Cisplatin Sensitivity of Ovarian Cancer in the Histoculture Drug Response Assay Correlates to Clinical Response to Combination Chemotherapy with Cisplatin, Doxorubicin and Cyclophosphamide

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Abstract. *The histoculture drug sensitivity assay (HDRA) has been demonstrated to have high predictability for resistance, sensitivity, and survival for gastrointestinal cancer (Clin Cancer Res 1: 305-311, 1995; Clin Cancer Res 1: 1537-1543, 1995). In this report, we evaluated the clinical usefulness of the HDRA in ovarian cancer. HDRA was performed on tumors from patients with ovarian cancer. Eighty-five cases (97%) were evaluable. Tumor fragments were cultured on collagen-sponge gels. The cultures were incubated with cisplatin (CDDP) for seven days. Cell viability were assessed with the MTT end point. The optimal cut off concentration of CDDP was determined to be 25 μg/ml by correlation with the historical clinical response rate to CDDP. HDRA results were correlated to clinical response of 15 patients who received CDDP-based therapy that included doxorubicin and cyclophosphamide (CAP therapy). The true positive rate was 88%, the true negative rate was 86%, the sensitivity was 88%, the specificity was 86%, and the accurate prediction rate was 87% when HDRA results were compared to the response of the treated patients. The data suggest that the HDRA is capable of predicting the response to antitumor chemotherapy in patients with ovarian cancer and that measuring response to CDDP can be useful for optimization of CAP chemotherapy for patients with this disease.*

The cisplatin, doxorubicin and cyclophosphamide (CAP) combination is current front-line therapy for ovarian cancer in which cisplatin (CDDP) is the key drug (1,2). However, the use of this therapy includes patients who do not respond to CDDP. This may have contributed to the increasing number of patients who have developed resistance to this combination chemotherapy (3). Therefore, the five-year survival rate of patients with ovarian cancer is still low (23% for stage III and 8% for stage IV) (4).

To improve the response rate of ovarian cancer, it is essential to select the anti-cancer agents that are effective for individual case. To this end, an *in vitro* drug sensitivity test is appropriate. A number of approaches to assess the sensitivity of cancer to drugs *in vitro* have been attempted. Among these methods, the human tumor clonogenic assay (HTCA) was reported to be clinically useful in predicting the responses of cancer to treatment (5,6,7). Inoue et al. used the HTCA on ovarian cancer and reported a true positive rate of 29%, a true negative rate of 77%, and an accuracy of 60% (8). Thus, the HTCA allowed identification of ineffective drugs to some extent but had a low probability of identifying effective drugs (9). Furthermore, the HTCA involves complex manipulation and takes a relatively long period of time (2-3 weeks). This technique can evaluate drug sensitivity at a 60-75% rate in ovarian cancer (8,9). This percentage is higher than that for other types of cancer, but it is still not sufficiently high. For these reasons, the HTCA has not yet been extensively adopted in clinical practice.

Hoffman *et al.* have developed the histoculture drug response assay (HDRA) which combines three-dimensional culture of tissue fragments of the tumor (histoculture) using a collagen gel matrix with a [^{3}H]thymidine-uptake end point (10-14). Unlike many conventional drug sensitivity tests which use isolated tumor cells obtained after enzyme treatment, this technique uses cancer cells in their native tissue architecture which can grow in three dimensions, maintaining intercellular contact and interactions with stromal cells. This technique can thus assess the sensitivity of tumor cells in conditions similar to those *in vivo.*

Kubota *et al.* demonstrated that *in vitro* response in the HDRA with the [^{3}H]thymidine end point correlates to survival of patients with gastric cancer (21). Furukawa *et al.*

