Factors of the Ovarian Cancer Resistance to Combined Chemotherapy with Platinum Preparations

A. G. Blyumenberg, L. B. Gorbacheva, V. A. Gorbunova, V. P. Kozachenko, and V. Z. Lankin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 12, pp. 651-654, December, 1996
Original article submitted June 20, 1995

The glutathione content and the activities of glutathione reductase, glutathione peroxidase, and glutathione S-transferase were measured in biopsy samples of epithelial ovarian cancer. It is shown that in stages 3-4 of ovarian cancer the effectiveness of chemotherapy correlates with the glutathione content and activity of glutathione S-transferase.

Key Words: *glutathione S-transferase; glutathione peroxidase, glutathione reductase; ovarian cancer; refractority*

Platinum complexes have been successfully used for the treatment of ovarian cancer for more than two decades. However, refractority occurring in 20-25% patients [3] lowers the effectiveness of the therapy.

Although the mechanisms of refractority to platinum derivatives have been extensively investigated, they are not fully understood. It was reported that drug resistance is associated with changes in the plasma membrane permeability, rates of formation and reparation of DNA adducts, glutathione (GSH) content, and activity of some GSH-dependent enzymes participating in the detoxication of platinum derivatives [6-11].

The development of drug resistance may result from cloning of preexisting cells with certain GSH content and activity of GSH-dependent enzymes as well as from a potent mutagenic activity of cis-platinum.

The aim of this study was to identify some biochemical indicators for the effectiveness of combined chemotherapy of patients with ovarian cancer.

MATERIALS AND METHODS

The results of clinical and laboratory examinations of 20 patients are summarized in Table 1. Biopsy material was collected during surgery (extirpation of the uterus and adnexa, resection of the greater omentum). The tumor diameter was <4 cm. The bioptates were stored at -70°C. Nineteen patients had serous cystadenocarcinoma and one patient had endometrial cystadenocarcinoma.

2-[^{14}C]Thymidine (1.9 TBq/mol) was from Izotop (Russia), NADPH was from Reanal (Hungary), all other reagents were from Sigma (USA). The patients were treated with platidiam, cycloplatam, and cyclophosphane according to the following scheme: 100 mg/m^2 platidiam + 600 mg/m^2 cyclophosphane on day 1 (18 patients) and 100 mg/m^2 cycloplatam on days 1-5 + 600 mg/m^2 cyclophosphane on day 1 (2 patients).

The activities of GSH enzymes and the total GSH content were determined in an FP-901 analyzer (Finland) using a thermostatically controlled 9-channel cuvette. For determination of GSH content, tumor cell suspension (0.5 ml) was incubated with 1.5 ml 0.2 M Tris-HCl (pH 8.2) and 0.1 ml 0.01 M 5,5'-DITHIO-bis(2-nitrobenzoic acid) in methanol for 30 min at room temperature. The mixture was then filtered, and its light absorbance was measured at 412 nm. The coefficient of molar extinction was 13,600 M^{-1}cm^{-1}. Proteins were precipitated with 5% trichloroacetic acid. The GSH content was calculated in nmol/mg protein. The activity of GSH reductase was assayed by the rate of NADPH oxidation at 30°C [11]. A unit of enzyme activity was defined as the

Oncology Research Center, Russian Academy of Medical Sciences, Moscow

0007-4888/96/0012-1213$15.00

TABLE 1. Clinical Characteristics of Patients

Patient No.	Stage of disease (FIGO)	Age	Tumor differentiation	Treatment	Effect	GSH, mo/g protein×10⁻⁵	Activity, U/mg protein		
							GSH reductase	GSH peroxidase	GST
1	3	63	poor	Pt+CPS	FR	2.08	0.039	0.044	0.281
2	3	54	moderate	Pt+CPS	FR	2.31	0.019	0.03	0.204
3	3	62	moderate	Pt+CPS	FR	2.37	0.07	0.157	0.204
4	4	55	high	CP+CPS	FR	2.9	0.009	0.051	0.197
5	3	53	high	Pt+CPS	FR	3.2	0.023	0.029	0.111
6	3	53	high	Pt+CPS	FR	4.2	0.022	0.047	0.248
7	3	25	high	Pt+CPS	FR	5.2	0.033	0.134	0.215
8	3	42	moderate	CP+CPS	PR>50%	6.0	0.03	0.061	0.164
9	3	49	poor	Pt+CPS	PR>50%	1.32	0.091	0.014	0.185
10	3	49	moderate	Pt+CPS	PR>50%	1.66	0.089	0.105	0.2
11	3	55	moderate	Pt+CPS	PR>50%	2.29	0.069	0.378	0.145
12	3	28	moderate	Pt+CPS	PR>50%	1.85	0.58	0.59	0.129
13	3	2	moderate	Pt+CPS	PR>50%	1.8	0.021	0.081	0.211
14	4	62	moderate	Pt+CPS	PR<50%	1.55	0.24	0.056	0.077
15	3	52	moderate	Pt+CPS	PR<50%	1.6	0.016	0.053	0.034
16	3	53	poor	Pt+CPS	PR<50%	1.7	0.018	0.022	0.208
17	3	59	moderate	Pt+CPS	PR<50%	1.5	0.001	0.019	0.116
18	3	54	moderate	Pt+CPS	Pr	0.55	0.01	0.12	0.042
19	4	48	high	Pt+CPS	Pr	0.5	0.014	0.18	0.078
20	3	51	moderate	Pt+CPS	Pr	1.1	0.091	0.036	0.019

