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Chemosensitivity Testing of a Novel Platinum Analog, Nedaplatin (254-S), in Human Gynecological Carcinomas: a Comparison with Cisplatin

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Abstract. *Background: The tetrazolium dye (MTT) assay is useful for predicting chemosensitivity. Materials and Methods: Using the MTT assay, an in vitro chemosensitivity test was designed for nedaplatin (cis-diammine glycolato platinum; 254-S) and the results were compared with the sensitivity to cisplatin in 137 resected gynecological carcinomas. Results: The mean tumor inhibition rate [I.R.; %] for nedaplatin was equal or superior to cisplatin in 15 cervical [70.7% vs. 63.9%], 65 ovarian [61.7% vs. 54.8%] and 57 endometrial carcinomas [52.1% vs. 47.7%]. In ovarian carcinomas, the I.R.s for nedaplatin were significantly higher than cisplatin in poorly-differentiated, serous and endometrioid adenocarcinomas [80.7% vs. 56.4% ($p<0.05$), 77.0% vs. 64.9% ($p<0.01$), and 68.2% vs. 54.6% ($p<0.05$), respectively]. Conclusion: Our data suggest that nedaplatin has equivalent or superior antitumor activity to cisplatin in cervical, ovarian and endometrial carcinomas. In particular, nedaplatin showed a siginificantly better antitumor activity among the histological subtypes of ovarian carcinomas.*

There are many adjuvant chemotherapies for patients with gynecological malignancies, such as ovarian, cervical and endometrial carcinomas. Among them, cisplatin-based chemotherapies, especially cisplatin, are the most important drugs used.

Nedaplatin (cis-diammine glycolato platinum; 254-S) is one of the new platinum analogs, a second-generation platinum-coordination complex developed in Japan (1) and marketed recently. It has a novel structure involving a glycolate ring bound to the platinum atom as a bidentate ligand. This product has a higher water solubility than cisplatin and shows very limited binding to plasma proteins. Therefore, it causes less renal, gastrointestinal and neurotoxicity (2, 3), and there is no need for hydration in its administration. Recently, nedaplatin has been used against gynecological carcinomas, such as ovarian carcinomas (4, 5) and cervical carcinomas (6, 7). However, the efficacy of nedaplatin, as compared with cisplatin, has not been well recognized in endometrial carcinomas.

In this study, a comparative examination was performed of the *in vitro* activity of nedaplatin and cisplatin against 137 human gynecological malignancies, comprising 65 ovarian, 15 cervical and 57 endometial carcinomas, using the tetrazolium dye (MTT) assay.

Materials and Methods

Tissues. The tumor specimens were obtained from patients during primary surgery at the Department of Obstetrics and Gynecology of Himeji Medical Center and Tenri Hospital, Japan. All tissues were obtained from untreated patients after informed consent has been given. Sixty-five samples of ovarian carcinomas, 15 samples of cervical carcinomas and 57 samples of endometrial carcinoma were collected. The specimens were collected directly into tissue-culture medium for cell culture.

According to the International Federation of Gynecology and Obstetrics (FIGO) classification, the 65 ovarian carcinomas consisted of 20 stage 1a, 9 stage 1c, 4 stage 2 , 21 stage 3 and 11 stage 4 specimens. Histologically, there were 34 serous cystadenocarcinomas, 4 mucinous cystadenocarcinomas, 13 clear cell adenocarcinomas, 8 endometrioid adenocarcinomas and 6 poorly-differentiated adenocarcinomas. With respect to the 15 cervical carcinomas, 5 stage 1b, 5 stage 2b, 4 stage 3b and 1 stage 4b specimens were identified. Histologically, all 15 were squamous cell carcinomas. The 57 endometrial carcinomas consisted of 41 stage 1, 3 stage 2 and 13 stage 3 specimens. Histologically, all 57 endometrial carcinomas were of the endometrioid type, with 30

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Key Words: Nedaplatin, MTT assay, ovarian carcinoma, cervical carcinoma, endometrial carcinoma.

Table I. *The tumor inhibition rates for nedaplatin and cisplatin in 65 ovarian carcinomas and the differences among the different histological subtypes.*

	n=65	serous	endometrioid	poorly-differ.	mucinous	clear cell
I.R. for nedaplatin (%)	61.7±25.6	77.0±11.2	68.2±4.5	80.7±3.1	38.6±24.6	49.7±28.9
I.R. for cisplatin (%)	54.8±24.4	64.9±22.5	54.6±19.6	56.4±31.1	44.2±15.5	36.5±21.9
P-value	NS	$p<0.01$	$p<0.05$	$p<0.05$	NS	NS

well-differentiated (G1), 15 moderately-differentiated (G2) and 12 poorly-differentiated (G3) adenocarcinomas.