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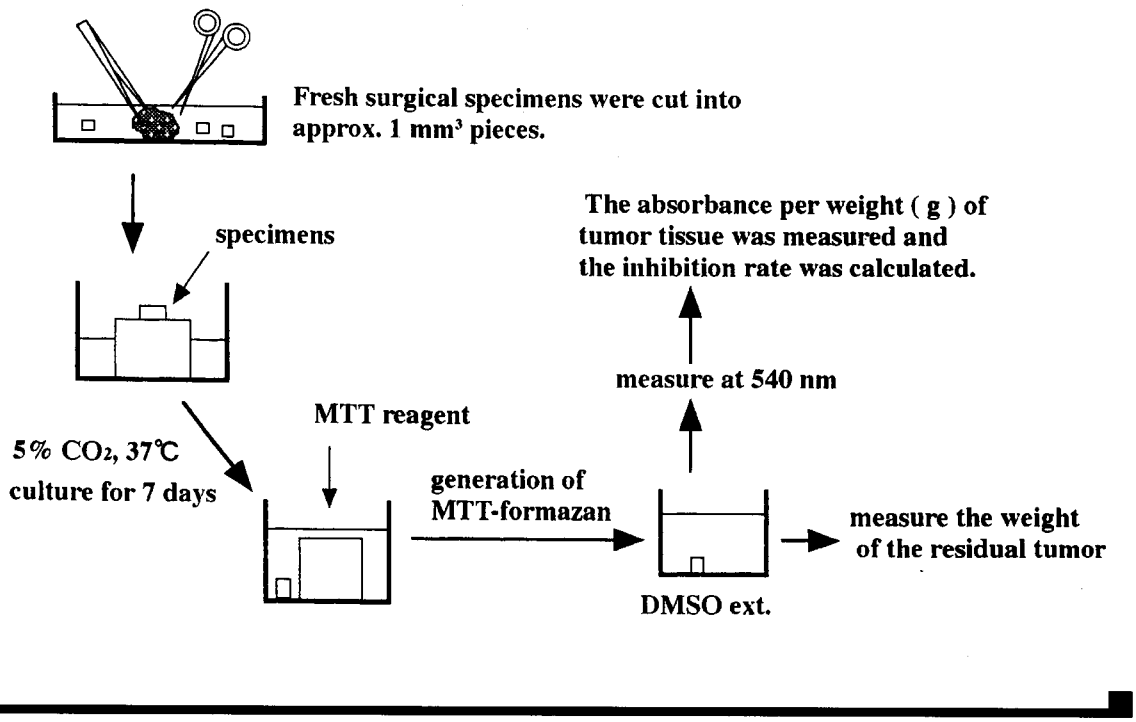

Figure 1. *HDRA procedure.*

demonstrated that response in the HDRA with the 3-(4,5-dimethyl2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reduction as an end point (15,16) correlated with patient drug response and survival (17).

The present study was undertaken to determine the predictability of the HDRA for response of individual patients with ovarian cancer. A cumulative response curve for CDDP was prepared from a series of HDRA assays of tumor specimens from patients with ovarian cancer. Historical clinical response rates to CDDP were compared with this curve to calculate the optimum cut off concentration of CDDP. The HDRA was then used on a series of 88 ovarian cancer patients. The *in vitro* results were correlated with the clinical response of these patients to CAP.

Table I. *Ovarian cancer histologies evaluated in the HDRA.*

Histology	Number of patients
Serous cystadenocarcinoma	23
Mucinous cystadenocarcinoma	9
Clear cell adenocarcinoma	28
Endometrioid adenocarcinoma	19
Others	9
Total	88

Materials and Methods

Subjects. The specimens were obtained from 88 patients with ovarian cancer treated surgically at the Keio University Hospital and related hospitals between April 1994 and July 1996. The histologies types of the individual cases are shown in Table I.

HDRA procedure. The HDRA method reported by Furukawa *et al.* (16) was used with some modification (Figure 1). Collagen-sponge gels (Gelfoam, Upjohn, Kalamazoo, MI) were cut into approximately one cm^3 pieces with a surgical knife. One piece was then placed in each well of a 24-well plate. CDDP was dissolved at varying concentrations in Ham's F-12 medium (Gibco BRL), containing 20% heat-immobilized fetal calf serum (Nakashibetsu Fetal Calf Serum, Mitsubishi Chemical Industries, Ltd.) and kanamycin (80 μg/ml). The medium was pipetted into each well (1 ml/well), taking care to avoid covering the sponge gel completely. Five concentrations of CDDP were used (100, 50.0, 25.0, 12.5, and 6.25 μg/ml). Four samples were prepared at each concentration.

Fresh surgical specimens, collected aseptically, were cut with scissors into approximately 1 mm^3 fragments in Hanks' solution. The pieces were put on sponge gels in culture medium.