Note. Poor: poorly differentiated tumor; moderate: moderately differentiated tumor; high: highly differentiated tumor; CPS: cyclophosphane; CP: cycloplatam; Pt: platidiam; FR: full regression; PR: partial regression; Pr: progression.

amount of enzyme that reduces 1 μM GSH per minute.

The activity of GSH peroxidase was assayed by oxidation of NADPH in a GSH peroxidase coupled system using tert-butyl peroxide as a substrate (30°C, λ=340 nm). The amount of GSH peroxidase required for oxidation of 1 μM GSH was taken as a unit of enzyme activity which was expressed in U/mg

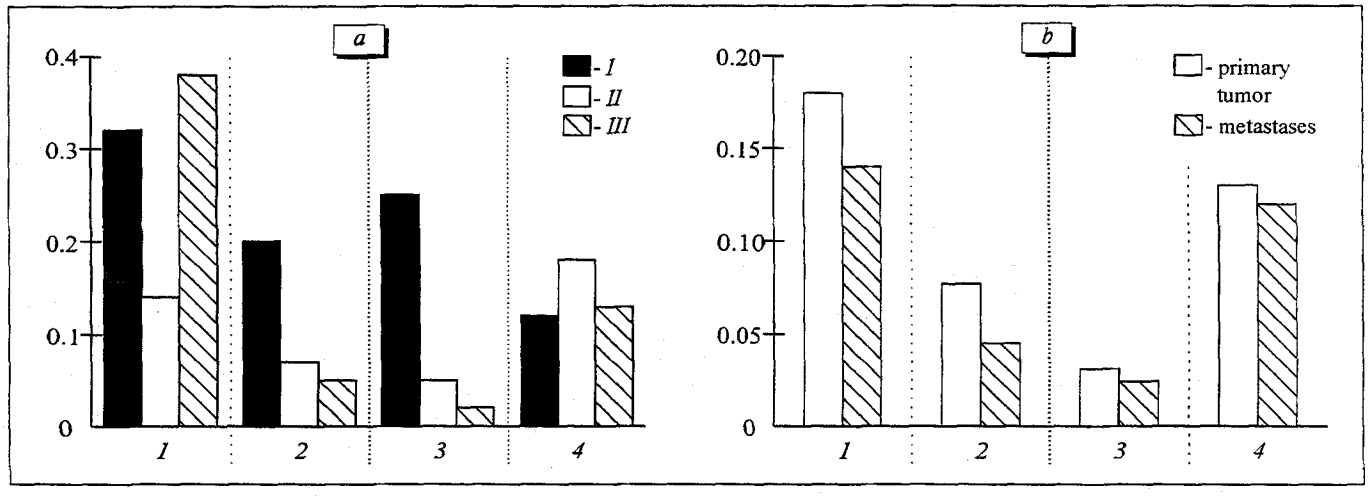

Fig. 1. Relationship between the glutathione content (*1*) and the activities of glutathione reductase (*2*), glutathione peroxidase (*3*), glutathione S-transferase (*4*) and tumor differentiation (*a*) in primary cancer and metastases into the greater omentum (*b*). Ordinate: mmol/mg protein×10⁻⁵ (*1*) and U/mg protein (*2-4*). Poorly (*I*), moderately (*II*), and highly (*III*) differentiated tumor.

