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Glutathione concentration and glutathione S-transferase activity in gynecological normal and tumor tissues: A preliminary report

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Drug resistance is a major problem in cancer therapy. In vitro *studies have suggested that glutathione (GSH) and glutathione transferase (GST) may be associated with alkylating agents resistance. In this study, we determined GSH concentrations and GST activity in 41 samples of gynecological tissues, which include: normal tissue of cervix uteri (7 samples), normal tissue of corpus uteri (14 samples), benign tumors of corpus uteri (4 samples) malignant tumors of corpus uteri (7 samples), the normal tissue of ovary (5 samples), benign ovarian tumors (2 samples) and 2 malignant ovarian tumors. The GSH concentrations were similar in normal tissue of cervix uteri, corpus uteri and ovary. Similar levels of GSH were also found in malignant tumors of corpus uteri, but these levels were lower in benign tumors. In the ovarian tissue, lower levels of GSH were found in benign and malignant tumors. The GST activities were similar in the normal tissue of cervix uteri, corpus uteri and ovary. In corpus uteri, similar values were obtained for normal tissue and benign tumors, but higher ones for malignant tumors. This difference was statistically significant if two malignant Muller mixed tumors (with very low GST levels) were excluded from the analysis. Similar GST activities were obtained for the normal ovarian tissue and benign ovarian tumor, but higher activities for ovarian malignant tumors. In spite of a modest number of samples, our data nevertheless suggest that the activity of glutathione transferase is increased in tumor tissues. The GST activity may contribute to resistance of tumor cells to alkylating drugs in chemotheraphy.*

Key words: ovarian neoplasms-analysis; uterine neoplasms-analysis; glutathione; glutathione transferases; drug resistance

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Introduction

The intrinsic and acquired drug-resistance are rate-limiting step in successful antineoplastic

therapy. Until recently the mechanisms underlying drug-resistance have received little attention, in spite of the fact that this phenomenon was observed as early as in 1948. A wide variety of factors are now implicated as causes of the resistance.[1,2,3] One mechanism may involve overexpression of plasma membrane P-glycoprotein P170. It decreases the drug-accumulation in cells by facilitating the drug efflux from cells, leading to multidrug resistant phenotype. The other mechanism may involve the increased protection by cellular detoxification systems: glutathione (GSH), glutathione transferases (GST), gluthathione peroxidases, and/or metallothioneins. DNA repair, the altered activity of topoisomerase II, changes in drug metabolism and drug transport are also implicated in drug resistance. In many cases, several different mechanisms were found in resistant cells.[4,5,6] In patients, the values of these various parameters might allow us to predict the response of tumors to the particular therapy.

The thiol-mediated detoxification of anticancer drugs is of considerable interest. GSH is the main intracellular nonprotein sulphydryl compound. It has a variety of functions, such as the transport of amino acids into the cells, detoxification of xenobiotics, scavenge of free radicals, biosynthesis of deoxyribonucleotides and biosynthesis or metabolism of prostaglandins and leukotriens. GSH has been shown to have an important role in cell resistance to different drugs.[7,8,9] It affects drug-efficiency by non-catalytic nucleophilic reactions, or by the reactions catalyzed by GST. GSH can express its activity at two levels: cytoplasmatic – by increasing drug inactivation and elimination, or nuclear – by affecting formation and repair of DNA lesions.[10]

GSTs are a group of multifunctional enzymes that catalyze the conjugation of GSH with the various electrophilic agents. This reaction is the initial step in the formation of mercapturic acids, a pathway important in eliminating potentially cytotoxic or mutagenic compounds from the body. GST also act as intracellular blinding proteins for many hydrophobic compounds.[11] In humans, there are three main classes of GSTs in cytosol: α, π and μ; they differ in structural and functional characteristics. Regarding GST in anticancer drug resistance, the following data are well documented: a) tumors express the high level of GST, especially GSTπ; b) nitrogen mustards are good substrates for GSTα; c) most drugs associated with multidrug resistant phenotype are not GST substrates; d) transfection with GST complementary DNAs have produced some lines with increased resistance to alkylating agents.[12,13]

In addition to being important for chemotherapy, GSH[7,9,14] and GST[15] may also play a role in the cellular response to oxidative stress generated by ionizing radiation.

Chemotherapy alone or combined with surgery or radiotherapy, is the usual strategy for treatment of patients with gynecological cancers. Therefore, we compared GSH concentrations and GST activities in gynecological tissues (cervix uteri, corpus uteri and ovary) in an attempt to determine whether they can be used as diagnostic and prognostic factors.

