Biochemistry (Moscow), Vol. 66, No. 2, 2001, pp. 221-224. Translated from Biokhimiya, Vol. 66, No. 2, 2001, pp. 273-278.

Role of Glutathione-Dependent Peroxidase in Regulation of Lipoperoxide Utilization in Malignant Tumors

E. G. Gorodzanskaya*, V. B. Larionova, G. N. Zubrikhina, N. G. Kormosh, T. V. Davydova, and K. P. Laktionov

Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Kashirskoe Shosse 24, Moscow, 115478 Russia; fax: (095) 323-5788

Received May 3, 2000
Revision received June 6, 2000

Abstract—Glutathione content, the activity of glutathione-dependent enzymes (glutathione reductase, glutathione peroxidase, and glutathione S-transferase), and also SOD (superoxide dismutase) and catalase were studied in human malignant tumors (uterus, breast, and ovaries) and normal tissues. Glutathione level and the activity of glutathione-dependent enzymes were 2-3 times higher in the malignant tumors than in normal tissues. A negative correlation between the level of glutathione and glutathione-dependent enzymes (glutathione peroxidase and glutathione S-transferase) in tumors and the efficacy of postoperative chemotherapy may characterize the degree of tumor resistance to chemotherapy and therefore may have prognostic value. Low SOD and catalase activity and high activity of glutathione-dependent enzymes in tumors suggest that glutathione peroxidase and glutathione S-transferase play a major role in peroxide utilization in malignant tumors.

Key words: glutathione, glutathione-dependent enzymes, lipid peroxidation, malignant tumors

Glutathione (GSH) is a polyfunctional non-protein thiol. It is involved into numerous biochemical processes including protein synthesis, regulation and expression of cell cycle genes, hydroperoxide reduction during lipid peroxidation (LPO), and the detoxification of various xenobiotics [1, 2]. The level of reduced glutathione is controlled both by *de novo* synthesis and by reduction of oxidized glutathione by the coupled NADPH–glutathione peroxidase system [3]. In a normal cell increase in GSH level is usually associated with increased mRNA expression and catalytic activity of γ-glutamyl cysteine synthetase, the first enzyme of GSH biosynthesis [2, 4]. Under pathological conditions glutathione level is significantly influenced by enzyme activities regulating the content of its oxidized and reduced forms; this may influence anti-peroxidation processes [5]. Being involved in the utilization of peroxides and hydroxyl radicals, glutathione is one of the major components of the antioxidant protective system of the cell [1, 2]. Glutathione, glutathione reductase, glutathione peroxidase, and glutathione S-transferase are the main components of the glutathione-dependent anti-peroxide system, a part of the basic antioxidant system in humans [4, 5]. Using NADPH, these enzymes protect cells against peroxide stress. Reactive oxygen species and LPO processes play an important role in the development of various diseases, including cancer [2, 4-7]. Changes in the activity of the antioxidant system are not only responsible for the accumulation of toxic LPO intermediates influencing body homeostasis; they can promote proliferation of tumor cells, which is directly linked to regulatory functions of biological membranes and the intracellular content of O_2^- radicals [8]. Glutathione also promotes tumor resistance to chemotherapy [9]. So, in the present study we investigated the activity of glutathione-dependent enzymes as one of the possible mechanisms regulating glutathione level in malignant tumors.

MATERIALS AND METHODS

Glutathione content and activity of glutathione-dependent enzymes were investigated in 64 malignant tumors of the female reproductive system (uterus body, 16; breast, 21; ovaries, 27). The material was obtained from patients who had not received antitumor treatment and who underwent surgery at clinics of the Blokhin Cancer Research Center. The diagnosis was verified after histological examination according to the WHO histological tumor classification. In most cases, tumor morphology was characterized as adenocarcinoma of various degrees of differentiation. Normal tissues taken

Abbreviations: GP) glutathione peroxidase; GR) glutathione reductase; GT) glutathione S-transferase.

* To whom correspondence should be addressed.