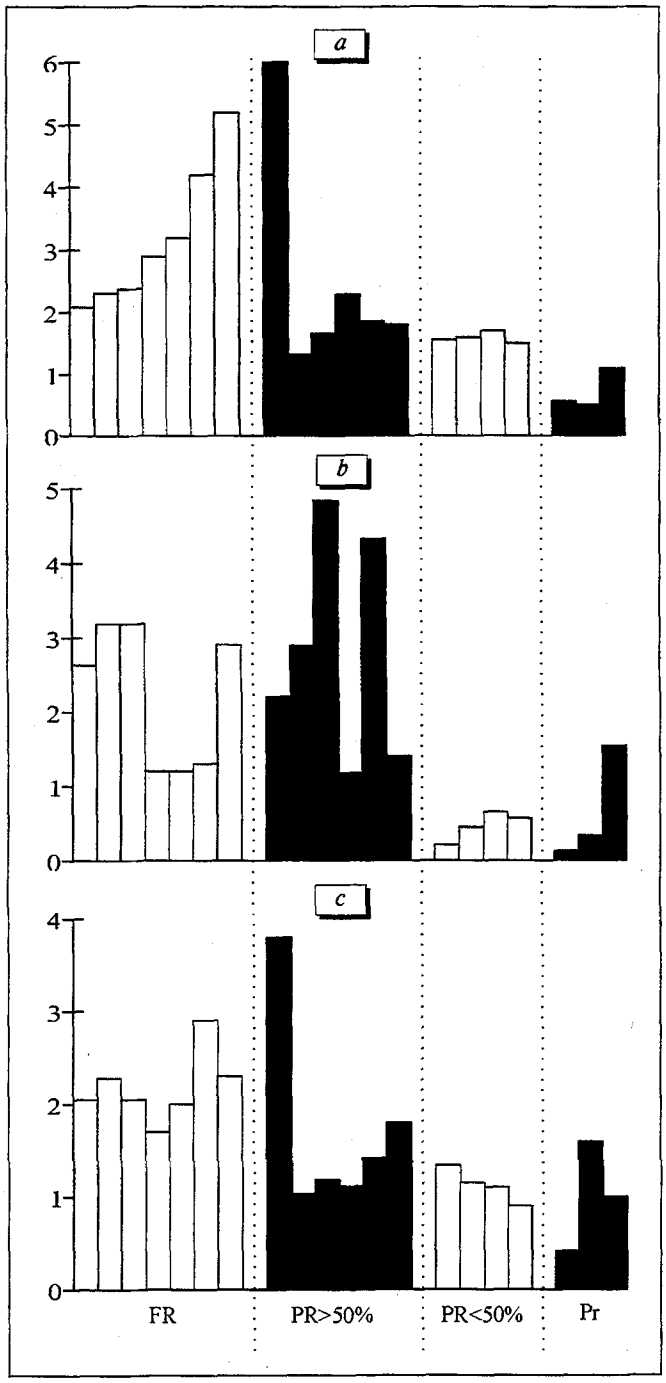

Fig. 2. Relationship between the effectiveness of chemotherapy and concentration of glutathione groups: total (*a*), nonprotein (*b*), and protein-bound (*c*). Here and in Fig. 3: FR — full regression, PR — partial regression, Pr — progression. Ordinate: glutathione level, mmol/mg×10^{-5}.