Materials and methods

Tissues

The tissues were obtained from fresh specimens removed during surgery or biopsy at the Department of Obstetrics and Gynecology, School of Medicine, University of Zagreb. Each sample was divided in two halves, for histological and biochemical studies.

The samples were frozen and maintained at – 196°C.

Fortyone samples of normal, benign and malignant tumor tissue from cervix uteri, corpus uteri and ovary were analyzed (Table 1). They were divided as: gradus I (normal), gradus II (benign) and gradus III (malignant).

The protocol was approved by the Ethics of Research Committee at the School of Medicine, University of Zagreb.

Glutathione determination

The total intracellular glutathione (GSH) level was measured by modified Tietze's method.[16]

Briefly, the samples were cut into small pieces, covered with buffer (50 mM TRIS, 250 mM saccharose, 134 mM KCl, pH = 7.6) and homogenized on ice (Ika-Kunkel, Labor Technick, Germany; three strokes for 5 sec). The suspension was centrifugated at 4°C for 45 mit at 15000 g. The total GSH content in the supernatant was determined by the enzymatic recycling assay.[16] The absorbance of 5,5-dithiobis-2-nitrobenzoic acid (Boehringer Mannheim GmbH, Germany) at 412 nm was monitored spectrophotometrically. Values were normalized according to the total protein assessed by Bradford's method.[17] Each sample was divided into two halves and GSH was determined in each of them two times.

Glutathione transferase determination

The intracellular glutathione transferase (GST) activity was determined as described by Habig and Jacoby.[18] GST activity was measured in the supernatant prepared for GSH determination, using 1-chloro-2,4-dinitrobenzene as the electrophilic substrate. GST activity was expressed as nanomoles of GSH-1-chloro-2,4-dinitrobenzene conjugate formed per min per mg protein. In each clinical sample GST activity was determined four times.

Statistics

The significance of differences between the particular groups was tested by analysis of Mann-Whitney or Kruskal-Wallis. The level of significance was set to 0.05.

Results

In this study, we examined the glutathione concentrations and glutathione S-transferase activities in 41 samples that originated from the normal tissues (26 samples), benign tumors (6 samples) and malignant tumors (9 samples) (Table 1). Glutathione concentrations in normal and tumor tissues are given in Figure 1. The data reveal that the concentration of glutathione was about the same in the normal tissue of cervix uteri, corpus uteri and ovary. It contrast, the values obtained for benign tumors of corpus uteri were significantly lower than that for the normal tissue. However, GSH concentration in normal and malignant tumors was not statistically different (Figure 1b).

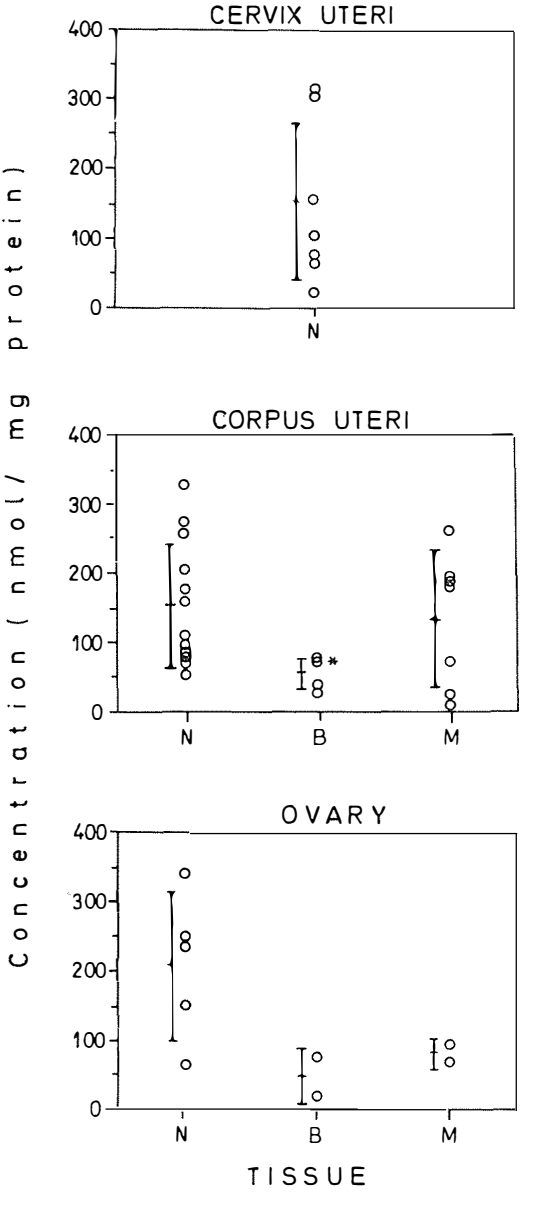

Figure 1. Glutathione concentrations in cervix uteri, corpus uteri and ovary. Individual values are given for N = normal tissues, B = benign tumors, M = malignant tumors. The mean values ± SD are presented.
* Statistically different from normal tissue.