0006-2979/01/6602-0221$25.00 ©2001 MAIK "Nauka/Interperiodica"

from 18 women who died from accidental traumas were used as controls.

Tissues were homogenized in the cold for 30 sec in 0.04 M Tris-HCl buffer, pH 7.4. The homogenate (1 : 4 w/v) was centrifuged under refrigeration (4°C) for 30 min at 100,000*g*. The supernatant was used for determination of enzyme activities. Superoxide dismutase (SOD) activity (EC 1.15.1.1) was assayed spectrophotometrically at 560 nm using the xanthine–xanthine oxidase system [10]. One unit of SOD activity was defined as the amount of SOD required for 50% inhibition of the xanthine oxidase reaction. Activity of catalase (EC 1.11.1.6) was assayed by H_2O_2 utilization [11]. GSH content was determined spectrophotometrically at 340 nm in the reaction with 5,5-dithio-bis(2-nitrobenzoic acid) after preincubation with glutathione reductase and NADPH for 20 min [12]. Activity of glutathione reductase (GR; EC 1.6.4.2) was determined spectrophotometrically at 340 nm by NADPH oxidation in the presence of oxidized glutathione [13]. One unit of GR activity was defined as the amount of the enzyme required for reduction of 1 μmole of oxidized glutathione per min. Activity of glutathione peroxidase (GP; EC 1.11.1.9) was assayed spectrophotometrically at 340 nm using H_2O_2 as the substrate; NADPH oxidation was registered in the coupled glutathione reductase system in the presence of reduced glutathione [14]. One unit of GP activity was defined as the amount of enzyme required for oxidation of 1 μmole of NADPH per min. Activity of glutathione S-transferase (GT; EC 2.1.5.18) was determined spectrophotometrically at 340 nm by formation of a conjugate with 1-chloro-2,4-dinitrobenzene in the presence of reduced glutathione [15]. One unit of GT activity was defined as the amount of enzyme required for conjugation of 1 μmole of GSH per min.

All measurements were carried out using a Beckman DU-650 spectrophotometer (USA). Results are expressed per g of tissue.

All chemicals were purchased from Sigma (USA).

The statistical analysis of the data was carried using the PC program Statistica for Windows and Student's *t*-test. Correlation was evaluated using Pearson's criterion.

RESULTS AND DISCUSSION

Healthy organs of the female reproductive system (uterus, breast, and ovaries) are characterized by low levels of glutathione and glutathione-dependent enzymes (Table 1). No significant changes were found in glutathione content and glutathione-dependent enzymes in these organs. There were no significant tissue variations of SOD and catalase activity (Table 1). This suggests good coupling between formation and utilization of H_2O_2 in normal tissues.

Malignant tumors of uterus, breast, and ovaries possess significantly lower activity of antioxidant enzymes: SOD (by 34.3, 34.7, and 47.1%, respectively) and catalase (35.9, 55.1, 40.4%, respectively). Similar changes were observed in malignant tumors of lungs, stomach, and kidneys [16]. Human malignant tumors are also characterized by reduced content of the main natural antioxidants (α-

Table 1. Parameters of the antioxidant system in human normal tissues and malignant tumors

Material	Glutathione	Glutathione reductase	Glutathione peroxidase	Glutathione S-transferase	SOD	Catalase
Uterus						
normal (18)	0.24 ± 0.03	0.48 ± 0.04	1.01 ± 0.03	1.31 ± 0.03	460.5 ± 31.8	2.31 ± 0.06
tumor (16)	0.43 ± 0.05*	0.81 ± 0.07*	1.19 ± 0.05*	1.50 ± 0.04*	302.6 ± 28.3*	1.48 ± 0.09*
Breast						
normal (18)	0.26 ± 0.02	0.42 ± 0.03	0.80 ± 0.02	0.76 ± 0.03	402.6 ± 14.5	2.54 ± 0.02
tumor (21)	0.49 ± 0.06*	0.68 ± 0.04*	0.95 ± 0.03*	1.05 ± 0.04*	262.9 ± 28.1*	1.14 ± 0.06*
Ovaries						
normal (18)	0.29 ± 0.02	0.57 ± 0.02	1.06 ± 0.03	1.46 ± 0.03	451.2 ± 21.4	1.71 ± 0.04
tumor (27)	0.65 ± 0.08*	0.96 ± 0.04*	1.25 ± 0.04*	1.85 ± 0.05*	238.8 ± 26.1*	1.02 ± 0.05*

