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Original Article

# Efficacy of taxane and platinum-based chemotherapy guided by extreme drug resistance assay in patients with epithelial ovarian cancer

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**Objective:** To evaluate the efficacy of taxane and platinum-based chemotherapy guided by extreme drug resistance assay (EDRA) in patients with epithelial ovarian cancer.
**Methods:** Thirty-nine patients were enrolled, who were diagnosed as epithelial ovarian cancer, tubal cancer or primary peritoneal carcinoma and received both debulking surgery and EDRA in Asan Medical Center between August 2004 and August 2006. Another thirty-nine patients were enrolled, who did not receive EDRA as control. Paclitaxel 175 mg/m$^2$ and carboplatin AUC 5 were administered as primary combination chemotherapy to both EDRA group and the control group. In the EDRA group, paclitaxel was replaced by docetaxel 75 mg/m$^2$ if a patient showed extreme drug resistance (EDR) to paclitaxel and not to docetaxel. Carboplatin was replaced by cisplatin 75 mg/m$^2$ if a patient showed EDR to carboplatin and not to cisplatin. If only one drug showed low drug resistance (LDR), it was allowed to add another drug which showed LDR such as gemcitabine 1,000 mg/m$^2$. CT scan was performed every three cycles and CA-125 was checked at each cycle.
**Results:** There was no significant difference in overall response rate between EDRA group and the control group (84.5% vs. 71.8%, p=0.107). However, 93.8% of patients in EDRA group did not show EDR to at least one drug and its response rate was significantly higher than that of the control group (93.3% vs. 71.8%, p=0.023).
**Conclusion:** we could choose a combination of taxane and platinum which did not show EDR and could obtain a good response in the patients with ovarian cancer.

**Key Words:** Ovarian neoplasms, Antineoplastic combined chemotherapy protocol, Drug resistance, neoplasm, Biologic assay

## INTRODUCTION

Epithelial ovarian cancer is one of the most common causes of death among gynecologic malignancies. The principle of treatment is cytoreductive surgery followed by adjuvant chemotherapy, in which taxane and platinum-based combination chemotherapy is the treatment of choice. However, 27-40% of the advanced epithelial ovarian cancer patients do not respond to such a primary chemotherapy and the 5-year survival rate is still less than 50%, which is responsible for chemoresistance.[1]

Therefore, several types of *in vitro* drug response assays, which measure the potential activities of various chemotherapeutic agents to individual patient before administration, have been developed to overcome the limitation of chemotherapy and to improve response and survival. Such *in vitro* assays, if reliable, can make it possible to build individualized plan for treatment according to biologic characteristics of the tumor, save time and cost, and avoid unnecessary adverse effects. Those assays are usually called 'chemotherapy sensitivity tests', but the formal term is 'chemotherapy sensitivity and resistance assay (CSRA)'.[2]

Since Black and Speer, the pioneers of CSRA, developed the tetrazolium dye reduction assay in 1950's,[3,4] a variety of *in vitro* techniques have been developed, including the recent extreme drug resistance assay (EDRA). Extreme drug resistance (EDR) is defined as the tumor cell growth which is larger than

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a standard deviation over the median after administration of a chemotherapeutic agent. EDRA is the only chemotherapy 'resistance' test among the CSRAs with an accuracy of 99.2% that predicts chemoresistance.[5,6] EDRA was commercialized as the Extreme Drug Resistance Assay (EDR)® by Exiqon Diagnostics, Inc. (Tustin, CA, USA), and it is the only test approved by the College of American Pathologists, one of the Center for Medicare and Medicaid Services (CMS).

In this study, we utilized EDRA in choosing chemotherapeutic agents in patients with epithelial ovarian cancer. The objective is to assess feasibility of individualized chemotherapy guided by EDRA.

## MATERIALS AND METHODS

### 1. Patients and control

Patients with epithelial ovarian cancer, tubal cancer, or primary peritoneal carcinoma who received cytoreductive surgery at Asan Medical Center between August 2004 and August 2006 were eligible. Eligibility criteria included age ≥ 18 years; a diagnosis of stage ≥ Ic; Gynecologic Oncology Group performance status of 0, 1, or 2. We prospectively enrolled those patients who voluntarily received EDRA at their own expense and thirty-nine patients were enrolled as the EDRA group. These patients were matched with those who received surgery but did not receive EDRA with regard to age, histology, stage, and residual tumor size during the same period, and another thirty-nine patients were enrolled as the control group retrospectively.

