

## Preclinical *in vivo* Antitumor Efficacy of Nedaplatin with Gemcitabine against Human Lung Cancer

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The antitumor efficacy of the combination of nedaplatin (NDP) with gemcitabine (GEM) was evaluated. We also compared the antitumor activity of NDP plus GEM with that of cisplatin (CDDP) plus GEM or carboplatin (CBDCA) plus GEM. Ma44, which is a human lung cancer sensitive to GEM, and NCI-H460, which is a human lung cancer refractory to GEM, were used in this study. GEM was injected i.v. once followed by i.v. injection of NDP at an interval of approximately 30 min into tumor-bearing athymic mice. GEM was administered again 3 or 4 days thereafter. Combined dosing of NDP with GEM resulted in synergistically enhanced inhibition of tumor growth in the Ma44 tumor model. NDP plus GEM was also effective against Ma44 cells when given late in the therapy, a model for advanced disease. Potent augmentation of growth inhibition by NDP with GEM was also found with the NCI-H460 tumor model. The combination effect of NDP plus GEM appeared to be superior to that of CDDP plus GEM or CBDCA plus GEM in both tumor models. Toxicity in terms of blood cell numbers was not enhanced by the combination of NDP with GEM. These results suggest the effectiveness of combination of NDP with GEM for clinical therapy.

Key words: Nedaplatin — Gemcitabine — Combination chemotherapy — Lung cancer

Nedaplatin (NDP) has been developed as a second-generation platinum complex with pronounced preclinical antitumor activity against solid tumors but with lower nephrotoxicity than cisplatin (CDDP) in preclinical<sup>1–5)</sup> and clinical studies.<sup>6–10)</sup> NDP was launched in Japan in 1995. In a series of studies with our combination therapy model, we have demonstrated that antitumor activity was augmented when NDP was combined with etoposide,<sup>11)</sup> 5-fluorouracil<sup>12)</sup> or cyclophosphamide<sup>13)</sup> against lung, squamous or ovarian cancer, respectively.

Gemcitabine (2',2'-difluorodeoxycytidine, GEM) has been developed as a deoxycytidine analogue, which, unlike cytosine arabinoside, shows potent antitumor activity against panels of solid tumors.<sup>14–16)</sup> In clinical studies, GEM has demonstrated antitumor activity against a variety of solid tumors, including pancreatic, colorectal, lung, head and neck, ovarian, urothelial, breast, and renal cancer.<sup>17–22)</sup> In recent preclinical models, the CDDP-GEM combination appeared to show synergy between the two drugs.<sup>23, 24)</sup> Many clinical trials of the CDDP-GEM combination in non-small-cell lung cancer (NSCLC) have been performed<sup>25–29)</sup> and a recent randomized phase III study demonstrated that this combination gave a significantly higher response rate with a significant delay in time to disease progression than the CDDP-etoposide regimen.<sup>30)</sup> Other platinum analogs, such as carboplatin (CBDCA) also have been used in combination with GEM in NSCLC.<sup>31)</sup>

The purpose of the present study was to evaluate antitumor activity of combination therapy with NDP plus GEM against human NSCLC cell lines which show different sensitivity to GEM. We also compared the therapeutic efficacy of NDP plus GEM with that of CDDP plus GEM or CBDCA plus GEM.

### MATERIALS AND METHODS

**Animals** BDF<sub>1</sub> and athymic BALB/c nude mice (female, 7–9 weeks old) were purchased from Japan SLC Inc. (Shizuoka) and CLEA Japan Inc. (Tokyo), respectively.

**Tumors** Ma44 human squamous lung carcinoma was provided by Dr. T. Komiya (Kinki University Medical School, Osaka). NCI-H460 human squamous lung carcinoma was purchased from the American Tissue Culture Collection (Rockville, MD). These cell lines were maintained by *in vitro* passage using Eagle's MEM (Nissui Pharmaceutical Co., Tokyo) supplemented with 10% fetal calf serum (Life Technologies Inc., Rockville, MD).

**Drugs** NDP was obtained from Shionogi & Co., Ltd. (Osaka). CDDP and CBDCA were purchased from Nippon Kayaku (Tokyo) and Bristol-Myers Squibb (Tokyo), respectively. GEM was synthesized in our laboratory. All drugs were dissolved in saline immediately before use.