Each plate was incubated for 7 days at 37°C under 5% CO_2. At the end of the incubation, MTT dissolved in phosphate buffered saline (5 mg/ml), containing 100 mM sodium succinate, was aseptically added to each well of the 24-well plate (100 μ/well). The plate was then

Table II. *Relationship of surgical outcome and HDRA sensitivity to CDDP.*

Resection	IC_{50} value (μg/ml)			
	≤25	25 ~ 70	70≥	Total
Complete	12	14	8	34
Incomplete	12	13	8	33
Total	24	27	16	67

Of the 88 cases for which HDRA was conducted, 67 received chemotherapy including CDDP after surgery. Table II shows the distribution of sensitivity, as assessed by the HDRA, in relation to the degree of surgery. The sensitivity distribution did not depend on the degree of surgery. Of the 33 cases which received incomplete surgery, 15 had some residual lesions suitable for evaluation after surgery.

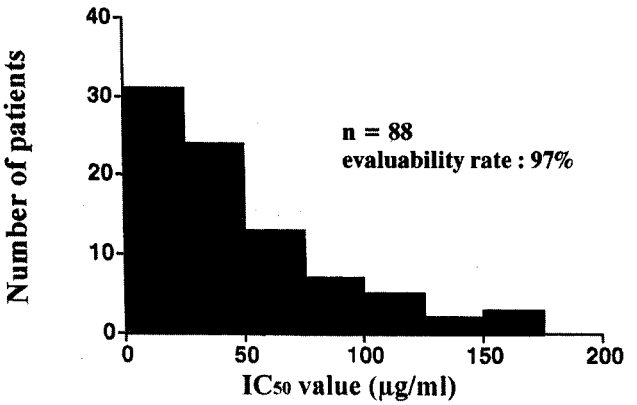

Figure 2. *Histogram of IC_{50} values of CDDP for ovarian cancer. HDRA was performed on tumor tissue from 88 patients with ovarian cancer, 86 of which were evaluable. The mean IC_{50} was 48.9 μg/ml.*

incubated for 4 hours at 37°C under 5% CO_2. The stained tumor pieces were then transferred to another 24-well plate containing dimethyl sulfoxide (1ml/well) to extract MTT-formazan. The extract was then placed into the 96-well microplate (100 μl/well). The absorbance in each well at 540 nm was measured, using a microplate reader (Model 450, Bio-Rad, Hercules, CA). The weight of the residual tumor after extraction of MTT-formazan was measured, and the absorbance per g tumor was calculated. The tumor inhibition rate (%), relative to the untreated control group, was then calculated using the following equation: Tumor inhibition rate (%) = [1 - (absorbence per g tumor in the treated group / absorbence per g tumor in the untreated group)] x 100.

At each concentration, the inhibition rates for the 4 wells were averaged to construct a dose-response curve. The concentration that caused 50% inhibition of tumor growth (IC_{50}) was then calculated. A 50% or greater inhibition at the cut-off concentration of 25 μg/ml CDDP was scored as "sensitive" in the HDRA.

The clinical responses to CDDP-based chemotherapy were assessed in 15 cases whose residual tumor lesions were suitable for evaluation after surgery. The criteria of the Japanese Society of Cancer Therapy were employed in evaluating the direct effects of chemotherapy. The response rate, that is the percentage of cases showing complete response (CR) or partial response (PR), was analyzed. Cases in which tumor tissue was determined by the HDRA to be sensitive and which responded clinically to chemotherapy were regarded as true positive (S/S) cases. Cases in which tumor tissue was determined by the HDRA to be resistant to chemotherapy were regarded as true negative (R/R) cases. Cases in which tumor tissue was determined by HDRA to be resistant but responded to chemotherapy were regarded as false negative (R/S) cases. Cases in which the tumor tissue was determined to be sensitive in the HDRA but clinically resistant were regarded as false positive (SIR). The accurate prediction rate (%) was calculated using the following equation:

Accurate prediction rate (%) = (No. of true positive cases + No. of true negative cases) / Total No. of cases.

