Drug resistance in epithelial ovarian cancer: P-glycoprotein and glutation S-transferase. Can they play an important role in detecting response to platinum-based chemotherapy as a first-line therapy

T. Şimşek[1], *M.D., Asso. Prof.*; **G. Özbilim**[2], *M.D., Assist. Prof.*; **H. Gülkesen**[2], *M.D.*; **H. Kaya**[1], *M.D.*; **F. Sargin**[2], *Biolog*; **Ş. Karaveli**[2], *M.D. Prof.*

[1]*Department of Obstetrics and Gynecology and* [2]*Pathology, Akdeniz University School of Medicine, Antalya (Turkey)*

Summary

Objective: Drug resistance is important for the treatment of ovarian cancer. P-glycoprotein and glutation S-transferase as resistance markers play an important role in the effectivity of chemotherapeutical agents. The role of P-glycoprotein and glutation S-transferase in the treatment of epithelial ovarian cancer is not well understood. We investigated the relation between P-glycoprotein and glutation S-transferase level for response to platinum-based chemotherapy in epithelial ovarian cancer.

Material and Methods: We reviewed 30 cases diagnosed as epithelial ovarian cancer and treated with platinum-based chemotherapy in the Department of Obstetrics and Gynecology, Akdeniz University School of Medicine. The material was attained from initial parafin-embeded blocks stained for P-glycoprotein and glutation S-transferase. The cases that were diagnosed and treated before attending our clinic were not enrolled in the study.

Results: Mean age was 58.2 (25-70) and mean gravida 4.1 (0-10). Twenty-four patients (80%) were glutation S-transferase positive. Three cases (10%) out of 30 had positive reaction for P-glycoprotein. No difference was revealed regarding chemotherapy response rate among the cases showing glutation S-transferase positivity and P-glycoprotein negativity.

Conclusion: Detection of glutation S-transferase and P-glycoprotein levels in epithelial ovarian cancer tissue is not important for response to platinum-based chemotherapy as a first line.

Key words: Glutation S-transferase; P-glycoprotein; Ovarian Cancer; Drug Resistance.

Introduction

Ovarian cancer is the fifth most common gynecologic malignancy. It is responsible for the most cancer deaths in woman because the disease is usually incurable surgically at the time of laparotomy or laparoscopy. Besides surgical intervention cisplatin-based chemotherapy is an important part of treatment, but 40-60 of cases have an incomplete response. On the other hand, the great majority of patients relapse and develop resistance to further chemotherapy. The primary factor that has limited the success of chemotherapy in ovarian cancer is drug resistance. About 50% of patients with ovarian carcinomas are intrinsically resistant to chemotherapy. At the cellular level several biochemical changes contributing to drug resistance have been identified [1]. Changes in detoxification pathways, altered drug transport and changes in DNA repair are some of the resistance mechanisms [2].

Glutation (GSH), a nonprotein sulfhydryl and its associate enzymes, glutathione S-transferases (GST), acts by detoxification of some chemotherapeutics such as alkylating and platinium-based agents. Elevated levels of GSH have been found in cisplatin-resistant ovarian cancer cell lines [3].

P-glycoprotein (Pgp), a 170000 Da transport protein, functions as an energy-dependent efflux pump for a number of drugs and is responsible for decreas drug accumulation within cells. Drug accumulation within cells is required for treatment.

The purpose of this study was to evaluate the role of GST and Pgp in response to platinum-based chemotherapy.

Materials and Methods

We reviewed 30 cases diagnosed as epithelial ovarian cancer and treated with platinum-based chemotherapy (cisplatin and cyclophosphamide) in the Department of Obstetrics and Gynecology, Akdeniz University School of Medicine. The material was attained from initial paraffin-embedded blocks stained for P-glycoprotein and Glutation S-transferase, immunohistochemically. Response for treatment was evaluated by clinical and histopathological findings of second-look laparatomy. Response rate (complete, incomplete and nonresponder) was accepted according to the WHO criteria. Cases that were diagnosed and treated before being admitted to our clinic were not enrolled in the study. All the patients were in stage 3 and underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, pelvic and paraaortic lymphadenectomy. Benign and borderline tumors were excluded. All patients underwent optimal cytoreductive surgery (less then 1 cm). Patients were divided into two groups according to degree of immunohistological positive staining. The patients with lower than 30% of positive staining formed one group and above 30% the other group (Table).

This article was presented in part at the 11th International Meeting of Gynaecological Oncology, ESGO 11.