***In vivo* therapeutic experiments** The experimental procedure was described previously.<sup>12, 13)</sup> In all experiments, 5 to 7 mice per group were used. On day 0, tumor cells ( $5 \times 10^5$  for Ma44 and  $1 \times 10^6$  for NCI-H460) were implanted s.c. into the back of BALB/c nude mice. Treatment was started when the tumor volume reached 100–

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300 mm<sup>3</sup>. In the advanced tumor model, the tumor was allowed to grow to approximately 1000 mm<sup>3</sup> before treatment was started. GEM was administered i.v. once and then the platinum compounds were administered i.v. once at an interval of approximately 30 min. GEM was injected i.v. again at 3 or 4 days thereafter. The total doses of drugs used for combinations were 15 or 120×2 mg/kg for GEM, 10 or 20 mg/kg for NDP, 6 mg/kg for CDDP and 57 mg/kg for CBDCA. All compounds were administered at the volume of 0.1 ml/10 g of body weight. Saline was injected i.v. into the untreated control group. The maximum tolerated doses (MTDs) of GEM, NDP, CDDP and CBDCA in the route and schedule used in this study were 240, 40, 12 and 114 mg/kg, respectively. All experiments were performed with the approval of the Shionogi Animal Care and Use Committee.

**Evaluation of antitumor efficacy** Tumor size and body weight were scored throughout each experiment. Relative growth inhibition (RV) was calculated as  $RV = V_n/V_0$ , where  $V_n$ : tumor volume on day  $n$ , and  $V_0$ : initial tumor volume. The growth inhibitory effect was estimated using the treated/control ratio (T/C). For the evaluation of combination therapies, the combination ratio (CR) was used. CR was calculated using the T/C value with  $CR = M_{A+B}/(M_A \times M_B)$ , where  $M_A$ ,  $M_B$ : the mean T/C of drug A, drug B alone, and  $M_{A+B}$ : the mean T/C of drug A with drug B. A CR less than 1 indicated synergy (i.e. the effect of the combination was greater than expected from the product of T/C of the component agents), a CR equal to 1 indicated additivity and a CR greater than 1 indicated antagonism.

**Statistics** In this study, the statistical significance of differences from the untreated group or between treated groups was evaluated using Welch's test and Dunnett's test, respectively.<sup>32, 33)</sup>

**Hematotoxicity study** NDP (22 mg/kg, day 0) and/or GEM (120×2 mg/kg, days 0 and 4) were injected i.v. into BDF<sub>1</sub> mice and blood samples were collected from the heart of anesthetized mice on 2, 4, 6, 8, 11, 14 and 18 days thereafter. Nucleated bone marrow cells (BMC) were collected from the right femur by flushing the bone marrow cavity with saline using a syringe with a 22G needle. Single cells were prepared by vigorous pipetting. The number of white blood cells (WBC), platelets (PLT) and BMC were counted with an automatic cell counter (Sysmex K-1000 and CDA-500, Sysmex, Kobe).<sup>13)</sup> The experiment was conducted with 3 mice per time point.

## RESULTS

**Different *in vivo* sensitivities of Ma44 and NCI-H460 human lung cancer to GEM** In the present study, the two human NSCLC cell lines, Ma44 and NCI-H460, with different sensitivities to GEM, were used in therapeutic

Table I. Different *in vivo* Sensitivity of Ma44 and NCI-H460 Human Lung Cancer to Gemcitabine (GEM)

Cancer	Group	GEM (mg/kg) <sup>a)</sup>	RV <sup>b)</sup> (Mean±SD)	T/C <sup>c)</sup> (Mean±SD)
Ma44	Untreated control	0	8.8±2.0	
	GEM only	240×2	1.5±0.8 <sup>d)</sup>	0.17±0.09
		120×2	1.8±0.8 <sup>d)</sup>	0.20±0.09
		60×2	2.9±1.0 <sup>d)</sup>	0.33±0.11
NCI-H460	Untreated control	0	4.0±1.3	
	GEM only	240×2	2.6±1.6	0.65±0.40
		120×2	2.8±1.0	0.70±0.25
		60×2	3.1±1.4	0.78±0.35

a) i.v.×2 (days 10 and 14).