Results

HDRA was conducted on fresh surgical specimens from 88 patients with ovarian cancer. The HDRA was successfully carried out in a very high percentage of cases (97%). Evaluation in the HDRA was not possible for 3 cases because

Table III. *HDRA and clinical responses in patients with measurable residual tumor.*

Patient	Stage	IC_{50} value of CDDP (μg/ml)	Clinical response
S.G.	III c	6.4	PR
M.M.	III c	11.3	PR
F.K.	III c	12.0	CR
Y.W.	III c	22.4	PR
K I.	III c	23 4	PR
R.O.	III c	23.4	PR
K.T.	III b	23.7	CR
K.O.	III c	24.1	NC
SN.	IV	36.2	CR
K.A.	IV	39.5	NC
N.T.	IV	49.5	NC
S.H.	IV	75.8	PD
E.M.	IIIc	106	NC
R.K.	IIIc	112	NC
T.K.	IIIc	137	NC

Of the 88 cases for which HDRA was conducted, 33 cases had residual disease after surgery and received chemotherapy including CDDP. Of these 33 cases, 15 cased shown in this table had some residual lesions suitable for evaluation after surgery. NC: no change, PR: partial response, CR: complete response, PD: progressive disease.

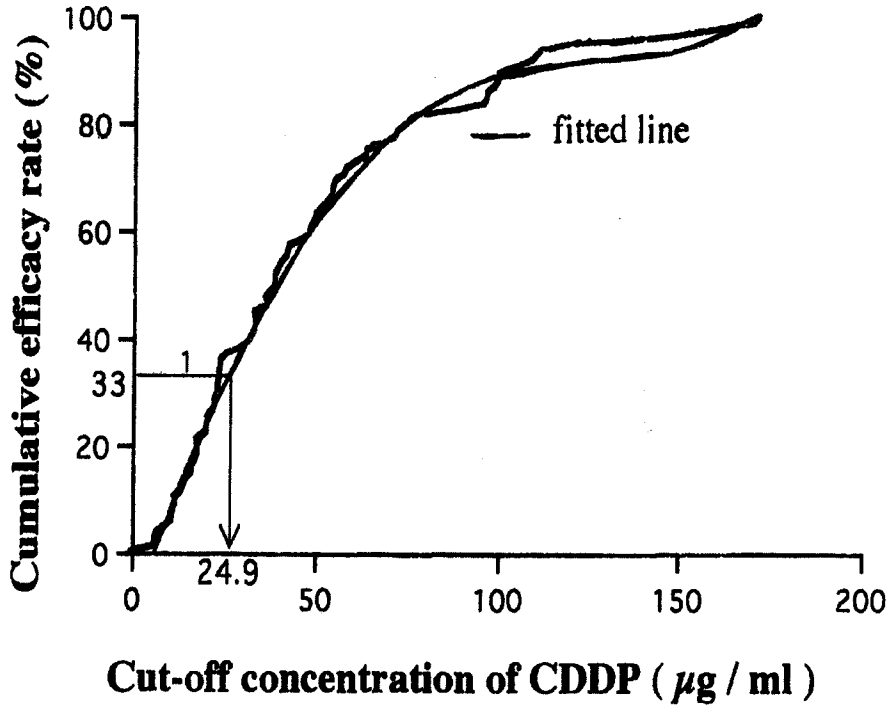

Figure 3. *Cumulative efficacy rate curve of CDDP for ovarian cancer. A cumulative response curve was prepared from the distribution of CDDP IC_{50}'s as determined by the HDRA. An approximate curve was obtained from this cumulative response curve, using the curve. The historical clinical response rate of 33%, reported by Tobias and Griffiths, et al. (18), was used as the clinical response rate for treatment with CDDP alone. The resultant optimum cut-off dose level for CDDP was 25 μg/ml.*

of insufficient color development of the controls in the assay.

The HDRA yielded IC_{50} levels for CDDP which ranged from 6.4 to 132, with a mean 48.9 μg/ml (Table III). The sensitivity did not differ significantly with histologic type, clinical stage or degree of tumor differentiation (data not shown). Figure 2 shows a histogram of the distribution of the CDDP IC_{50}'s. A cumulative response curve was prepared from the distribution of CDDP IC_{50}'s as determined in the HDRA (Figure 3).

An approximate curve was obtained from this cumulative response curve, using the curve fit method. Clinical response rates were applied to this equation, to calculate the optimum cut off IC_{50} level. The response rate of 33%, reported by Tobias and Griffiths *et al.* (18), was used as the historical clinical response rate to treatment with CDDP alone. The resultant optimum cut off dose level for CDDP was determined to be 25 μg/ml. Therefore, cases for which the IC_{50} was below 25 μg/ml were scored as sensitive to CDDP.

