Multidrug resistance gene-1 (Pgp) expression in epithelial ovarian malignancies

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Summary

Objective: To assess the value of P-glycoprotein (Pgp) expression in advanced epithelial ovarian cancer with regard to clinicopathological findings and disease prognosis.

Methods: Twenty-four cases diagnosed as primary epithelial ovarian malignancies, between 1993-1999, were enrolled in this study. All of the cases had undergone cytoreductive surgery and an optimal staging procedure. Following cytoreductive surgery, in 18 patients, cisplatin+cyclophosphamide, and in six patients, cisplatin+paclitaxel combination chemotherapy regimens were initiated. After six courses of chemotherapy, cases were evaluated by pelvic examination, transvaginal ultrasound, pelvi-abdominal tomography and serum Ca-125 levels for the presence of residual disease. Following this evaluation residual tumor was detected in 14 cases and secondary cytoreductive surgery was undergone. In ten cases without any clinical and laboratory confirmation of the presence of tumor, second-look laparotomy was performed. In 24 epithelial ovarian cancer cases, both in primary or secondary cytoreductive surgery, Pgp expression was determined by immunohistochemical methods.

Results: Following primary surgery, in 25% (6/24) of cases, analysis of tumor specimens showed presence of Pgp expression. In cases recurring after first-line chemotherapy, Pgp expression was not statistically different in regard to chemotherapy regimen ($p = 0.098$). Pgp expression in tumoral tissues after chemotherapy did show a higher Pgp expression than before chemotherapy ($p = 0.016$). No significant correlation was relevant between Pgp expression and Ca-125 levels, histopathological differentiation, histologic subgroups of tumor, primary and residual tumor sizes and overall survival.

Conclusion: In epithelial ovarian cancer, Pgp expression has no effect on overall disease survival.

Key words: P-glycoprotein; Multidrug resistance; Epithelial ovarian cancer.

Introduction

Epithelial ovarian cancer (EOC) has been ranked as the fourth leading cause of death among women all over the world [1]. The development of acquired resistance limits the effectiveness of chemotherapy in the treatment of ovarian cancer. Many model systems have emerged to study the mechanisms associated with primary resistance to chemotheurapeutic agents and cross-resistance (multidrug resistance) which is characteristic of human ovarian cancer cell lines [2].

Mostly cited pathways to explain this resistance state were increased intracellular gluthathion-S transferase enzyme activity, changes in topoisomerase II activity, structural changes in tubulin molecules, high thimydylate synthetase and dihydrofolate reductase activity, increased DNA repair activity, somatic mutations in gene structures and multidrug resistant gene-associated P-glycoprotein (Pgp) expression [3-6]. Convenient chemotherapeutic combinations have not yet been available to get an optimal response and to decrease residual tumor burden. Hence, early recognition of chemotherapy-resistant tumor cell lines could possibly initiate new therapeutic strategies to cope with recurrent ovarian tumors. Pgp, a transmembrane glycoprotein, 170,000 kDa with 1,380 aminoacids, is encoded by multidrug resistance gene-1 (MDR-1) and plays an important role in the efflux or toxic agents out of the cell [7].

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The scope of this study was to assess the degree of Pgp expression in both primary and recurrent tumors following six courses of standard chemotherapy and its possible prognostic and predictive significance of the expression of P-glycoprotein in patients with advanced epithelial ovarian cancer.

Materials and Methods

Twenty-four patients, diagnosed with primary epithelial ovarian cancer between 1993-1999, at Osmangazi University Faculty of Medicine, Department of Obstetrics and Gynecology, Gynecologic Oncology Unit, were enrolled in this study. All cases had persistent tumor following six courses of combined chemotherapy within six months. All patients underwent primary optimal cytoreductive surgery including midline vertical incision, peritoneal washing, total abdominal hysterectomy, bilateral salpingo-oophorectomy and retroperitoneal (pelvic, paraaortic) lymph node sampling as the surgical staging procedure proposed by FIGO. Three weeks following the operation, 18 patients were assigned to PC (cisplatin 75 mg/m^2/IV and cyclophosphamide 750 mg/m^2 1-day course) and six patients to PT (cisplatin 75 mg/m^2/IV and paclitaxel 135 mg/m^2 IV, 1-day course) with a maximum of six courses of chemotherapy. During follow-up, all cases were evaluated by pelvic examinations, abdominopelvic ultrasound and computerised tomography and serum Ca-125 levels. Fourteen cases with persistent residual tumor on imaging scans or elevated serum Ca-125 levels were termed as 'having clinical recurrence and underwent secondary cytoreduction procedures. Ten cases with laboratory and clinical signs for clinical remission were sub-

