & Andrews, D.F. (1990) *Am. J. Med. Genet.* **35**, 459–467.
11. Torlinski, L. (1972) *Ann. Med. Sect. Pol. Acad. Sci.* **18**, 233–248.
12. Beauchamp, Ch. & Fridovich, I. (1971) *Anal. Biochem.* **44**, 276–287.
13. Beutler, E. (1984) in *Red Cell Metabolism;* 3d edn. pp. 89–90, Grune & Stratton, Philadelphia.
14. Paglia, D.E. & Valentine, W.N. (1967) *J. Lab. Clin. Med.* **70**, 158–169.
15. Benöhr, H. Chr., Tigges, F.J. & Waller, H.D. (1974) *Klin. Wschr.* **52**, 474–477.
16. Anderson, M.E. (1985) *Methods Enzymol.* **113**, 548–555.
17. Drabkin, D.L. & Austin, J.M. (1935) *J. Biol. Chem.* **112**, 52–65.
18. Fujii, S., Dale, G.L. & Beutler, E. (1984) *Blood* **63**, 1096–1101.
19. Gonzales, R., Auclair, Ch., Voisin, E., Gautero, H., Dhermy, D. & Boivin, P. (1984) *Cancer Res.* **44**, 4137–4139.
20. Warchoł, T. (1975) *Ann. Med. Sect. Pol. Acad. Sci.* **20**, 39–46.
21. Batko, J., Płotast-Necas, B., Warchoł, T. & Karoń, H. (1992) *Acta Biochim. Polon.* **39**, 317–326.
22. Batko, J. & Karoń, H. (1994) *Acta Biochim. Polon.* **41**, 13–16.