Of the 88 cases on which HDRA was conducted, 67 received themotherapy including CDDP after surgery. Table II shows the distribution of sensitivity, as assessed using the HDRA, in relation to the degree of surgery. The sensitivity distribution did not depend on the degree of surgery. Of the 33 cases which received incomplete resection 15 had residual lesions suitable for evaluation after surgery. For these 15 cases, we analyzed the relationship between clinical responses and sensitivity in the HDRA (Table III).

. Of the 8 cases in which the IC_{50} was determined by the HDRA to be 25 μg/ml or lower, 2 had CR, 5 had PR, and 1 had NC (no change). Of the 3 cases with an IC_{50} between 25 and 70 μg/ml, 1 had CR and 2 had NC. Of the 4 cases with an IC_{50} over 70 μg/ml, all had NC.

Table IV summarizes the results of the analysis of the relationship between clinical responses and sensitivity in the HDRA. When the cut off level was set at 25 μg/ml (a level determined from the historical clinical response rate to CDDP), the true positive rate was 88%, the true negative rate was 86%, the sensitivity was 88%, the specificity was 86%, and the accurate prediction rate was 87%.

Discussion

A number of drug sensitivity tests have been used to predict the responses of individuals to anticancer agents. However, none of these tests satisfy the criteria of predicting clinical outcome, simplicity, and rapidity. The HDRA, which was used in the present study, is based on three-dimensional culture. The most important characteristic of this method is

Table IV. *Correlation between clinical response and sensitivity in the HDRA.*

Cut-off value (μg/ml)	Sensitivity with HDRA / clinical response			
	S/S	S/R	R/S	R/R
25	7	1	1	6

S: Sensitive, R: Resistant

Cases in which tumor tissue was determined by the HDRA to be sensitive and which responded clinically to chemotherapy were regarded as true positive (S/S). Cases in which tumor tissue was determined in the HDRA to be resistant and which did not respond to chemotherapy were regarded as true negative (R/R). Cases in which tumor tissue was determined by the HDRA to be sensitive but which did not respond to chemotherapy were regarded as false positive (S/R). Cases in which tumor tissue was determined by the HDRA to be resistant but which responded to chemotherapy were regarded as false negative (R/S) cases.

that it maintains inter-cellular contact in a native three-dimensional architecture. The accurate response and a high rate of evaluability of the HDRA is most probably based on this feature.

In the present study, the use of the HDRA determined drug sensitivity in 7 days without causing any significant delay in the start of drug therapy. Furthermore, the HDRA evaluated a very high percentage (97%) of the cases.

Front line therapy in ovarian cancer usually involves CDDP as the key drug in combination with cyclophosphamide and doxorubicin (CAP therapy). The efficacy of multiple drug chemotherapy is not always equal to the total of the efflcacy of individual drugs used. Furthermore, the extent of contribution by individual drugs and their interactions are often unknown. We attempted in this study to predict the response to CAP chemotherapy by assessing the sensitivity to CDDP alone. The cut-off dose of 25 μg/ml CDDP correlated to the historical clinical response rate of 33% to CDDP alone (10).

When the response to CDDP-based CAP therapy was correlated on the basis of this cut-off level, the true positive rate was 88%, the true negative rate was 86%, the sensitivity was 88%, the specificity was 86%, and the accuracy rate was 87%. The true-positive rate was particularly high, compared with previously-used drug sensitivity tests. This suggests that the HDRA enables the identification of effective drugs in individual cases with ovarian cancer.

The high accuracy rate, obtained in the present study, indicates that the sensitivity to CDDP may be the sentinel for sensitivity to CAP therapy.

The average IC_{50} level obtained for CDDP in the present study (48.9 μg/ml) was high compared to the peak blood CDDP level in humans (about 2.5 μg/ml). Furukawa et al. have reported that when the same tumor cell line was used, the sensitivity to anti-cancer agents in the HDRA was lower than that as assessed by monolayer culture using isolated cells (19). Maehara *et al.* noted that the activity of succinate dehydrogenase measured by the MTT procedure remained even after the cells lost their viability (20). The MTT end point may also be less sensitive than cell proliferation assays. Therefore, three-dimensional culture with the MTT end point has a lower sensitivity to drugs than monolayer or other culture methods.

The goal of a drug sensitivity assay is to accurately predict the response of tumors to chemotherapy not to attempt to be a scale model of the patient. Prospective studies will be carried out to determine to what degree the HDRA can improve clinical outcome in ovarian cancer.

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