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Acquired Disturbance of Erythrocyte Glutathione Reductase in Experimental Tumors

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Summary. Erythrocyte glutathione reductase activity was assayed in animals bearing Walker, Ehrlich, and Krebs tumors, 21 days after implantation. It was noticed that the activity was significantly enhanced in all three tumors.

Key words: Erythrocyte glutathione reductase – Acquired enzyme defects

Introduction

Acquired defects of erythrocyte enzymes have been described in human malignancies, especially in hematological disturbances (Kahn 1981).

Some possible mechanisms have been considered, such as partial reversion to a fetal form of erythropoiesis (Rochant et al. 1972), quantitative and qualitative disturbances (Valentine et al. 1973), somatic mutations (Kahn et al. 1972), and posttranslational alteration of some enyzmes in malignant cells (Arnold et al. 1974), so that increases and decreases in erythrocyte enzymes have been reported (Boivin et al. 1975).

It is well known that tumor cells depend more on the glycolytic pathway to match their energy requirements and that reduced glutathione is important for protein synthesis (Kosower and Kosower 1978), which led us to study the activity of glutathione reductase.

In this study we monitored the erythrocyte glutathione reductase in animals bearing Ehrlich, Krebs, and Walker tumors to search for the possible effects on erythrocyte metabolism in experimental conditions.

Material and Methods

Three kinds of experimental tumors were used, namely Ehrlich and Krebs tumors implanted in Swiss mice and Walker carcinosarcoma 256 implanted in Wistar rats. Tumors were obtained by injecting 0.1 ml of a suspension of 10^5 cells SC in the hind paw of the mice and the dorsal region of the rats.

For each tumor studied the animals were divided into four groups: a control group, a group that received riboflavin phosphate dissolved in the drinking water (0.1 mg/ml), a tumor group, and a tumor group receiving vitamin B_2 (0.1 mg/ml) in the drinking water.

At 21 days after implantation, blood was collected in ACD by aspiration from the occular sinus of mice and from cardiac puncture of rats. The blood was kept at 4 °C for glutathione reductase assay within 5 days.

Glutathione reductase determination was performed by Beutler's method (1975) at 37 °C, using a Gilford 2451 recording spectrophotometer.

The results were submitted to statistical analysis of variance and groups were compared with the control group by Dunnet's test, $P<0.05$ being taken as statistically significant (Berquó et al. 1980).

Results

The results are presented in Table 1.

Animals bearing the three types of tumors studied showed a significant increase of erythrocyte glutathione reductase levels. The addition of riboflavin to the diet lowered enzyme activity in the Krebs tumor to the control level. The results after stimulation with flavin adenine dinucleotide (FAD) are not presented but correlated to those without FAD.

Discussion

In the three types of tumors used there was a significant increase of erythrocyte glutathione reductase levels compared with control groups. This enzyme also depends on the nutritional condition of each animal, being sensitive to deficiency of vitamin B_2 (Beutler 1969). Some groups received supplementary riboflavin, but in the Krebs tumor group the addition of this vitamin paradoxically lowered enzyme activity to that of the matching control group.

Boivin et al. (1975) studied the glutathione reductase in blood samples of 41 patients with myeloid leu-

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Table 1. Values of eryhtrocyte glutathione reductase: Analysis of variance of the results

Tumor	Glutathione reductase in I.U./g Hb/min (mean ± SEM)	No. of animals	Dunnett
Walker			
1. Control	1.16 ± 0.09	10	–
2. Control + riboflavin	0.97 ± 0.18	12	n.s.
3. Tumor	2.91 ± 0.28	09	*
4. Tumor + riboflavin	2.85 ± 0.25	09	*
Krebs			
1. Control	6.45 ± 0.62	12	–
2. Control + riboflavin	7.07 ± 0.52	15	n.s.
3. Tumor	10.78 ± 1.8	10	*
4. Tumor + riboflavin	8.85 ± 1.23	10	n.s.
Ehrlich			
1. Control	8.89 ± 0.69	20	–
2. Control + riboflavin	12.27 ± 1.05	20	n.s.
3. Tumor	25.27 ± 4.17	17	*
4. Tumor + riboflavin	17.61 ± 2.07	17	*

SEM, standard error of the mean; n.s., not significant; * significant ($P < 0.05$)

kemia and found an increase in 17 patients and a decreased level in 2. In 9 patients with myelomonocytic leukemia the activity of glutathione reductase was enhanced. The author did not find significant variation of the enzyme in other hematological diseases.

As glutathione reductase activity is directed at maintaining reduced glutathione levels, it is feasible to suppose that in our series the animals presented high levels of reduced glutathione (GSH). The increase in glutathione reductase could be a consequence of the neoplastic process, due to a metabolite perhaps acting against the suppressive effect on the synthesis of the enzyme. Acquired erythrocyte enzyme defects have been encountered in hematological diseases, even suggesting preleukemia states. Our experiments have shown up a certain erythroid disturbance in nonhematological neoplasias, suggesting that cancer has a very broad spectrum of action in the whole organism, leading to metabolic changes in tissues not directly affected by tumor growth.

In hematological diseases the activity of some erythrocyte enzymes increases while that of others decreases. In the experimental tumors studied we observed an increase of glutathione reductase, suggesting that tumoral proliferation induces a disturbance in the gene control mechanism or, by posttranslational action, in the erythrocyte precursors, increasing glutathione reductase synthesis.

Our results in the three groups of animals show that the activity of erythrocyte glutathione reductase was lower in animals receiving riboflavin after tumor implantation than in animals with tumor inplants that did not receive riboflavin. The explanation of this finding requires further studies.

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