mitted to second-look laparotomy (SLL), where peritoneal washing and multiple biopsies were taken. Out of ten cases with SLL, microscopic and macroscopic tumor burden were observed in four and six cases, respectively.

Pgp expression was determined both in primary and secondary cytoreductive surgery and SLL specimens, via the immunohistochemical method described by Beck *et al.* [8], by a copathologist. Paraffinized blocks of 4 μ from tumor tissues were retrieved, treated by 10% formol solution and spread on glass slides. These specimens were then deparaffinized in xylol, three times for five min, following a stay in the incubator at 70°C. Having been processed with 96% alcohol and distilled water, deparaffinized tissue blocks were then washed with EDTA enriched solution for 20 min, at 121°C. After a 20 min incubation period at room temperature, blocks were then processed with hydrogen peroxidase solution for 10 min. Following EDTA-enriched waterbath and Pepsin, glass slides were then treated with one drop of monoclonal primary antibody p170/P glycoprotein/MDR (human), Ab-2, Clone 4 [8]. After 60 min incubation and washing with tamponated solutions, secondary antibody (biotinylated antimouse immunoglobulin, Link, Biotinylated goat anti-mouse) was added and slides were then incubated at room temperature for 10 min. Following a wash with buffer solutions and being treated with streptavidin enzyme and EDTA-enriched solutions, consecutively, specimens were then preserved in a hematoxylin-eosin solution for 1 min and then washed with distilled water, 1% NH_3 solution was added to the slides and washing procedures with distilled water were processed. Slides were then covered and prepared for light microscopic examination (x10). A staining percentage < 30% of total slide area was considered to be Pgp negative, while between 30-100% staining was considered to be Pgp positive expression. Histopathologic types, degree of differentiation, preoperative serum Ca-125 levels, residual tumor volume and survival period were then analysed in association with degree of Pgp expression.

Statistical methods include the Fisher's exact test, chi-square test, Spearman rank correlation and Kaplan-Meier life table analysis via the SPPS 8® Package Programs; p values < 0.05 were considered statistically relevant.

Table 1. — *Pgp expression and clinicopathological variable distribution in 24 EOC cases.*

Parameters	Pgp (-)	Pgp (+)	P value
Histologic subtypes			
Serous	13	4	NS*
Non serous	5	2	
Ca- 125 levels (mIU/ml)			
< 35	4	1	NS
> 35	14	5	
Tumor differentiation			
Well differentiated	5	2	NS
Moderately differentiated	9	2	
Poorly differentiated	4	2	
Inital chemotherapy			
PC	1	5	NS (p = 0.098)
CC	10	8	
Residual tumor			
< 2 cm	6	11	NS
> 2 cm	0	7	
Residual tumor following 2nd surgery			
< 2 cm	7	4	NS
> 2 cm	6	7	

* Not significant

Table 2. — *Pgp expression and its changes before and after chemotherapy.*

	Pgp (+) after chemotherapy	Pgp (-) after chemotherapy	Total
Before chemotherapy			
Pgp (-)	11	7	18
Before chemotherapy			
Pgp (+)	0	6*	6
Total	11	13	24

