HIGH ANTIOXIDATIVE ENZYME ACTIVITY IN TUMORS IS A FACTOR MAKING THE IMMUNE SYSTEM "OUT OF CONTROL"

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UDC 616-006-008.931-07-092.9

KEY WORDS: malignant tumors; catalase; glutathione peroxidase; glutathione-S-transferase; glutathione reductase; glucose-6-phosphate dehydrogenase; glutathione.

Among the characteristic properties of metabolism of malignant tumors of varied histogenesis and degree of differentiation are the virtually complete absence of lipoperoxides in them and the extremely low rate of formation of lipid peroxidation (LPO) products, even under the influence of activators of this process [3, 4]. Meanwhile lipids extracted from tumors can undergo peroxidation in vitro, and inhibition of the enzyme system by sulfhydryl groups also leads to an increase in the velocity of LPO in tumor cell homogenates [3, 4]. It has accordingly been postulated [3, 4] that the cause of the resistance of tumor cells to LPO is high activity of antioxidative enzymes: superoxide dismutase, catalase (CAT), glutathione peroxidase (GP), glutathione-S-transferase (GT), and glutathione reductase (GR), which participate in the utilization of active forms of oxygen and of lipoperoxides [9, 10]. Since the cytotoxicity of effector cells of the system of natural antitumor resistance (NATR), such as macrophages and neutrophils, includes the generation of active forms of oxygen and also, probably, activation of LPO [6, 14], the resistance of tumors to LPO may be the result of selection of clones of transformed cells for this feature, under the influence of the effector cells of NATR. In fact, the sensitivity of tumor cells to the action of active forms of oxygen and of activated macrophages rises sharply during inhibition of the coupled enzymic system for utilization of lipoperoxides and H_2O_2, including GR and GP [14]. The presence of molecular oxygen is essential for the realization of oxygen-dependent cytotoxicity, and for that reason the action of macrophages and neutrophils ought to be expressed mainly in the peripheral regions of growing solid tumors.

With the above considerations in mind, it was decided to study activity of antioxidative enzymes, and concentrations of reduced and oxidized glutathione in the peripheral and internal zones of a chemically induced tumor in rats.

EXPERIMENTAL METHOD

As the carcinogen we used 9,10-dimethyl-1,2-benzanthracene (DMBA), a solution of which in dimethyl sulfoxide (DMSO) was injected subcutaneously into Wistar rats weighing 160 ± 10 g, 3 times at weekly intervals in a dose of 5 mg DMBA in 1.5 ml DMSO per rat. The rats were decapitated 3 months after the beginning of the injections, when the average size of the tumors was 1.5 × 1.5 cm. Histologically the tumors were identified as fibrosarcomas. To determine activity of the various enzymes, peripheral parts of the tumor tissue not more than 3 mm thick, including the capsule, cental parts of the tumor with no visible signs of necrosis, and areas of skeletal muscle adjacent to the tumor were taken. The tissues were homogenized at 4°C in 50 mM phosphate buffer, pH 7.4, containing 1 mM EDTA (1:9, w/v). The resulting homogenate was centrifuged at 4°C (30,000g) for 40 min. Enzyme activity was determined in the supernatant. CAT activity was measured on the basis of hydrogen peroxide utilization [7], GP activity in a coupled glutathione reductase system on the basis of oxidation of NADPH with tert-butyl hydroperoxide as the substrate [12], GT activity as the formation of conjugates of 1-chloro-2,4-dinitrobenzene (CDNB) with glutathione [5], GR activity as oxidation of NADPH in the presence of oxidized glutathione [8], and glucose-6-phosphate dehydrog-

Laboratory of Tumor Immunology, Research Institute of Oncology, Tomsk Scientific Center, Academy of Medical Sciences of the USSR. Department of Human Cardiovascular Pathology, Institute of Clinical Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 4, pp. 467-469, April, 1989. Original article submitted June 30, 1988.