### 2. Collection of malignant tissue

We examined the tumor grossly and harvested tissues three times larger than required for EDRA from the central portion of the tumor, which was divided into three pieces. One was sent to pathologist for frozen biopsy. The other was sent for EDRA and the third was fixed with 10% buffered formaldehyde for paraffin block and hematoxylin and eosin staining. If the result of frozen biopsy was 'carcinoma', we regarded the other two pieces of the tissue as the same 'carcinoma'. More than 2.0 grams of tumor tissue was harvested for EDRA by an aseptic technique, rinsed out with normal saline to remove blood, put in a transport media and packed immediately, which was send to Exiqon Diagnostics, Inc., the name of which was Oncotech Co. previously, to be tested.

### 3. EDRA

Fresh viable tumor tissue was minced and enzymed to disaggregate the tumor cells. The tumor cells were plated in soft agar which preferentially favors tumor cell proliferation. Cells were exposed to chemotherapeutic agents, such as carboplatin, cisplatin, docetaxel, paclitaxel, etoposide, gemcitabine and ifosphamide, for five days in a carefully controlled environment. Drug exposures in excess of the maximum tolerated doses were used. Due to the reduced rate of drug metabolism, *in vitro* tumor exposure is 5 to 80 times greater than *in vivo*.[6,7] Tritiated thymidine was introduced during the last two days of culture as a measure of cell proliferation. Treated cells were compared to untreated controls.

### 4. Assessment of chemoresistance

Assay results were divided into three categories; EDR was defined as tumor cell growth greater than 1 standard deviation above the median. Intermediate drug resistance (IDR) was defined as tumor cell growth greater than the median growth but less than 1 standard deviation above the median. Low drug resistance (LDR) was defined as tumor cell growth of less than the median growth.[6]

### 5. Chemotherapy

Paclitaxel 175 mg/m$^2$ and carboplatin AUC 5 were administered as primary combination chemotherapy to both the EDRA group and the control group. In the EDRA group, paclitaxel was replaced by docetaxel 75 mg/m$^2$ if a patient showed EDR to paclitaxel and not to docetaxel. Carboplatin was replaced by cisplatin 75 mg/m$^2$ if a patient showed EDR to carboplatin and not to cisplatin. If only one drug showed LDR, it was allowed to add another drug which showed LDR, such as gemcitabine 1,000 mg/m$^2$.

### 6. Assessment of clinical response

We assessed those patients who received three cycles or more of taxane-platinum combination chemotherapy. Abdomen and pelvis CT scan was performed every three cycles and CA-125 was checked at each cycle. Clinical response was assessed by REICEST criteria in measurable disease[8] and by CA-125 criteria in unmeasurable disease.[9]

### 7. Analysis of data and statistics

$\chi^2$ test and Fisher's exact test were utilized to compare the response rate. We assessed that it was statistically significant when p-values were less than 0.05. Statistical Package was SPSS for Windows ver. 13.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

### 1. Demographics of the patients

The median age of patients was 49 years (range, 27 to 78 years) in the EDRA group, and 52 years (range, 33 to 80 years) in the control group. Their diagnosis, histologic type, FIGO stage, and size of residual tumor are described in Table 1. There was no difference in the patient demographic characteristics between the two groups.

### 2. Chemotherapy

In the EDRA group, twenty-two patients received paclitaxel-carboplatin, nine patients received docetaxel-cisplatin, including two cases of docetaxel-cisplatin-gemcitabine triplet, four patients received docetaxel-carboplatin, including one

case of docetaxel-carboplatin-gemcitabine triplet, two patients received paclitaxel-cisplatin, one patient received docetaxel-gemcitabine, and one patient received cyclophosphamide-adriamycin-carboplatin. In the control group, all patients received conventional paclitaxel-carboplatin chemotherapy.

### 3. Comparison of EDRA results with clinical responses

In the EDRA group, ten out of thirty-nine patients (25.6%) showed LDR to both two chemotherapeutic agents (two of three agents in triplets). One patient died during chemotherapy due to septic shock and the other nine patients, whose clinical response could be assessed, showed seven complete responses (CR) and two partial responses (PR). Eleven patients (28.2%) showed LDR to one agent and IDR to the other agent. Their clinical responses were six CRs, three PRs, one stable disease (SD) and one 75% response of CA-125. Four patients (10.3%) showed LDR to one agent and EDR to the other agent and they all showed CR. Four patients (10.3%) showed IDR to both two agents and their clinical responses were two CRs, one PR and one SD. Two patients (5.1%) showed IDR to one agent and EDR to the other agent. They all showed CR, but one patient recurred. Two patients showed EDR to both two agents. One patient showed SD and the other was progressed, therefore there was no response in the patients who showed EDR to both two agents. In the control group, the clinical responses were twenty-four CRs (61.5%), four PRs (10.3%), three SDs and eight progressive diseases (PD) (Table 2).