b) Relative tumor volume on day 16.

c) Treated/control.

d)  $P < 0.01$  for untreated control by Welch's test.

experiments *in vivo* (Table I). GEM showed significant ( $P < 0.01$ ) and also effective tumor growth inhibition (T/C  $< 0.5$ ) against Ma44 tumor cells at least 60×2 mg/kg, but not against NCI-H460 even at 240×2 mg/kg, which is the MTD in the this schedule. These results indicated that Ma44 is sensitive, whereas NCI-H460 is rather refractory to GEM.

**Combination therapy with NDP plus GEM against Ma44 human lung cancer** Combination of NDP at 20 or 10 mg/kg with GEM at 15×2 mg/kg resulted in enhanced antitumor efficacy at both doses in comparison with NDP or GEM alone (Table II, Exp.1). In particular, combination of NDP at 20 mg/kg with GEM showed significantly higher tumor growth inhibition of Ma44 cells ( $P < 0.05$ ) than NDP or GEM monotherapy. The CR in this combination was 0.55, and it indicated that this combination effect was synergistic. No severe body weight losses were observed for this combination.

We next examined the combination of NDP with GEM against Ma44 tumor cells in the advanced stage. In this experiment, therapy was started when the tumor volume reached approximately 1000 mm<sup>3</sup>. At least 120×2 mg/kg of GEM or 40 mg/kg of NDP was required to exhibit effective antitumor activity (T/C  $< 0.5$ ) as a monotherapy, indicating that advanced Ma44 tumor was less sensitive to both agents (Table II, Exp. 2). When 20 mg/kg of NDP was combined with 120×2 mg/kg of GEM, however, the antitumor efficacy was significantly ( $P < 0.01$ ) augmented in comparison with either NDP or GEM alone. The CRs in the combination at 20 and 10 mg/kg of NDP were 0.81 and 0.67, respectively, indicating that these combination effects were synergistic. The body weight losses in this combination were 13% and 9% of initial body weight.

**Combination therapy with NDP plus GEM against NCI-H460 human lung cancer** Augmentation of antitu-

Table II. Augmentation of Antitumor Activity in Combination Chemotherapy with Nedaplatin (NDP) plus Gemcitabine (GEM) against Ma44 Human Lung Cancer

	Group	Total dose (mg/kg)		RV <sup>c)</sup> (Mean±SD)	T/C <sup>d)</sup> (Mean±SD)	Maximum BW loss (%) <sup>e)</sup>
		NDP <sup>a)</sup>	GEM <sup>b)</sup>			
Exp. 1 <sup>f)</sup>	Untreated control	0	0	20.0±8.2		0
	GEM only	0	15×2	8.8±6.2 <sup>g)</sup>	0.44±0.31	0
	NDP only	20	0	8.9±5.2 <sup>g)</sup>	0.45±0.26	4
		10	0	11.4±6.3	0.57±0.32	0
	Combination	20	15×2	2.1±0.6 <sup>h, i, j)</sup>	0.11±0.03	3
10		15×2	6.7±4.9 <sup>h)</sup>	0.34±0.25	3	
Exp. 2 <sup>k)</sup>	Untreated control	0	0	5.3±1.9		0
	GEM only	0	240×2	1.4±0.2 <sup>h)</sup>	0.26±0.04	1
		0	120×2	1.9±0.2 <sup>g)</sup>	0.36±0.04	0
		0	60×2	2.8±0.5 <sup>g)</sup>	0.53±0.09	0
	NDP only	40	0	2.0±1.4 <sup>g)</sup>	0.38±0.26	10
		20	0	3.1±1.3	0.58±0.25	1
		10	0	4.6±0.9	0.87±0.17	0
	Combination	20	120×2	0.9±0.3 <sup>h, l, m, o)</sup>	0.17±0.06	13
		10	120×2	1.1±0.5 <sup>h, l, n)</sup>	0.21±0.09	9

a) i.v.×1 (day 5).

b) i.v.×2 (days 5 and 8).

c) Relative tumor volume on day 12 for Exp. 1 and on day 21 for Exp. 2.

