Role of Glutathione-S-Transferase in Tumor Drug Resistance

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The content of glutathione and glutathione-S-transferase activity in erythrocytes are determined in patients with testicular and ovarian cancer and melanoma before and after chemotherapy. The initial levels of glutathione and glutathione-S-transferase activity in patients susceptible to drug therapy are substantially lower than in drug-resistant patients. Complex platinum-based chemotherapy increases the content of glutathione and inhibits glutathione-S-transferase in patients with drug-sensitive tumors; in patients resistant to chemotherapy, the content of glutathione decreases, while glutathione-S-transferase activity remains practically unchanged. High levels of glutathione and glutathione-S-transferase activity in tumor patients can be used as the measure and prognostic criteria of drug resistance.

Key Words: *glutathione; glutathione-S-transferase; tumor patients; resistance*

Primary and acquired resistance of tumor cells to cytostatics is a very important problem in treating tumor patients. This resistance is formed at the molecular level and involves various interrelated systems. It manifests itself in various disturbances in the structure and function of proteins, lipids, DNA, cell membranes, and membrane-bound enzymes. Mechanisms of drug resistance have been extensively studied. Special attention has been given to changes in glutathione content and glutathione-S-transferase activity (GT), which are directly involved in detoxication processes [2,7,15]. It has been demonstrated that SH-compounds take part in inactivation of some antitumor drugs and reduce their toxicity [1]. Platinum-containing preparations are now widely used in the treatment of tumor patients and are highly efficient in tumors of various location. These preparations are a usual component of highly efficient combinations including preparations with different mechanisms of action. However, some patients are resistant to platinum-based therapy. It is assumed that glutathione and GT assays in tumor patients before and during chemotherapy will allow us to elucidate the interrelationship between these parameters and drug resistance and will provide useful prognostic information.

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MATERIALS AND METHODS

A total of 20 healthy individuals and 34 patients with ovarian (20) and testicular (5) cancer and disseminated skin melanoma (9) were examined. All patients received antitumor drugs in various combinations, platinum derivatives being an obligatory component of these schemes.

Patients with ovarian cancer were treated with platidiam (cisplatin) and cyclophosphamide. Patients with testicular cancer were treated by the BEP protocol (bleomycin, etoposide, and platidiam). Patients with disseminated melanoma received platidiam, nidran, and dacarbazine.

Blood was obtained before and one day after treatment, and the content of glutathione and activity

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of GT in erythrocytes were measured in a Beckman DU-650 spectrophotometer. The content of glutathione was measured at 305 nm in trichloroacetic extract of lysed erythrocytes by its complexation with alloxan and expressed in μmol/ml erythrocytes [10]. Activity of GT (EC 2.1.5.18) in erythrocytes was assessed by the rate of glutathione conjugation with 1-chloro-2,4-dinotrobenzene (λ=340 nm) and expressed in μmol glutathione/min/ml erythrocytes [8]. The amount of the enxyme required for conjugation of 1 μM gluthione per minute was taken as a unit of activity and calculated per 10^6 erythrocytes.

Reagents were purchased from Sigma.

RESULTS

The content of glutathione in erythrocytes of healthy donors varied from 0.94 to 1.31 μmol/ml erythrocytes (mean 1.19±0.17 μmol/ml). In tumor patients this parameter varied considerably, so the patients were divided into two subgroups: with high and low concentration of glutathione (below and above 0.9 μmol/ml, respectively). When comparing these data with the efficiency of chemotherapy, we found that the initial glutathione level in patients with drug-sensitive tumors was considerably lower than in nonresponders. For instance, in 7 of 9 patients with disseminated melanoma the content of glutathione was below 0.9 μmol/ml (lower limit of the normal range); of them, partial regression was noted in 5 patients with a glutathione content of 0.2 (2 patients) and 0.3 μmol/ml (3 patients) and stabilization was achieved in one patient (glutathione level 0.8 μmol/ml). In 2 patients with high glutathione levels (1.1 and 1.74 μmol/ml) melanoma progressed despite intensive chemotherapy; tumor progression occurred in one patient with low glutathione content (0.2 μmol/ml). Thus, positive dynamics was observed primarily in patients with initially low glutathione levels (85%), while high concentrations of this metabolite correlated with melanoma progression (Fig. 1).