Revised manuscript accepted for publication February 26, 2001

Glutation S-transferase distribution among patients.

	Positive (under 30%) and negative patiens	Above 30% positive patients
Complete	7 (58.3%)	5 (41.6%)
Incomplete and non responder	8 (44.4%)	10 (55.5%)
Total	15 (50%)	15 (50%)

($p > 0.05$)

Statistical analysis was performed with Fisher's exact test; $p < 0.05$ was accepted as statistically significant.

Results

Mean age was 58.2 (25-70) and mean gravida 4.1 (0-10). Twenty-four patients (80%) were Glutation S-transferase positive (Figure 1). However positivity for P-glycoprotein was low (10%) (Figure 2). There was no relation between degree of glutation S-transferase positivity and response to chemotherapy.

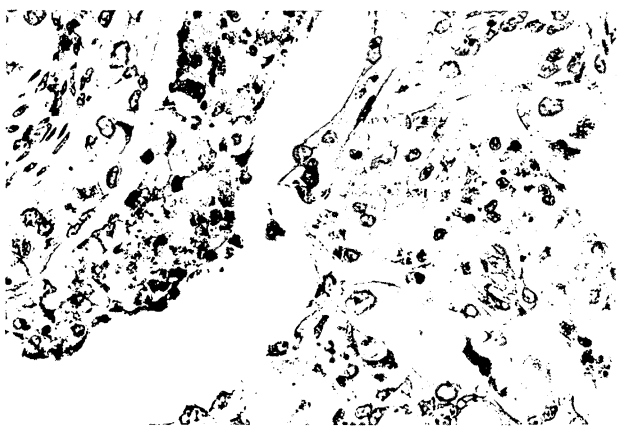

Figure 1. — Cytoplasmic staining with glutation S-transferase immunohistochemistry in serous adenocarcinoma (GST x 40).

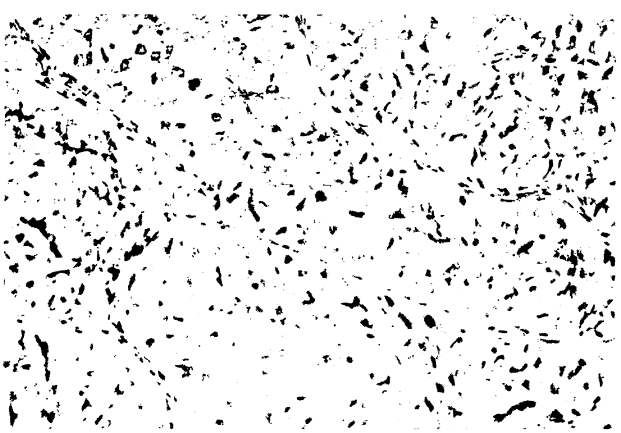

Figure 2. — Cytoplasmic staining with glutation S-transferase immunohistochemistry in serous adenocarcinoma (GST x 20).

Discussion

Glutation levels in cells reduces the amount of various cytotoxic drugs such a platinum in tissue. Thus drug resistance can develop. Glutation S-transferase plays a role in oxido-reduction of glutation for detoxifiying drugs within cells. The levels of glutation S-transferase would result in increased detoxification of drugs. There are many reports which include GSH and GST in vivo and in vitro in studies. Increased levels of GSH have been reported in a number of cisplatin- and doxorubicin-resistant cell lines derived from ovarian carcinomas [3, 4]. Joncourt *et al.* [5] found that GSH levels increase with stage, and tend to increase with grade. GSH levels in the tumor tissue does not predict treatment response. In our study there was a difference between strong positive and negative or silent positive patients. However the difference was not statistically significant.

Platinum-based chemotherapy is added to surgical therapy in epithelial ovarian cancer cases. However, drug resistanse develops in many of these patients. Response rates decrease from 70-80% to 20%. First, chemosensitive clones are destroyed with chemotherapy and resistants survive; these surviving clones may be included as high GST or GSH. Also Kigawa *et al.* [6] reported that glutathion concentrations may useful in predicting second-line chemotherapy response, however we studied it as a first-line therapy.