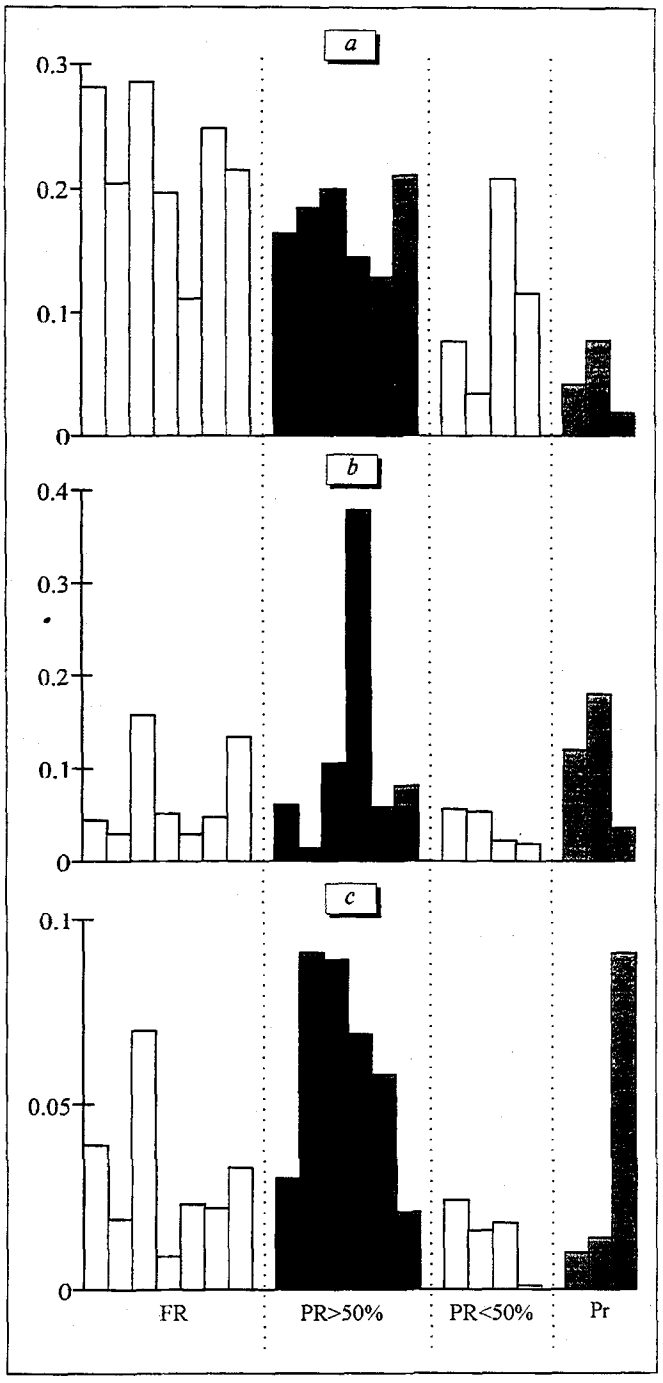

Fig. 3. Relationship between the effectiveness of chemotherapy and activity of glutathione S-transferase (*a*), glutathione peroxidase (*b*), and glutathione reductase (*c*). Ordinate: U/mg protein.

protein [2]. The glutathione S-transferase (GST) activity was measured by GSH conjugation with 1-chloro-2,4-dinitrobenzene [5] (25°C, λ=340 nm). A unit of enzyme activity was defined as the amount of GST conjugating 1 μM GSH per minute. The protein content was measured by the microbiuret method [1].

RESULTS

Individual variations of GSH content and GSH enzyme activities were revealed in ovarian cancer specimens. These parameters did not correlate with the degree of tumor differentiation (Fig. 1, *a*). The GSH

content and activities of GSH enzymes were significantly higher in metastases (greater omentum) than in a primary tumor (Fig. 1, *b*).

A positive correlation between the effectiveness of combined chemotherapy and the SH-group content in the tumor was established (Fig. 2, *a-c*). A similar although weaker correlation was established for GST (Fig. 3, *a*). The sensitivity of the tumor to chemotherapy did not depend on the activities of GSH peroxidase and GSH reductase (Fig. 3, *b*, *c*).

The relationship between the activity of GSH-dependent enzymes and resistance of tumor cells to platinum preparations has been extensively studied in animal and tissue culture experiments. A correlation between these parameters was revealed by some researchers [10] and was not by others [7-9]. The information regarding the relationship between GSH content and GST activity in ovarian cancer bioptates and tumor refractority is scarce, neither confirming nor denying any correlation between these parameters. This may be due to heterogeneity of the studied group by histologic type of tumor, degree of tumor differentiation, stage of disease, and small number of patients.

We have found that the GSH content and GST activity are higher in malignant ovarian tumors treated with platinum preparations in combination with cyclophosphane. This finding cannot be explained in terms of the currently accepted mechanisms of action of these drugs.

REFERENCES

1. J. Bailey, *Methods of Protein Chemistry* [Russian translation from English], Moscow (1965).
2. V. Z. Lankin and S. M. Gurvich, *Dokl. Akad. Nauk. SSSR*, **226**, 705-710 (1976).
3. M. A. Livshits, in: *New Approaches to the Treatment of Gynecological Cancer* [in Russian], St. Petersburg (1993), pp. 88-101.
4. A. Habeeb, *Methods Enzymol.*, **25**, 457-464 (1972).
5. J. H. Keen and W. B. Jacoby, *J. Biol. Chem.*, **253**, No. 16, 5654-5657 (1978).
6. F. Y. F. Lee, D. J. Flannery, and D. W. Siemann, *Br. J. Cancer*, **63**, 217-222 (1991).
7. P. Mistry, L. R. Kelland, *et al.*, *Ibid.*, **64**, 215-220.
8. D. Murphy, A. T. McGown, A. Hall, *et al.*, *Ibid.*, **66**, 937-942 (1992).
9. S. A. Pellin and B. S. Davidson, *et al.*, *Cancer Lett.*, **85**, No. 2, 223-232 (1994).
10. M. W. J. Sark, H. Timmer-Bosscha, C. Meijer, *et al.*, *Br. J. Cancer*, **71**, 684-690 (1995).
11. A. G. J. van der Zee, B. van Ommen, C. Meijer, *et al.*, *Ibid.*, **66**, 930-936 (1992).