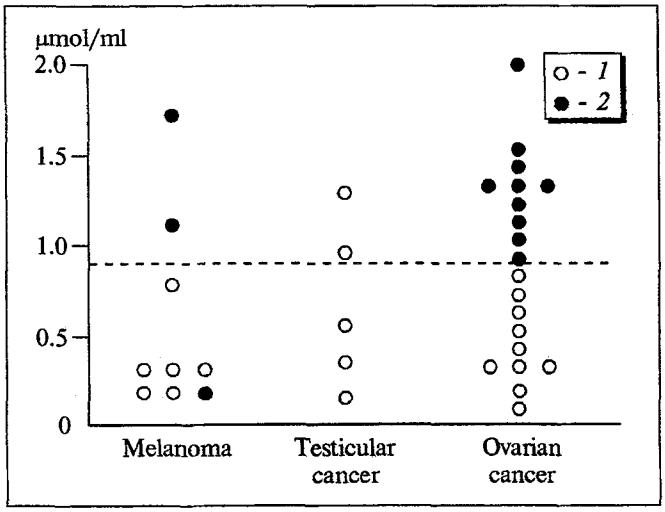

Fig. 1. Glutathione levels in patients sensitive (*1*) and resistant (*2*) to chemotherapy. The lower boundary of the normal range is shown by dotted line.

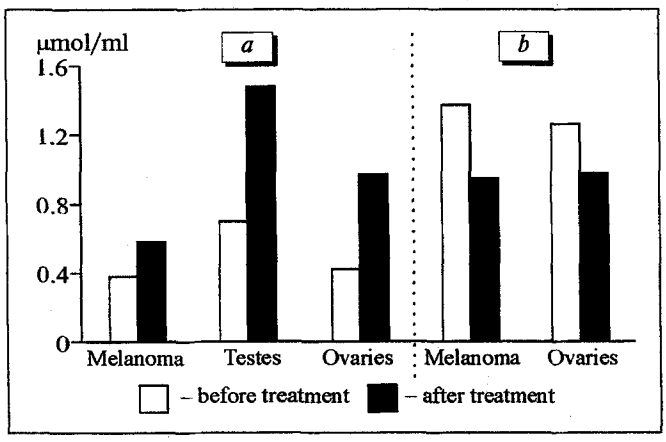

Fig. 2. Content of glutathione in erythrocytes from tumor patients before and after chemotherapy. Here and in Fig. 3: *a*) positive effect; *b*) no effect.

Similar results were obtained in patients with testicular cancer: regression was achieved in 3 out of 5 patients with glutathione contents of 0.16, 0.37, and 0.64 μmol/ml, and stabilization was noted in one patient (glutathione level of 0.98 mmol/ml). At the same, one patient with high glutathione content (1.28 μmol/ml) completely recovered.

Glutathione concentration in erythrocytes depends on various factors, and probably cannot be used as unique criterion of tumor resistance to chemotherapy. It should be noted that in patients sensitive to chemotherapy glutathione content increased on the first day after treatment, while in nonresponders it considerably decreased (Fig. 2). These changes were most pronounced in patients with testicular and ovarian tumors, who received 3-6 courses of chemotherapy. The content of glutathione measured before each route progressively increased in comparison with the control and before the 6th course it sometimes 5-6-fold surpassed the initial level. The content of glutathione probably plays a role in the development of tumor resistance to chemotherapy.

In 10 patients with ovarian cancer and with positive effect of chemotherapy and stabilization of tumor process, the content of glutathione varied from 0.1 to 0.78 μmol/ml. One day after chemotherapy, it increased in all patients and attained 0.95±0.06 μmol/ml (0.5-1.1 μmol/ml) and then increased with each new course. In 10 nonresponders, the initial level of glutathione was 1.3±0.08 μmol/ml (0.95-2.03 mmol/ml), while after the 1st course of chemother-

apy in decreased to 0.96±0.1 μmol/ml. The treatment was stopped because of progressive tumor growth and intoxication.