GST is a family of enzymes which form part of the GSH-associated detoxification system. GST levels have been found to be increased in tumor cells lines [7] and transfection studies have implicated them in platinum resistance [8]. The data on GST invivo studies are conflicting as reported by Green *et al.* [9]. GST-Il immunohistochemical staining is related to resistance to cytotoxic chemotherapy and survival, but Joncourt *et al.* [5] reported GST activity was not significantly different in responding and non-responding patients. However these two studies are a little different in that Green *et al.* studied only part II of GST as α, π and μ. We also studied the free part of GST with the same results as Joncourt *et al.*

P-glycoprotein (Pgp) functions as an energy-dependent efflux pump. It acts to decrease drug accumulation within cells. Increasing the effectivity of Pgp results in drug resistance. However Pgp expression has a different frequency in ovarian cancer. While some authors detected Pgp in only a minority of ovarian tumors [10, 11], others reported frequencies greater than 50% [12]. These differencies depend on methods used for detected Pgp, FISH and PCR which can be detected at even very low levels. However, immunohistochemistry has varying sensitivity depending on antibodies and the detection system used. We used immunohistochemistry and the positivity of Pgp was 10%.

Conclusion

It is not important to determine the level of glutation S-transferase in tumor cells to detect the response rate to platinum-based chemotherapy. Positivity of P-glycoprotein is low in epithelial ovarian cancer.

References

[1] Yokoyama Y., Sato S., Fukushi Y., Sakamoto T., Futagami M., Saito Y.: "Significance of multidrug-resistant proteins in predicting chemotherapy response and prognosis in epithelial ovarian cancer". *J. Obstet. Gynaecol. Res.*, 1999, *6*, 387.

[2] Perez R. P., Hamilton T. C., Ozols R. F., Young R. C.: "Mechanisms and modulation of resistance to chemotherapy in ovarian cancer". *Cancer*, 1993, *71 (suppl.)*, 1571.

[3] Godwin A. K., Meister A., Odwyer P. J., Huang C. S., Hamilton T. C., Anderson M. E.: "High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutation synthesis". *Proc. Natl. Acad. Sci. USA*, 1992, *89*, 3070.

[4] Lewis A. D., Duran G. E., Lau D. H. M., Sikic B. I.: "Sensitization of drug resistant human ovarian cancer cells to cyanomorpholino doxorubicin (MRA-CN) by modulation of glutathione metabolism". *Int. J. Radiat Oncol. Biol. Phys.*, 1992, *22 (4)*, 821.

[5] Joncourt F., Buser K., Altermatt H., Bacchi M., Oberli A., Cerny T.: "Multiple drug resistance parameter expression in ovarian cancer". *Gynecol. Oncol.*, 1998, *70*, 176.

[6] Kigawa J., Minagava Y., Kanamori Y., Itamochi H. *et al.*: "Glutathione concentration may be a useful predictor of response to second-line chemotherapy in patients with ovarian cancer". *Cancer*, 1998, *82 (4)*, 697.

[7] Meijer C., Mulder N. H., De Vries E. E.: "The role of detoxifying systems in resistance of tumor cells to cisplatin and adriamycin". *Cancer Treat. Rev.*, 1990, *7*, 389.

[8] Stelmack G. L., Goldenberg G. J.: "Increased expression of cytosolic glutathione S-transferases in drug resistant L5178Y murine lymphoblasts: Chemical selectivity and molecular mechanisms". *Cancer Res.*, 1993, *53*, 3530.

[9] Green J. A., Robertson L. J., Clark A. H.: "Glutathione S-transferase expression in benign and malignant ovarian tumours". *Br. J. Cancer*, 1993, *68 (2)*, 235.

[10] Rubin S., Finstadt C. L., Hoskins W. J., Saigo P. E., Provencher D. M., Federici M. G. *et al.*: "Expression of P-glycoprotein in epithelial ovarian cancer: Evaluation as a marker of multidrug resistance". *Am. J. Obstet. Gynecol.*, 1990, *69*, 163.

[11] Bourhis J., Goldstein L. J., Riou G., Pastan I., Gottesman M. M., Benard J.: "Expression of a human multidrug resistance gene in ovarian carcinomas". *Cancer Res.*, 1989, *49*, 5062.

[12] van der Zee A. G. J., Hollema H., de Jong S., Boonstra H., Gouw A., Willemse P. H. B., *et al.*: "P-glycoprotein expression and DNA topoisomerase I and II activity in benign tumors of the ovary and in malignant tumors of the ovary, before and after platinum/cyclophosphamide chemotherapy". *Cancer Res.*, 1991, *51*, 5915.

Address reprint requests to:
T. ŞIMŞEK, M.D.
Yıldız Mahallesi 217 sok
Culfalar apt. D 7 Antalya (Turkey)