Drugs. The drugs used were cisplatin (Bristol-Myers Squibb Co., Tokyo, Japan) and nedaplatin (Shionogi & Co., Ltd., Osaka, Japan) . The following reported peak plasma concentrations (PPC), which corresponded to 100% PPC, were used in accordance with previously reported data: cisplatin 2.49 μg/ml (clinical dosage 100.0 mg/m^2) (8) and nedaplatin 7.00 μg/ml (clinical dosage 100.0 mg/m^2) (9). One PPC was used for each chemotherapeutic agent in the MTT assay.

MTT assay. The tissues were minced by scissors in RPMI 1640 medium (Nissui Corp., Tokyo, Japan) containing 20% fetal calf serum and 0.3 g/l glutamine. The tumor cells were then incubated at 37˚C for 30 min in an enzyme cocktail containing 0.02% deoxyribonuclease I (Sigma, St. Louis, USA), 0.05% pronase (Calbiochem, Dormstadt, Germany) and 0.02% collagenase. The tumor cell suspension (5x10^5 cells/ml) was then strained through a 150-μm stainless steel mesh. The cells were centrifuged at 1000 rpm for 5 min and, after rinsing twice, the live carcinoma cells were verified by 0.25% trypan blue dye exclusion (Sigma). The cell number was adjusted to 1-2x10^5 cells/ml, and a 180-μl aliquot of the tumor cell suspension was plated into each well of 96-well cell culture plates (Nunc Inc., Rochester, USA), followed by the addition of 20 μl of 1PPC of each chemotherapeutic agent. The cells were then incubated at 37˚C for 72 h in a 5% CO_2 incubator. After the cells had been washed with phosphate-buffered saline, 25 μl of MTT (2 mg/ml) (Sigma) was added, and the mixture was allowed to incubate for 4 h at 37˚C. The plates were then centrifuged at 1800 rpm for 10 min, and the supernatant was removed and formazan was eluted with 150 μl of DMSO (Nakarai Tesque, Kyoto, Japan). The optical density (OD) was measured with an enzyme-linked immunosorbent assay (ELISA) reader (NJ-2000, Japanese Intermed.) at 540 nm. The tumor inhibition rate (I.R.) was calculated from the following equation:

$$\text{I.R. (\%)} = (1-T/C) \times 100\%$$

where $T=OD_{540}$ of the treated cells and $C=OD_{540}$ of the control cells. The drug was judged to be effective or ineffective when the I.R. was ≥50% or <50%, respectively.

Results

Tumor sensitivity to nedaplatin and cisplatin in ovarian carcinoma. A comparison of the sensitivities to nedaplatin and cisplatin in 65 ovarian carcinomas and the differences among the different histological subtypes are listed in Table I. The mean I.R.s for nedaplatin and cisplatin were 61.7% and 54.8%, showing no significant (NS) difference on comparison of the sensitivities to nedaplatin and cisplatin among the different histological subtypes, while better sensitivity for nedaplatin was detected in serous, endometrioid and poorly-differentiated adenocarcinomas [77.0% *vs.* 64.9%, $p<0.01$; 68.2% *vs.* 54.6%, $p<0.05$; and 80.7% *vs.* 56.4% $p<0.05$, respectively]. However, there were no significant differences in mucinous and clear cell carcinomas [38.6% *vs.* 44.2% and 49.7% *vs.* 36.5%, respectively]. Furthermore, comparing the I.R.s for nedaplatin itself among the different histological subtypes, serous and poorly-differentiated adenocarcinomas were both higher [77.0% and 80.7%, NS]. Based on the I.R. in serous adenocarcinomas, the I.R. in endometrioid adenocarcinomas was relatively lower [77.0% *vs.* 68.2% , $p<0.05$]. Moreover, the I.R.s in mucinous and clear cell adenocarcinomas were significantly lower [77.0% *vs.* 38.6 % and 77.0% *vs.* 49.7%, $p< 0.001$, respectively].

Table II. *The tumor inhibition rates for nedaplatin and cisplatin in 15 cervical carcinomas, all of which were squamous cell carcinoma.*

	n=15
I.R. for nedaplatin (%)	70.7±13.2
I.R. for cisplatin (%)	63.9±19.2
P-value	NS

Tumor sensitivity to nedaplatin and cisplatin in uterine cervical carcinoma. A comparison of the sensitivities to nedaplatin and cisplatin in 15 cervical carcinomas is given in Table II. The mean I.R.s for nedaplatin and cisplatin were 70.7% and 63.9% in cervical carcinomas, without a statistically significant difference (NS). Histologically, all cervical carcinomas were squamous cell carcinomas (Table II).