 0007-4888/89/0004-0530$12.50

TABLE 1. Activity of Antioxidative Enzymes and Glutathione Concentration in Central and Peripheral Parts of a Chemically Induced Fibrosarcoma and in Skeletal Muscle of Rats (M ± m)

Enzymes studied	Tumor		Skeletal muscle
	central part	peripheral part	
	1	2	3
CAT, μmoles H_2O_2/min/mg protein	2,9±1,0 (5) $p_{1,2}<0,01$	7,4±3,4 (5) $p_{2,3}<0,01$	1,9±0,9 (6)
GT, nmoles GSH/min/mg protein	49,4±20,0 (5) $p_{1,2}<0,01$	159,0±72,0 (5) $p_{2,3}<0,05$	90,6±37,0 (5) $p_{1,3}<0,05$
GP, nmoles GSH/min/mg protein	28,1±9,3 (19) $p_{1,2}<0,01$	56,8±14,0 (8) $p_{2,3}<0,01$	17,0±5,0 (8) $p_{1,3}<0,05$
GR, nmoles NADPH/min/mg protein	5,0±0,5 (4) $p_{1,2}<0,05$	10,3±3,0 (5) $p_{2,3}<0,01$	4,7±1,6 (7)
G6PDH, μmoles NADP/min/mg protein	4,4±1,2 (4) $p_{1,2}<0,05$	11,1±1,5 (5) $p_{2,3}<0,01$	0,6±0,1 (5) $p_{1,3}<0,01$
Reduced glutathione, μg/g tissue	571,7±106,9 (10) $p_{1,2}<0,01$	356,3±48,9 (5) $p_{2,3}<0,01$	416,3±96,4 (12) $p_{1,3}<0,01$
Oxidized glutathione, μg/g tissue	245,4±89,3 (11)	225,2±96,1 (5) $p_{2,3}<0,05$	146,9±22,9 (11) $p_{1,3}<0,01$

Legend. Number of experiments in parentheses.

enase (G6PDH) activity as reduction of NADP in the presence of glucose-6-phosphate [2]. Concentrations of oxidized and reduced glutathione were measured spectrofluorometrically [11]. The protein concentration in the samples was determined as in [13]. The following reagents were used: GR (Boehringer, West Germany), tert-butyl hydroperoxide (Merck, West Germany), sodium glucose-6-phosphate, oxidized and reduced sodium-NADP and oxidized and reduced glutathione (Reanal, Hungary), CDNB (Serva, West Germany). The results were subjected to statistical analysis by the nonparametric Wilcoxon–Mann–Whitney U test [1].

EXPERIMENTAL RESULTS

The results showed that activity of all the antioxidative enzymes studied (CAT, GP, GT, GR) was 2-3 times higher, and that of G6PDH 2.5 times higher, in the peripheral parts than in the central parts of the tumor (Table 1). Differences in activity of the antioxidative enzymes in the peripheral and central parts of the tumor, however, ought to have been even greater, because when material was taken from the peripheral zones of the tumor, removal of some tissue from the inner zone, with lower enzyme activity, could not be avoided. Activity of CAT and GP in the central zones of the tumor was nevertheless higher than the activity of these enzymes in the adjacent skeletal muscle, but GT activity was higher in the muscle than in the central parts of the tumor, and GR activity within the tumor and in the muscle was identical. The concentration of oxidized glutathione in the peripheral and central parts of the tumor was identical, whereas the concentration of reduced glutathione in the peripheral

part of the tumor was more than 33% lower than in the central part, and together with the topography of GR activity which was noted, this may be evidence of the more effective utilization of lipoperoxides in the peripheral parts of the tumor. The possibility of active bioregeneration of glutathione in the peripheral parts of the tumor also was reflected by characteristic differences in activity of the system for enzymic reduction of $NADP^+$ (G6PDH) the peripheral and central parts of the tumor. The results are evidence that during induction of LPO by active forms of oxygen, enzymic protection in the peripheral parts of the tumor must be much more effective than in the central parts.

The results thus are in accordance with the view that antioxidative enzymes play an important role in determining the resistance of tumor cells to the cytotoxic action of macrophages and neutrophils. Incidentally, one result of the selection of tumor cells for high resistance to LPO may be that they have cross resistance to pro-oxidative factors such as ionizing radiation and antitumor antibiotics. The topographic featurs of the antioxidative enzymes in solid tumors must therefore be taken into account when rational immuno-, radio-, and chemotherapy of tumors is planned.

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