d) Treated/control.

e) % of initial.

f) Therapy was started when tumor volume reached 100 mm<sup>3</sup>.

g, h)  $P < 0.05, 0.01$  for untreated control by Welch's test.

i)  $P < 0.05$  for GEM 120 mg/kg by Dunnett's test.

j)  $P < 0.05$  for NDP 20 mg/kg by Dunnett's test.

k) Therapy was started when tumor volume reached approximately 1000 mm<sup>3</sup>.

l)  $P < 0.01$  for GEM 120 mg/kg by Dunnett's test.

m)  $P < 0.01$  for NDP 20 mg/kg by Dunnett's test.

n)  $P < 0.01$  for NDP 10 mg/kg by Dunnett's test.

o)  $P < 0.05$  for GEM 240 mg/kg by Dunnett's test.

mor activity in the combination of NDP with GEM was examined using NCI-H460, which is refractory to GEM. Neither GEM nor NDP alone exhibited effective antitumor activity even at MTD dosing. However, antitumor efficacy of the combination of NDP at 10 or 20 mg/kg with GEM at 120×2 mg/kg was significantly ( $P < 0.05$  or 0.01, respectively) enhanced and both combinations were found to be effective (T/C < 0.5) (Table III). The CRs of the combination at 10 and 20 mg/kg of NDP were 0.81 and 0.69, respectively. These results indicated that the combination effect of NDP with GEM against the NCI-H460 tumor model was also synergistic. The body weight losses in this combination were 18% and 14% of initial body weight. These body weight losses, however, were not augmented by combination.

**Combination therapy with NDP plus GEM versus CDDP plus GEM or CBDCA plus GEM** Table IV shows the results obtained with NDP plus GEM, CDDP

plus GEM and CBDCA plus GEM against NCI-H460 human lung cancer. A 1/2 MTD of platinum compound (20 mg/kg of NDP, 6 mg/kg of CDDP and 57 mg/kg of CBDCA) was used in this experiment. While growth inhibition was significantly ( $P < 0.05$  or 0.01) enhanced by combined treatment in all three cases, only the growth inhibitory activity of NDP plus GEM was effective (T/C < 0.5). In particular, tumor regression was found only in NDP plus GEM therapy. CRs in NDP plus GEM, CDDP plus GEM and CBDCA plus GEM were 0.55, 0.86 and 0.83, respectively, indicating that these combination effects were synergistic. In this experiment, the body weight losses of NDP plus GEM and CDDP plus GEM reached the toxic range, but they were temporary and no mice died.

A comparative experiment was again performed against Ma44 in the advanced disease model. As shown in Table V, growth inhibition was significantly ( $P < 0.05$  or 0.01)

Table III. Augmentation of Antitumor Activity in Combination Chemotherapy with Nedaplatin (NDP) plus Gemcitabine (GEM) against NCI-H460 Human Lung Cancer

Group	Total dose (mg/kg)		RV <sup>c)</sup> (Mean±SD)	T/C <sup>d)</sup> (Mean±SD)	Maximum BW loss (%) <sup>e)</sup>
	NDP <sup>a)</sup>	GEM <sup>b)</sup>			
Untreated control	0	0	3.9±1.3		6
GEM only	0	240×2	2.6±0.9	0.67±0.21	13
	0	120×2	2.8±0.9	0.72±0.21	8
NDP only	20	0	2.4±0.7	0.62±0.17	19
	10	0	3.0±1.2	0.77±0.29	10
Combination	20	120×2	1.2±0.2 <sup>g, h, i)</sup>	0.31±0.05	18
	10	120×2	1.8±0.6 <sup>f)</sup>	0.46±0.14	14

- a) i.v.×1 (day 10).
- b) i.v.×2 (days 10, 14).
- c) Relative tumor volume on day 16.
- d) Treated/control.
- e) % of initial.
- f, g)  $P < 0.05, 0.01$  for untreated control by Welch's test.
- h)  $P < 0.01$  for GEM only by Dunnett's test.
- i)  $P < 0.05$  for NDP 20 mg/kg by Dunnett's test.