Table III. *The tumor inhibition rates for nedaplatin and cisplatin in 57 endometrial carcinomas and the differences between the various histological grades.*

	n=57	G1	G2	G3
I.R. for nedaplatin (%)	52.1±30.5	39.9±40.0	56.2±27.0	59.4±29.0
I.R. for cisplatin (%)	47.7±21.9	46.8±22.1	48.4±24.2	48.9±20.1
P-value	NS	NS	NS	NS

Table IV. *The relationship between the tumor inhibition rates for nedaplatin and cisplatin in ovarian, cervical and endometrial carcinoma.*

	ovarian carcinoma	cervical carcinoma	endometrial carcinoma	*P*-value
I.R. for nedaplatin	61.7±25.6*	70.7±13.2*	52.1±30.5	$p<0.05$
I.R. for cisplatin	54.8±24.4	63.9±19.2	47.7±21.9	NS

*= Significantly better than that in endometrial carcinoma.

Tumor sensitivity to nedaplatin and cisplatin in uterine endometrial carcinoma. A comparison of the sensitivities to nedaplatin and cisplatin in 57 endometrial carcinomas and the diferrences between the various histological grades are listed in Table III. The mean I.R.s for nedaplatin and cisplatin were 52.1% and 47.7% in endometrial carcinomas, with no statistically significant difference. Furthermore, no statistically different sensitivities to nedaplatin and cisplatin were found between the G1, G2 and G3 subtypes [39.9% *vs.* 46.8%, 56.2% *vs.* 48.4% and 59.4% *vs.* 48.9%, respectively]. Nor did the I.R.s for nedaplatin itself among the different histological grades show any statistically significant differences between G1, G2 and G3 carcinomas [39.9% *vs.* 56.2% *vs.* 59.4 %, NS]. However, there was a tendency for the I.R. to be relatively higher in G3 , followed by G2 and G1 adenocarcinomas.

On the other hand, the I.R. for nedaplatin itself in ovarian and cervical carcinomas was significantly better than that in endometrial carcinomas [61.7% and 70.7% *vs.* 52.1 %, $p<0.05$, respectively] (Table IV).

Discussion

The MTT assay is widely used for chemosensitivity testing because it offers the advantages of low cost and a short assay time. The overall accuracy of the MTT assay for predicting clinical effect has been reported to be 78% in 45 advanced gastric carcinomas (10) and 81.3% in 16 gynecological carcinomas (11). These results support the usefulness of the MTT assay for the *in vitro* chemosensitivity testing of fresh surgical specimens.

In phase II studies in Japan, nedaplatin generated a 46.3% response rate in 41 patients with cervical carcinomas and a 37.7% response rate in 61 patients with ovarian carcinomas (4). These data were consistent with our *in vitro* study in that the antitumor activity of nedaplatin was strongest in cervical carcinomas, followed by ovarian carcinomas and endometrial carcinomas. Previous reports suggested a strong antitumor efficacy of nedaplatin against cervical carcinomas using adjuvant chemotherapy (6, 7). In patients with cervical carcinomas, the 46.3% response rate of nedaplatin was superior to the 35% response rate of cisplatin (12). In our study, the chemosensitivities for nedaplatin were also equal or superior to cisplatin in cervical carcinomas. However, there were no statistically significant differences between them.

With regard to ovarian carcinomas, we obtained a significantly better sensitivity for nedaplatin in poorly-differentiated, serous and endometrial adenocarcinomas as compared to cisplatin. On the other hand, lower rates were shown in mucinous and clear cell adenocarcinomas. Cisplatin has been recognized to be the most active drug against ovarian carcinomas, although the combination of nedaplatin with cyclophosphamide has been reported to be more effective than cisplatin with cyclophosphamide (5). This supports the possibility of a superior antitumor activity of nedaplatin against ovarian carcinomas. Recently, we have been using taxol-carboplatin combined chemotherapy as first-line chemotherapy against ovarian carcinomas. In the near future, based on the results of our study, a new combination chemotherapy using nedaplatin will be our choice.

With regard to endometrial carcinomas, to our knowledge, there have been no reports on the clinical application of nedaplatin, nor any reports on the *in vitro* testing of nedaplatin in these subjects. In the present study, the *in vitro* antitumor activity of nedaplatin against endometrial carcinomas was moderate. Histologically, there were no statistically significant differences in the sensitivity to nedaplatin between G1, G2 and G3 adenocarcinomas, although there was a tendency for it to be relatively higher in G3 adenocarcinomas. This result was similar to the *in vitro* sensitivity for irinotecan (13). Based on these results, we have to devise how to use nedaplatin as an adjuvant chemotherapy after radical surgery, or against refractory or recurrent endometrial carcinomas.

Nedaplatin has less renal and gastrointestinal toxicity than cisplatin, but increased hematotoxicity, which is the dose-limiting factor. On the other hand, hydration is not required, which is an advantage over cisplatin. In view of the sensitivity results obtained in the study, we will determine how to use nedaplatin as a novel adjuvant chemotherapy in the place of cisplatin in the examined gynecological malignancies.

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