The statistical analysis of the data of the GSH concentrations in ovary was impossible because the number of samples was too low. Therefore, we were also unable to make statistical comparison of the data obtained for tumors of corpus uteri and ovary (Figure 1b and 1c). Figure 1c shows, however, that the mean values for GSH concentrations were lower in benign and malignant tumors than in the normal ovarian tissue.

We next examined the activity of glutathione transferase in gynecological tissues. The results are given in Figure 2. We found no significant differences in the GST activity in normal tissues (cervix uteri, corpus uteri or ovary). The GST activities in benign and malignant tumors were similar to those found in the normal tissue of corpus uteri. If, however, the two lowest values are excluded from the analysis (marked with arrows in Figure 2b), significant differences in the GST activity between normal and malignant tumor tissue are found. These two exceptions are two carcinosarcomas (MMMT, see Table 1) that differ from malignant adenocarcinoma tumors by their composition: they have less glandular and more stromal components, indicating a possible explanation for lower GST activities. Again, we are unable to make the statistical analysis of the GST activity in ovary because of a low number of samples. As can be judged from Figure 2c, however, is the GST activity similar in the normal tissue and benign tumors, but higher in malignant tumors.

The comparison of the GST activity in corpus uteri and in ovarian carcinomas points to higher GST activities in ovary, although it is still in the range of values obtained for carcinomas of corpus uteri. When the data for MMMT are excluded, the GST activities in ovarian and endometrial tumor are quite similar.

Discussion

Resistance to chemotherapy is a serious problem in the management of patients with cancer. Some of the most active anticancer-drugs are electrophiles that damage DNA either directly by alkylation (melphalan, phenylalanine mustard, BCNU, cyclophosphamide, cisplatin) or indirectly through free-radical mechanism (Adriamycin). *In vitro* studies have suggested that GSH can participate in detoxification of antineoplastic drugs like alkylating agents and Adriamycin.[8,9] These studies have been extended to clinic to see whether tumor and normal tissues display phenotypic differences relevant to potential cytostatic drug resistance.

In this preliminary study we examined the level of GSH in different normal gynecological tissue (cervix uteri, corpus uteri and ovary) and found similar values. If these values were compared to those obtained for tumor tissue, similar (corpus uteri) or even lower (ovary) concentrations were found. Therefore, our data suggest that GSH concentrations cannot be used as a diagnostic and prognostic factor, at least not for tumors of corpus uteri and ovary.

The literature data concerning clinical studies suggest that differences in the GSH level between normal and tumor tissue depend on the tumor type. No significant difference between the GSH concentration in the tumor and the normal tissue of lung was obtained.[19] Moreover, a certain decrease in the GSH concentration in adenocarcinoma was found as compared to the

Table 1. Normal and tumor samples analyzed in this study

	Normal	Benign tumors	Malignant tumors
Cervix uteri	7	–	–
Corpus uteri	14	3 myomas	5 endometrial adenocarcinomas
		1 endometrial polyp	2 malignant Muller mixed tumors*
Ovary	5	1 mucinous ovarian cystadenoma	2 serous ovarian carcinoma
		1 ovarian endometriotic cyst	
Total	26	6	9