Similar changes in the content of glutathione were reported in patients with malignant ovarian tumors treated with adriamycin [12]. It should be noted that low glutathione content was associated with high sensitivity to adriamycin, cisplatin, and radiotherapy, but not to methotrexate, etoposide, and 5-fluorouracil [9], which indicates some differences in the interaction of SH-groups with different cytotoxic preparations.

The mechanisms underlying cytoprotective effect of glutathione remain poorly understood. It was assumed that cells resistant to chemopreparations, in particular, to adriamycin are characterized by a higher rate of glutathione synthesis in comparison to adriamycin-sensitive cells [12]. Buthionine-S-sulfoximine, an inhibitor of γ-glutamylcysteine synthetase, potentiates the antitumor effect of cisplatin due to inhibition of glutathione synthesis [4,14]. However, it should be noted that glutathione level depends not only on the rates of its synthesis and degradation, but also on the activity of glutathione-dependent enzymes and NADPH concentration. A special role is played by GT. This enzyme protects the cells by catalyzing conjugation of cytotoxic agents with glutathione. Higher GT activity in malignant tumors in comparison with normal tissues was reported by many authors. Moreover, the combination of high glutathione content and high GT activity is probably responsible for inherent tumor resistance to alkylating agents [1,7].

There is no consensus regarding the relationship between GT activity and efficiency of chemotherapy: many investigators believe that high GT activity is associated with resistance of tumor cells to alkylating agents and can serve as a prognostic criterion [11,13]. However, some authors revealed no correlation between GT activity and the efficiency of chemotherapy [3,6]. Retrospective analysis showed that in patients with ovarian cancer positive effect of chemotherapy was achieved primarily in individuals with low initial activity of GT. Similar relationships were observed in patients with melanoma. These data suggest that high GT activity characterizes tumor resistance to chemotherapy and prove its prognostic value.

Of particular importance is the assessment of GT activity one day after chemotherapy. Effective treatment was associated with a decrease in GT activity, while progression of tumor process was accompanied by gradual rise of this parameter (Fig. 3). Thus, in patients sensitive to chemotherapy the content of glutathione and GT activity were below the normal; one day after chemotherapy, the content of glutathione rose, while GT activity decreased. On the contrary, in nonresponders the initially elevated content of glutathione decreased, while elevated GT activity remained unchanged or slightly rose after chemotherapy. Our findings suggest that changes in glutathione level and GT activity serve as reliable prognostic criteria.

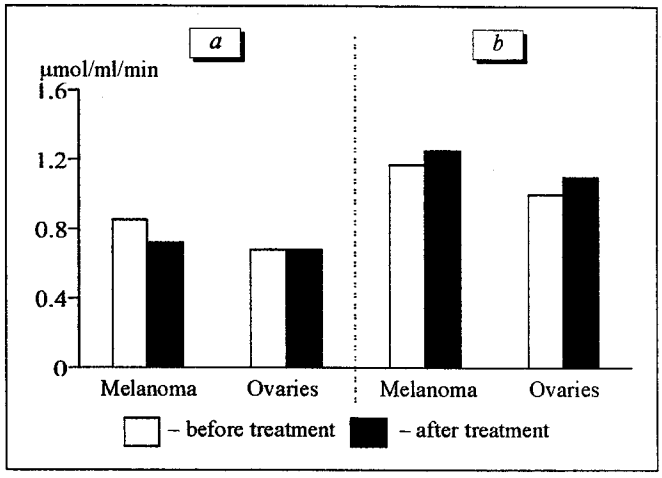

Fig. 3. Activity of glutathione-S-transferase in erythrocytes of tumor patients before and after chemotherapy.

It is clear that complex biochemical mechanisms underlying the resistance to antitumor drugs are not restricted by the glutathione system. Nevertheless, reduced glutathione content in tumor cells as well as impaired antioxidant defense previously observed by us in different cell lines of the same origin [5] can be of value for understanding the mechanisms of different tumorigenicity of various malignant cells and the role of these biochemical shifts in the development of drug resistance of tumor cells.

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