* p = 0.016

Results

Patient ages ranged from 24 to 70 (mean 51). Histopathological evaluation demonstrated that 77% of the cases were serous, two cases mucinous (8.7%), two cases endometrioid (8.3%), one case clear cell and one case malignant Brenner tumor (4.1%), respectively. According to FIGO surgical staging classification, two cases were stage I, two cases were stage II and 20 cases were stage III-IV. As depicted, 96% of cases were of high stages (III-IV). In terms of degree of differentiation, well, moderately and poorly differentiated tumors comprised 29.2%, 45.8% and 25% of all cases, respectively. Mean tumor volume was 8.7 ± 5.3 cm (2-23 cm). Following primary surgery, in 17 cases residual tumor volume was < 2 cm (optimally debulked) and in seven cases > 2 cm (suboptimally debulked). In tumor specimens following primary surgery, 25% of cases (6/24) demonstrated Pgp expression. Associations between the degree of Pgp expression and various clinicopathological findings are shown in Table 1. As shown all parameters, except type of initial chemotherapy, have not been associated with Pgp expression (p > 0.05). Patients treated with PC chemotherapy, although not statistically significant, showed a trend for lower Pgp expression values (p = 0.098). Pgp expression before and after chemotherapy has been analyzed in Table 2. Pgp positivity observed in six cases of prior chemotherapy was consistent after chemotherapy, which was statistically relevant (p = 0.016).

Pgp expression was assessed at primary cytoreductive surgery and for overall survival with mean survival in cases with and without Pgp positivity being 27.2 ± 2.9 months and 29.0 ± 3.6 months, respectively (Figure 1a) (> 0.05). In persistant tumors, following six courses of chemotherapy, Pgp expression status did not reveal any statistically important changes with regard to overall survival (Figure 1b). Moreover, degree of Pgp expression, before and after chemotherapy, was examined in the three groups: Pgp -/- (n = 11), Pgp -/+ (n = 7) and Pgo +/+ (n = 6). Mean overall survival in Pgp -/-, -/+ and +/+ groups was 29.8 ± 3.7, 25.1 ± 4.9 and 29.0 ± 3.6 months, respectively; a statistically irrelevant association is shown in Figure 1c.

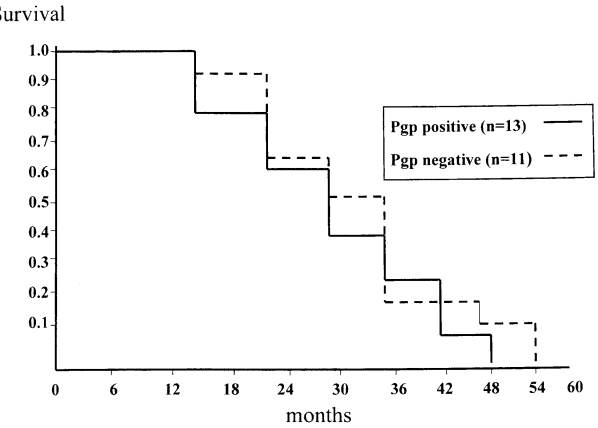

Figure 1a. — Life table analysis of Pgp expression in primary cytoreductive surgery.

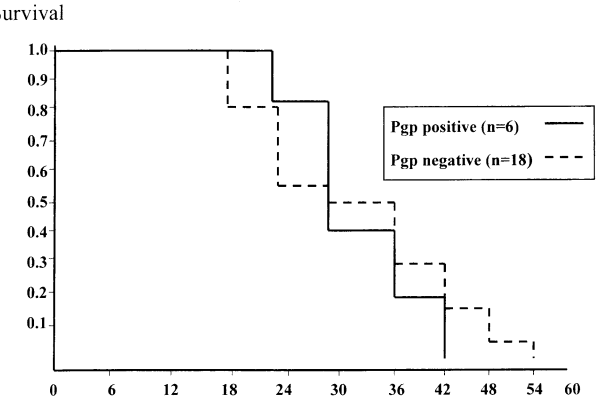

Figure 1b. — Life table analysis of Pgp expression status in persistant tumors following six courses of chemotherapy.

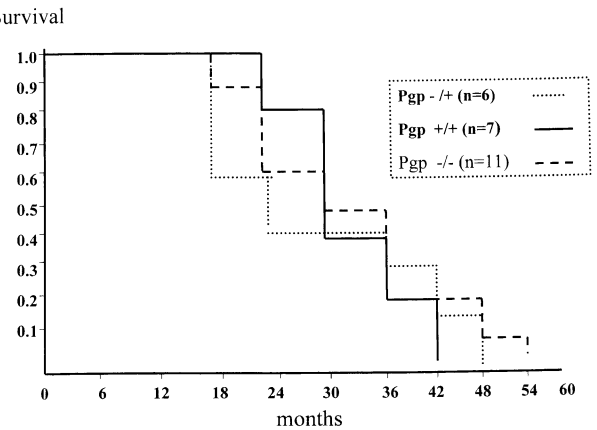

Figure 1c. — Life table analysis of tumor Pgp expression status before and after chemotherapy.

