Acta Biochimica Polonica

Vol. 44 No. 4/1997

767–770

QUARTERLY

This work was presented at the VIII Biennial Meeting of International Society for Free Radical Research in Barcelona on 1–5 October, 1996
Short communication

The effect of experimental neoplastic disease on malondialdehyde level and glutathione status in erythrocytes of rats

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Received:06 May, 1997; accepted: 12 October, 1997

Key words: malondialdehyde, glutathione, erythrocytes, Morris hepatoma

The level of lipid peroxidation products and the content of glutathione in erythrocytes of rats with Morris 5123 hepatoma at different stages of tumor development were examined. The content of endogenous malondialdehyde (MDA) was increased throughout all periods of tumor development as compared to the results for healthy rats. From the extent of MDA generation under oxidative stress we concluded that erythrocytes of Morris 5123 hepatoma bearing rats were more susceptible to autoxidation than those from control rats. The content of reduced glutathione (GSH) and oxidized glutathione (GSSG) was increased at the early stage of tumor growth. At the advanced stage of the disease both the content of GSH and the GSH/GSSG ratio were decreased while the content of GSSG remained at the elevated level.

The development of neoplastic disease is accompanied by increased levels of free radicals within cells, among them within erythrocytes, which eventually can lead to their destruction [1]. Erythrocytes are known to be exposed to high concentrations of oxygen and its reduction products. Free oxygen radicals and hydrogen peroxide, when present at concentrations exceeding physiological values, can cause damage to cellular membranes by oxidation of polyunsaturated fatty acids. They can also damage membrane proteins, mainly by oxidation of their thiol groups [2]. The glutathione redox system is one of the most important intracellular antioxidant system. The objective of this study was to determine the impact of an experimental neoplastic disease on lipid peroxidation and on glutathione content in red blood cells (RBC).

MATERIAL AND METHODS

Buffalo rats about 10 weeks of age were used in the experiments. Homogenized Mor-

Abbreviations: GSH, reduced glutathione; GSSG, oxidized glutathione; MDA malondialdehyde; RBC, red blood cells.

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ris hepatoma 5123 tissue was injected bilaterally into their thigh muscles. The compounds to be evaluated were measured on the 10th and 20th day after tumor transplantation in erythrocyte suspension prepared according to Stocks *et al.* [3]. The results were compared with those obtained for control, healthy rats of matched age.

The susceptibility of erythrocyte lipids to autoxidation (peroxidation potential) was assayed by the method of Stocks *et al.* [3] by addition of hydrogen peroxide solution to erythrocyte suspension and measuring the MDA level after 2 h.

Endogenous malondialdehyde content of erythrocyte suspension for control rats and for rats on the 10th and 20th day after tumor transplantation was measured spectrophotometrically as described by Stocks *et al.* [3].

Reduced and oxidized glutathione concentrations in erythrocytes were measured spectrophotometrically according to Anderson [4].

For the experiments, groups of 6 animals each were used. The results were evaluated statistically by means of the Student's *t*-test; mean values are presented together with standard deviations (±S.D.).

RESULTS AND DISCUSSION

The results obtained point to differences in the contents of endogenous MDA, glutathione and susceptibility of RBC lipids to autoxidation between rats with a neoplastic disease and healthy animals.

Malondialdehyde determination has showed that in animals with the transplanted tumor tissue the contents of both the endogenous MDA and MDA formed during *in vitro* treatment with hydrogen peroxide for 2 h were increased 10 days after transplantation of neoplastic cells (Table 1), and remained high till the 20th day of experiment. These results show that RBC of neoplastic rats are more susceptible to lipid peroxidation.

Erythrocyte GSH and GSSG contents and the GSH/GSSG ratio of control rats and from rats with Morris hepatoma are shown in Table 2. On the 10th day after tumor transplantation the contents of GSH and GSSG were raised in erythrocytes of rats with neoplastic disease. However, the GSH/GSSG ratio did not change. On the 20th day of hepatoma growth, the level of reduced glutathione decreased even below control values while the level of oxidized glutathione remained increased. The GSH/GSSG ratio in erythrocytes of tumor-bearing rats was significantly decreased on the 20th day after tumor transplantation.

Table 1. The effect of Morris hepatoma 5123 on the levels of endogenous malondialdehyde (MDA) and susceptibility of red blood cell (RBC) lipids to autoxidation (peroxidation potential)

	Endogenous malondialdehyde		Peroxidation potential	
	nmol/ml RBC	nmol/g Hb	nmol MDA/ ml RBC	nmol MDA/g Hb
Control animals	0.5 ± 0.02	1.2 ± 0.1	172 ± 10	465 ± 16
10 Days after tumor transplantation	0.8 ± 0.06[b]	2.4 ± 0.1[b]	219 ± 16[b]	712 ± 30[b]
20 Days after tumor transplantation	1.0 ± 0.06[b]	3.2 ± 0.1[b]	242 ± 16[b]	808 ± 42[b]

[b] Significantly higher ($P < 0.01$) than for control rats.

Our earlier studies on the effect of experimental neoplastic disease on the structure of erythrocytes demonstrated changes in the lipid and carbohydrate composition of RBC membranes [5, 6]. Erythrocyte membranes from tumor-bearing rats had a lower content of cholesterol than those from control rats. The phospholipid content of the membranes was similar to that of the control group. The molar ratio of cholesterol to phospholipid was significantly decreased (from 1.03 down to 0.70) both 10 and 20 days after transplantation.

Table 2. The effect of Morris hepatoma 5123 on the levels of reduced (GSH) and oxidized (GSSG) glutathione and their molar ratios in red blood cells (RBC)

	Reduced glutathione		Oxidized glutathione		GSH/GSSG ratio
	µmol/ml RBC	µmol/g Hb	nmol/ml RBC	nmol/g Hb	
Control rats	1.5 ± 0.1	4.4 ± 0.1	97.0 ± 5.1	278.8 ± 12.2	15.7 ± 1.6
10 Days after tumor transplantation	1.9 ± 0.1[b]	5.3 ± 0.2[b]	116.2 ± 4.0[b]	317.0 ± 7.8[b]	16.5 ± 0.9
20 Days after tumor transplantation	1.0 ± 0.1[a]	3.4 ± 0.1[a]	126.3 ± 5.1[b]	417.4 ± 8.6[b]	8.0 ± 0.2[a]

[a]Significantly less ($P < 0.01$) than for control rats. [b]Significantly higher ($P < 0.01$) than for control rats.

The abnormal increase of endogenous MDA and MDA after 2 h under oxidative stress in RBC of rats with a neoplastic disease could be the result of a high initial unsaturated lipid peroxidation or an impairment of antioxidant protective mechanisms. Increased lipid peroxidation in RBC during the neoplastic disease can reflect a shift in the delicate balance that normally exists between free radical production and the antioxidant defense systems. Glutathione plays an important role in the protection of cells against oxidation by free radicals and reactive oxygen intermediates [7]. The concentration of GSH in tissues is dependent on both its synthesis and regeneration. Glutathione reductase and glutathione peroxidase play the most important roles in regulating GSH and GSSG concentrations. Changes of GSH and GSSG levels in RBC of rats with Morris hepatoma 5123 were accompanied by changes in activities of glutathione reductase and glutathione peroxidase [8]. Similar observations were made by Gonzales *et al.* [9].

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.

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