Note: Glutathione content is expressed as μmole per g of tissue. Activity of glutathione-dependent enzymes is expressed as μmole/min per g of tissue. Activity of SOD and catalase is expressed as U per g of tissue. Number of analyzed samples is given in brackets.

* $p < 0.0005$ (tumor versus normal).

tocopherol, retinol) [17]. Reduced activity of enzymatic and non-enzymatic pathways of utilization of reactive oxygen species suggests that the glutathione-dependent antiperoxide system plays an important role in the reduction of lipid hydroperoxides in tumor cells.

The study of glutathione content and activity of glutathione-dependent enzymes revealed significant changes of these parameters in the malignant tumors. Glutathione level was 2-3 times higher in malignant tumors than in normal tissues (Table 1). The highest glutathione level was found in ovary malignant tumors (Table 1). However, it should be noted that glutathione content could vary significantly in the same type of malignant tumors [2, 18]. GT plays an important role in the regulation of glutathione level in cells; this enzyme catalyzes the formation of glutathione conjugates with cytotoxic agents and therefore protects cells against various cytotoxic effects [1]. GT activity in malignant tumors of uterus, breast, and ovaries was 14.5, 38, and 26.7% higher than in the controls. Activity of other glutathione-dependent enzymes (GR and GP) was also 1.5-2 times ($p < 0.05$) higher in these tumors than in the normal organs. This suggests that GSH level in tumor cells is significantly influenced by changes in GP activity.

Significant variations in glutathione content can be subdivided into two groups and may be related to the efficacy of chemotherapy. Postoperative progression of the malignant process during chemotherapy was usually associated with a high level of glutathione in tumor cells. On the contrary, low glutathione level in malignant tissues corresponded to successful postoperative chemotherapy (Table 2). However, glutathione content was always higher in tumors than in normal organs.

Study of glutathione content in tumors obtained during surgical operations and subsequent clinical examination of patients with ovarian cancer receiving chemotherapy suggest that irrespectively of tumor localization, in its tissue origin and histological features there was a negative correlation between low level of glutathione in tumors and effectiveness of chemotherapy ($r = -0.81$, $p = 0.0003$). Similar results were obtained in the case of breast cancer ($r = -0.77$, $p = 0.005$). In all uterus tumors there was a high level of glutathione, and this could be one of the reasons of low efficacy of postoperative chemotherapy for this disease.

Most authors believe that inborn resistance of tumor cells to alkylating agents can be attributed to the combination of high level of glutathione and high GT activity rather than to high level of glutathione alone [9]. In fact, comparison of glutathione level and GT activity with efficacy of chemotherapy revealed highly significant negative correlation between biochemical parameters and efficacy of chemotherapy ($r = -0.88$, $p = 0.0005$).

Thus, results of the present study confirm that the combination of high level of glutathione and high GT activity suggests resistance of tumor cells to chemotherapy (Table 2). Taking into consideration similar data obtained on erythrocytes from patients with malignant tumors [19], this parameter (glutathione level + GT activity) predicts resistance/sensitivity to chemotherapy. No correlation was found between efficacy of chemotherapy and activity of SOD and catalase (Table 2). It is possible that reduction of enzymes of the first line of cell defense against free radicals, SOD and catalase [6], does not influence mechanism(s) of tumor cell resistance to chemotherapy. It is also possible that reduction of glutathione concentration in tumor cells and low GT activity may be the key factor reflecting simultaneous impairments of two processes, detoxification of anti-tumor drugs and reduction of hydroperoxides, each of which can significantly influence the effectiveness of chemotherapy.