* = MMMT, carcinosarcoma

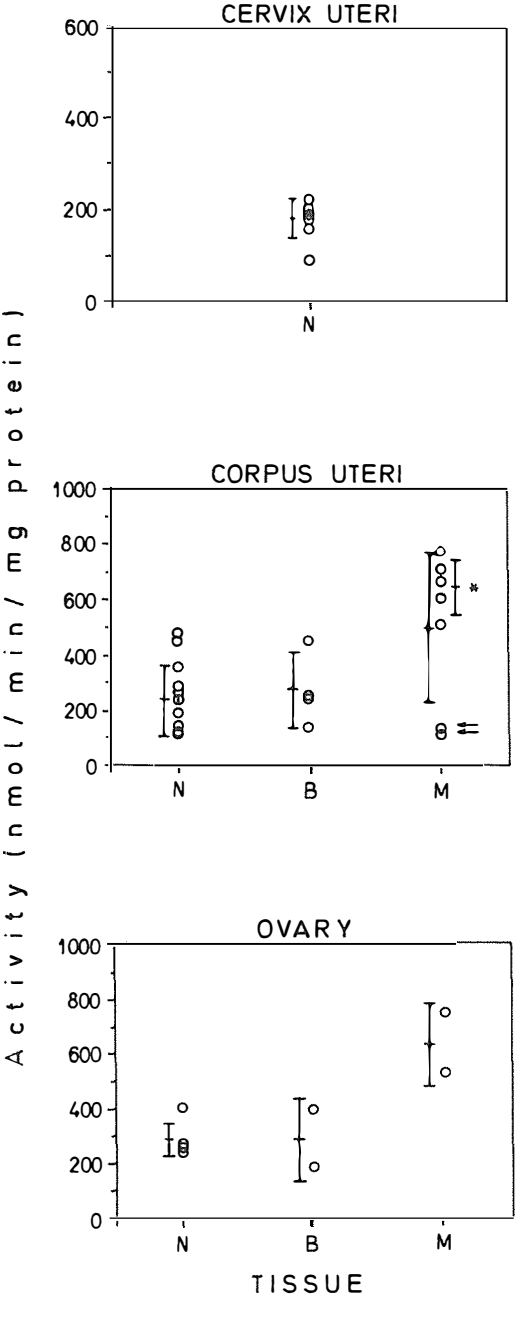

Figure 2. Activity of glutathione S-transferases in cervix uteri, corpus uteri and ovry. Individual values are given for N = normal tissues, B = benign tumors, M = malignant tumors. The mean values ± SD are presented.
* Statistically different from normal tissue.

normal lung specimens. A drop in the GSH concentration in tumors of sigmoid colon was also reported.[20] In contrast, the increased levels of GSH were found in the tumors of colon[21] and rectum.[22]

The activity of GST enzymes in normal and tumor tissues is of great importance. The levels in normal tissue may determine the susceptibility of the tissue to cytotoxic damage from chemical toxins, carcinogens and some anticancer drugs. In tumors, by contrast, the GST level may cause the resistance to chemotherapy.

Anticancer drugs that are substrates for GST can be divided in two categories: those for which convincing substrate/kinetic data are known (chlorambucil melphalan, nitrogen mustard, phosphoramide mustard, acrolein, BCNU, hydroxyalkenals, ethacrynic acid and steroids) or those for which only some indirect evidence exist (bleomycin, hepsulfan, mitomycin C, adriamycin, cisplatin, carboplatin).[12] So far, the involvement of GST in the resistance to classical alkylating agents, cisplatin based drugs and anthacyclines has been documented.[12,13]

In this study, we examined the activity of GST in normal cervix uteri, corpus uteri and ovary, and found similar values in these tissues. If the activity of GST for corpus uteri (with the exception of MMMT) and ovary were compared, similar values were again obtained. If, however, the GST activities in normal tissues were compared to the tumor tissue, a significant increase in the tumor tissue was observed. This is the main finding of our study. It suggests that for the gynecological tissue, GST activity may be used as a diagnostic and prognostic factor.

Our findings that gynecological tumors have higher GST activities than the corresponding normal tissues, are in agreement with the literature data. Higher GST activities have been found in various cancers, including cancer of colon, rectum, stomach, lung and breast, but not of kidney and liver.[21–26] In these studies, as well as in ours, GST activities varied considerably (1.3 to 2.7 -fold), and even higher variations were observed among individual tumors.

Promising strategies to overcome resistance are emerging from basic and preclinical studies. They seem likely to lead to the improved therapeutic regimens based on the modulation of chemotherapy. One approach could involve the elevation of the host cells GST enzyme level. On the other hand, inhibition or inactivation of GST enzymes of the tumor can be the principal goal. For this, the specific GST inhibitors can be used: inhibitory peptide analogues of GSH, the quinone inactivators of GST, the prostaglandin I_1 analogue piriprost or the diuretic agent ethacrynic acid. Ethacrynic acid is of particular interest, because it is applied in Phase II preclinical trial for chlorambucilrefractory chronic lymphatic leukemia.[12]

To sum up, in this preliminary study we did not find the elevated glutathione levels in tumor of corpus uteri and ovary as compared to the corresponding normal tissue. We found an increase in the activity of glutathione transferase in tumor tissues. This GST activity may contribute to resistance of tumor cells to alkylating drugs in chemotherapy. We started the clinical followup study to examine the correlation between the high tumor GST activity and prognosis of the ilness.

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