Discussion

Pgp has various functions in human tissues. It protects gastrointestinal mucosa against toxic materials accumulating in the lumen, it transports steroid hormones in the adrenal glands and it acts as an efflux pump to prevent intracellular toxin accumulation [8-10]. Van der Zee *et al.* [11], through their analysis on primary and recurrent epithelial ovarian tumors, declared a Pgp expression rate of 15% and 48%, respectively. In the same study, following chemotherapy, Pgp expression increased. In our study, there was a lack of significant differences in Pgp expression before and after chemotherapy. There are controversial results in the literature in terms of degree of Pgp expression. In our series, the expression rate was 25% (6/24). Goldstein *et al.* [12] in their study on ovarian malignancies, did not detect any Pgp staining. This variety of results could partially be explained by the method used to determine Pgp expression. The majority of cases in this study showed an uneven distribution trend toward higher stages. Singe most of the stages were stage II-IV, we were not able to find any association between Pgp expression and tumor stage. Schneider *et al.* [13], in their analysis of 38 epithelial ovarian tumors demonstrated higher expression rates in higher stages. In terms of histological subtypes, no significant differences in Pgp expression were found among various subtypes. There are however reports in the literature mentioning that degree of Pgp expression was higher in serous and mucinous types than in clear cell and endometriod subtypes [14]. Arao *et al.* [15] noticed higher expression in well-differentiated tumors, a finding that has not been reproducible by other studies or our study. Baekelandt *et al.* [16] also showed that Pgp expression is an independent predictor of both overall and progression-free survival. In our series, only in cases initially expressed as Pgp - and which developed Pgp positivity following chemotherapy had low overall survival time.

Pgp expression has been linked to acquired resistance to certain drugs named as MDR-associated drugs (taxanes, vinca alkaloids, anthracyclines and epipodophyllotoxins, although in cisplatin and cyclophospamide, methotrexate resistance, other than Pgp mechanisms have been found [5]. In MDR-associated chemotherapeutic regimens, Pgp expressivity varies between 30-100%, while, in non-MDR associated drugs such as cisplatin, expressivity rate is reported to be 0-25% [17]. Hence, different chemotherapeutics result in various degrees of Pgp expression that could affect clinical response rates. This result brings about the role of non-MDR activation mechanisms for partial clinical responses. Tumor cells may display a multidrug resistance phenotype by overexpression of ATP-binding cassette transporter genes such as MDR-1 and multidrug-resistance associated proteins- 1, 2, 3, 5 and MDR-3 P glycoproteins and lung resistance protein (LRP), that can be detected with a panel of specific monoclonal antibodies [18]. This knowledge, in the future will help us to change the genetic configuration of the genes and introduce a novel gene therapy for early recognition of epithelial ovarian cancers. In regard to MDR-associated chemotherapeutic use (especially taxanes), in recent years some MDR modulators (verapamil, cyclosporin A, etc.) have been experimented on and documented to be partially effective in alleviating cross resistance to these kinds of drugs [18-20]. In this study, P-glycoprotein expression was assessed in the tumors of

24 patients with ovarian carcinoma. In these patients, six patients (25%) had P-glycoprotein expression. This is in agreement with what has previously been reviewed in another recent study [19].

Conclusion

In this study we aimed to assess the expression of Pgp in ovarian cancers observed at first-line and second-look surgery to investigate whether this glycoprotein could be used as a predictor of response to treatment and survival. It is concluded that Pgp expression plays no role in overall survival and has no obvious association between various clinicopathological findings described in this study. However, an important drawback of this study is based on the small sample size which makes it difficult to assess any meaningful correlations. Further studies are needed to elaborate on the importance of Pgp or non-Pgp related resistance mechanisms on disease processes and to develop new MDR-modulator systems to overcome drug resistance against most chemotherapeutics, including taxanes.

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