Reduced glutathione, GSH, is involved in the detoxification of H_2O_2 and organic hydroperoxides. Its level depends not only on the rates of synthesis and degradation, but also on GR activity, which determines GSH regeneration. The biological importance of GR consists in the recovery of GSH level in cells without changes in its synthesis. This is especially important under conditions of malignant growth because some evidence exists that such protein kinases as protein kinase C involved in proliferative processes are stimulated by reactive oxygen species and are highly sensitive to LPO products [20]. Increased GSH content inhibits protein kinase C; the latter abolishes inhibition of γ-glutamyl cysteine synthetase (a key enzyme in GSH biosynthesis) by phosphorylation [21]. It is reasonable to suggest

Table 2. Dependence of effectiveness of chemotherapy of patients with ovary cancer on the activity of antioxidant system found in ovary tumors

Treatment	Glutathione	Glutathione reductase	Glutathione peroxidase	Glutathione S-transferase	SOD	Catalase
Effective	0.36 ± 0.03	1.07 ± 0.04	1.06 ± 0.05	1.05 ± 0.04	245.3 ± 18.8	1.05 ± 0.05
Ineffective	0.94 ± 0.03*	0.82 ± 0.05	1.39 ± 0.07*	2.65 ± 0.05*	232.4 ± 11.6	0.98 ± 0.06

Note: Glutathione content is expressed as μmole per g of tissue. Activity of glutathione-dependent enzymes is expressed as μmole/min per g of tissue. Activity of SOD and catalase is expressed as U per g of tissue.

* $p < 0.001$ (effective versus ineffective).

that under high activity of protein kinase C (typical for malignant cells) and consequent inhibition of GSH biosynthesis, the activities of glutathione-dependent enzymes are primarily responsible for changes in glutathione content.

However, comparison of GR activity with glutathione content did not show a reliable correlation. In some cases high glutathione content was found with a modest increase of the enzyme activity and *vice versa* (data not shown). Such uncertainty of these results suggests possible involvement of many factors into the control of GSH level. Besides GR (which depends on NADPH concentration), they obviously include other components of the glutathione anti-peroxide system such as Se-GP; the latter plays the major role in peroxide utilization under pathological conditions characterized by accumulation of products of phospholipase hydrolysis (polyunsaturated fatty acids) [22].

Low activity of catalase in tumor cells can result in accumulation of H_2O_2 and therefore in inactivation of SOD. This may cause accumulation of reactive oxygen species in the tumor. Under these conditions, the role of GP responsible for peroxide utilization increases. GP-dependent reduction of hydroperoxides prevents progression of LPO and appearance of toxic metabolites. The rate of this reaction depends on the concentrations of thiol compounds (especially of GSH) and so increase in tissue thiols may promote increase in GP activity and reduction of oxygen radical content.

Comparison of GP activity in tumors and normal tissues revealed that in all tumors the enzyme activity was significantly higher than in normal control sample tissues ($p < 0.05$). There was a correlation between GP activity and GSH content (correlation coefficient for ovary, breast, and uterus cancers was 0.36, 0.37, and 0.40, respectively; $p < 0.05$). Similar data were obtained during studies of experimental tumors [23].

The dependence of GSH content on GP and GT activity suggests that only a combination of glutathione-dependent peroxidases can influence glutathione level and provide protection of tumor cells against LPO products (Table 1). The known resistance of tumor cells to free radical lipid oxidation may stem from high activity of their lipid peroxidation systems [24].

The increase in GSH content and activity of glutathione-dependent enzymes in malignant tumors may be important for regulation of peroxide utilization. Since the activity of SOD and catalase and also the content of natural antioxidants regulating non-enzymatic LPO [17] are reduced, it is relevant to suggest that the glutathione system plays the major role in peroxide inactivation in tumor cells. Impairments of the functioning of the antioxidant system may influence cell proliferation and differentiation and promote malignant transformation of cells. Numerous data suggest the involvement of reactive oxygen species and radical-dependent oxidative reactions in cell proliferation, tumor cell transformation, and programmed cell death (apoptosis) [25]. The polyfunctional properties of the glutathione system and its direct involvement in the regulation of free radical processes should be employed for the development of new approaches for the improvement of effectiveness of chemotherapy of patients with malignant tumors, especially during the development of protocols of high-dose chemotherapy.