The response rate of the patients who showed LDR to both two agents was 100% (9/9). The response rate of the patients who showed LDR to at least one agent was 95.8% (23/24). The response rate of the patients who showed IDR to at least one agent was 93.3% (28/30). Overall response rate was 87.5% in the EDRA group and 71.8% in the control group, but there was no significant difference (p=0.107, $\chi^2$ test). However, 93.8% of patients in the EDRA group did not show EDR to at least one drug and its response rate (93.3%) was significantly higher than that of the control group (p=0.023, $\chi^2$ test).

**Table 1.** Demographics of the patients

| Characteristics | EDRA group (N=39) | Control group (N=39) |
|---|---|---|
| Median age (range) | 49 yr (27-78) | 52 yr (33-80) |
| Diagnosis | | |
| Epithelial ovarian Ca. | 36 (2 recurrent cases) | 36 |
| Epithelial tubal Ca. | 1 | 0 |
| Primary peritoneal Ca. | 2 | 3 |
| Histology | | |
| Serous | 29 | 33 |
| Mucinous | 2 | 1 |
| Clear cell | 3 | 0 |
| Endometrioid | 4 | 1 |
| Transitional cell | 0 | 3 |
| Others | 1 | 1 |
| FIGO stage | | |
| I | 7 Ic | 5 Ic |
| II | 2 IIc | 1 IIa, 1 IIb |
| III | 1 IIIb, 28 IIIc | 1 IIIb, 29 IIIc |
| IV | 1 | 2 |
| Residual tumor | | |
| <2 cm | 36 | 36 |
| >2 cm | 3 | 3 |

EDRA: extreme drug resistance assay

## DISCUSSION

After two decades since Black and Speer,[3,4] the pioneers of CSRA, developed the tetrazolium dye reduction assay in the 1950's, Hamburger and Salmon[10] developed the human cancer stem cell assay in the 1970's. Their success aroused investigators' interest in the chemosensitivity test in solid tumors and it lead them to develop a variety of *in vitro* CSRAs. The potential benefits of CSRA are, though they are not achieved yet, as follows; a screening tool for new chemotherapeutic agents, optimizing chemotherapy for individual patients, excluding ineffective agents that can reduce unnecessary complications,

**Table 2.** The relationship between EDR assay and clinical response

| EDR assay | no. (assessable) | Response | Response rate (%) |
|---|---|---|---|
| LDR/LDR | 10 (9) | 9 (7 CR, 2 PR) | 100 |
| LDR/IDR | 11 | 11 (6 CR, 3 PR, 1 SD, 1 75% R) | 91 |
| LDR/EDR | 4 | 4 (4 CR) | 100 |
| IDR/IDR | 4 | 3 (2 CR, 1 PR. 1 SD) | 75 |
| IDR/EDR | 2 | 2 (2 CR, 1 recur) | 100 |
| EDR/EDR | 2 | 0 (1 SD, 1 PD) | 0 |
| LDR to both two drugs | 9 | 9 (7 CR, 2 PR) | 100 |
| LDR to at least one drug | 24 | 23 (17 CR, 5 PR, 1 75% R) | 95.8 |
| IDR to at least one drug | 30 | 28 (21 CR, 6 PR, 1 75% R) | 93.3* |
| Overall (EDRA group) | 32 | 28 (21 CR, 6 PR, 1 75% R) | 87.5† |
| Control | 39 | 28 (24 CR, 4 PR, 3 SD, 8 PD) | 71.8 |

EDR: extreme drug resistance, LDR: low drug resistance, IDR: intermediate drug resistance, CR: complete response, PR: partial response, SD: stable disease, PD: progressive disease, 75% R: 75% response
*p=0.023, $\chi^2$ test, †p=0.107, $\chi^2$ test

chemosensitivity profile according to histology and subtype, establishing profile of cross-resistance and sensitivity before treatment or after recurrence, finding genes and proteins related to chemoresistance and chemosensitivity and matching preclinical *in vitro* assay and clinical response.

However, CSRA has a few limitations which should be resolved before it is applied clinically. First, is the tissue sample of the tumor able to represent the whole tumor? Second, the result of CSRA does not correlate to survival. Third, the cost of CSRA is so expensive that the cost-effectiveness should be proved.