This work was supported by the Russian Foundation for Basic Research (grants No. 98-04-49186 and 00-04-49461).

REFERENCES

1. Golikov, S. N., Sanotsky, I. V., and Tiunov, L. A. (1986) *General Mechanisms of Toxic Action* [in Russian], Meditsina, Leningrad.
2. Vinam, J. (ed.) (1990) *Glutathione Metabolism and Physiological Functions*, Boston.
3. Meister, A. (1973) *Science*, 33-39.
4. Kulinsky, V. I., and Kolesnichenko, L. S. (1990) *Usp. Sovr. Biol.*, **110**, 20-33.
5. Mazo, V. K. (1998) *Ros. Z. Gastroenterol. Gepatol. Koloproktol.*, **1**, 47-53.
6. Lankin, V. Z., Tihaze, A. K., and Belenkov, Yu. N. (2000) *Kardiologiya*, No. **7**, 58-71.
7. Schwartsburd, P., and Lankin, V. (1995) *Medical Oncol.*, **11**, 101-110.
8. Oberley, L., and Buettner, G. (1979) *Cancer Res.*, **39**, 1141-1149.
9. Belousova, A. K. (1993) *Molecular Biology Approaches to Tumor Chemotherapy* [in Russian], VINITI, Moscow.
10. Beauchamp, Ch., and Fridovich, I. (1971) *Anal. Biochem.*, **44**, 276-287.
11. Beers, R., and Sizer, J. (1952) *J. Biol. Chem.*, **195**, 133-140.
12. Tietze, F. (1969) *Anal. Biochem.*, **27**, 502-522.
13. Yusupova, L. B. (1989) *Lab. Delo*, **4**, 19-21.
14. Paglia, D., and Valentine, W. (1967) *J. Lab. Clin. Med.*, **70**, 158-169.
15. Habig, W., Pabst, M., and Jakoby, W. (1974) *J. Biol. Chem.*, **249**, 7130-7139.
16. Mikhaevitch, O., Mikhaevitch, I., and Gorodzanskaya, E. (1995) *J. Exp. Clin. Canc. Res.*, **14**, 247-253.
17. Gorodzanskaya, E. G., Patutko, Yu. I., and Sagaidak, I. V. (1995) *Vopr. Onkol.*, **41**, 47-51.
18. Lee, F., Vessey, A., Rofstad, E., Siemann, D., and Sutherland, R. (1989) *Canc. Res.*, **49**, 5244-5248.
19. Gorodzanskaya, E. G., Koroleva, E. Yu., Egorova, N. I., Larionova, V. B., Garin, A. M., and Kushlinsky, N. E. (1998) *Byul. Eksp. Biol. Med.*, **125**, 562-565.
20. Maltseva, E. L., Kurnakova, N. V., Burlakova, E. B., and Palmina, N. P. (1990) *Biokhimiya*, **55**, 471-479.
21. Kalinina, E. V., Novichkova, M. D., and Komissarova, I. A. (1999) *Byul. Eksp. Biol. Med.*, **128**, 56-59.
22. Lankin, V. A., Tihaze, A. K., Osis, Yu. G., Vikhert, A. M., Scheve, T., and Rapoport, S. (1985) *Dokl. Akad. Nauk SSSR*, **281**, 204-207.
23. Navarro, J., Obrador, E., Carretero, J., Petschen, I., Avino, J., Perez, P., and Estrela, J. (1999) *Free Rad. Biol. Med.*, **26**, 410-418.
24. Lankin, V. Z., and Gurevich, S. M. (1974) in *Lipids in Animal and Human Bodies* [in Russian], Nauka, Moscow, pp. 72-77.
25. Menshchikova, E. B., and Zenkov, E. B. (1997) *Usp. Sovr. Biol.*, **117**, 155-171.