Cortazar and Johnson[11] analyzed twelve reports which compare chemotherapy based on CSRA with those based on physician's experience. They concluded that the response rate of the chemotherapy based on CSRA is at least similar to that of the chemotherapy based on the physician's experience. The American Society of Clinical Oncology (ASCO) Working Group on CSRAs did not recommended the use of CSRAs to select chemotherapeutic agents for individual patients outside the clinical trial setting.[12] Therefore, we designed EDRA-guided chemotherapy in this study based on the taxane and platinum agents, which has been proved to be the most effective.

Cloven et al.[13] reported that the rates of EDR in taxanes and platinums are as follows; 22% in paclitaxel, 16% in carboplatin and 10% in cisplatin. Eltabbakh et al.[14] reported that one out of seventy-five patients with epithelial ovarian cancer showed EDR to both paclitaxel and cisplatin, and that the patient did not respond to primary chemotherapy with paclitaxel and cisplatin. In this study, we also experienced such results in two patients. Holloway et al.[7] reported a significantly lower 5-year survival rate in patients with EDR to platinum than the patients with LDR to platinum (19% vs. 68%).Therefore, it is reasonable to avoid such a combination as both agents show EDR. No significant difference in response is expected when we choose docetaxel instead of paclitaxel[15] and also when we choose cisplatin instead of carboplatin.[16] Therefore, it would be advisable to replace paclitaxel with docetaxel if the patient showed EDR to paclitaxel and not to docetaxel. We can also replace carboplatin with cisplatin if the patient showed EDR to carboplatin and not to cisplatin.

Though the high cost of EDRA is an obstacle to applying EDRA to every patient, it can be overcome if EDRA is proved to be cost-effective. Orr et al.[17] reported the cost-effectiveness of cytoreductive surgery and chemotherapy directed by EDRA in the treatment of women with advanced ovarian cancer. They chose either paclitaxel or cyclophosphamide in combination with platinum according to EDRA after cytoreductive surgery. Although there was no difference in survival whether chemotherapy was directed by EDRA or not, $6,000 of cost was saved when directed by EDRA.

In this study, we applied the strategy to patients receiving primary chemotherapy. If we apply this strategy to recurrent cases, there may be two theoretical reasons that *in vitro* resistance can be changed. One reason is that chemosensitive clones have been exterminated after primary chemotherapy but chemoresistant clones grows because of the tumor heterogeneity. The other reason is that tumor genes associated with resistance can be activated after primary chemotherapy. If the above are true, secondary cytoreductive surgery or at least open biopsies are necessary to obtain tissue for EDRA. Tewari et al.[18] compared the results of EDRA at primary cytoreductive surgery with the results at second cytoreductive surgery after recurrence. There was approximately 10% difference of the EDR profile in synchronous tumors (primary and metastatic tissues obtained from the same patient at diagnosis) among 119 patients, and there was no significant difference of the EDR profile in metachronous lesions (specimens from the same patient before and after chemotherapy) in 334 patients. The above authors therefore concluded that it is possible that assay results at diagnosis could be used to guide subsequent therapy at relapse, especially when recurrent tissue is not available for analysis. Loizzi et al.[19] reported a case-control study of EDRA-guided chemotherapy in recurrent cases. In the platinum-sensitive group, patients with EDRA-guided therapy had an overall response rate of 65%, compared with 35% in patients who were treated empirically ($p=0.02$). The overall and progression-free median survival rates were 38 and 15 months in the EDRA group compared with 21 and 7 months in the control group, respectively ($p=0.005$, overall; $p=0.0002$, progression free). In the platinum-resistant group, there was no improved outcome in the patients who underwent assay-guided therapy. In multivariate analysis, EDRA-guided therapy was an independent predictor for improved survival.

This study also showed that EDRA may be a possible tool to assess whether triplet chemotherapy is beneficial for a patient. The paclitaxel-carboplatin-gemcitabine triplet has failed to show benefits over the paclitaxel-carboplatin doublet.[20-22] If a patient shows EDR to gemcitabine or if a patient does not show EDR to both paclitaxel and carboplatin, the patient who receives triplet therapy may not show any survival benefit but additional toxicity. Therefore, a patient may benefit from a triplet therapy when the patient does not show EDR to gemcitabine or show EDR to both paclitaxel and carboplatin. There were only two cases of triplet in this study and this hypothesis should be evaluated in a larger clinical trial.

In conclusion, this trial was the first case-control study of first-line chemotherapy guided by EDRA in patients with epithelial ovarian cancer. It was possible to choose a combination of taxane and platinum which did not show EDR in most cases and to obtain a good response.

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