Table IV. Augmentation of Antitumor Activity in Combination Chemotherapy with Platinum Compounds plus Gemcitabine (GEM) against NCI-H460 Human Lung Cancer

Group	Total dose (mg/kg)		RV <sup>c)</sup> (Mean±SD)	T/C <sup>d)</sup> (Mean±SD)	Maximum BW loss (%) <sup>e)</sup>
	Platinums <sup>a)</sup>	GEM <sup>b)</sup>			
Untreated control	0	0	2.4±0.4		2
GEM only	0	120×2	1.9±0.4 <sup>g)</sup>	0.79±0.17	13
NDP only	20	0	2.1±0.4	0.88±0.17	12
NDP+GEM	20	120×2	0.9±0.2 <sup>g, i, j)</sup>	0.38±0.08	23
CDDP only	6	0	1.9±0.4 <sup>f)</sup>	0.79±0.17	15
CDDP+GEM	6	120×2	1.3±0.2 <sup>g, i, k)</sup>	0.54±0.08	20
CBDCA only	57	0	2.1±0.2	0.88±0.08	8
CBDCA+GEM	57	120×2	1.4±0.4 <sup>g, h, l)</sup>	0.58±0.17	14

- a) i.v.×1 (day 12).
- b) i.v.×2 (days 12 and 16).
- c) Relative tumor volume on day 19.
- d) Treated/control.
- e) % of initial.
- f, g)  $P < 0.05, 0.01$  for untreated control by Welch's test.
- h, i)  $P < 0.05, 0.01$  for GEM only by Dunnett's test.
- j)  $P < 0.01$  for NDP 20 mg/kg by Dunnett's test.
- k)  $P < 0.01$  for CDDP 6 mg/kg by Dunnett's test.
- l)  $P < 0.01$  for CBDCA 57 mg/kg by Dunnett's test.

enhanced, and was effective for combinations of all platinum compounds with GEM. However, the combination at a high dose of CDDP or CBDCA, but not NDP, caused toxic death of the treated mice (four of five in the case of CDDP plus GEM and one of five in the case of CBDCA plus GEM), indicating the safety of NDP plus GEM therapy. The calculated CR indicated that all combinations showed synergy.

**Toxicity study** The hematotoxicity of NDP with GEM was analyzed (Fig. 1). To minimize the physiological influence of the growing tumor, non-tumor-bearing BDF<sub>1</sub> mice were used for this study. NDP (22 mg/kg) and/or GEM (120 mg/kg) were injected i.v. and blood samples were collected 2, 4, 6, 8, 11, 14 and 18 days thereafter. No significant augmentation of hematotoxicity was detected with the combination of NDP and GEM for all parameters.

Table V. Augmentation of Antitumor Activity in Combination Chemotherapy with Platinum Compounds plus Gemcitabine (GEM) against Ma44 Human Lung Cancer

Group	Total dose (mg/kg)		RV <sup>c)</sup> (Mean±SD)	T/C <sup>d)</sup> (Mean±SD)	Toxic death	Maximum BW loss (%) <sup>e)</sup>
	Platinums <sup>a)</sup>	GEM <sup>b)</sup>				
Untreated control	0	0	4.0±0.4			0
GEM only	0	120×2	2.2±0.4 <sup>g)</sup>	0.55±0.10		8
NDP only	20	0	2.7±1.2	0.68±0.30		5
	10	0	3.1±0.7	0.88±0.17		2
NDP+GEM	20	120×2	0.8±0.3 <sup>g, i, j)</sup>	0.20±0.08		15
	10	120×2	1.2±0.2 <sup>g, i, j)</sup>	0.30±0.05		11
CDDP only	6	0	2.7±0.7 <sup>h)</sup>	0.68±0.18		5
	3	0	3.1±0.8	0.78±0.20		1
CDDP+GEM	6	120×2	0.5	0.16	4/5	22
	3	120×2	0.9±0.3 <sup>g, i, k)</sup>	0.23±0.08		11
CBDCA only	57	0	2.8±0.6	0.70±0.15		2
	29	0	3.4±1.2	0.85±0.30		0
CBDCA+GEM	57	120×2	1.4±0.6 <sup>g, l)</sup>	0.35±0.15	1/5	14
	29	120×2	1.0±0.4 <sup>g, h, m)</sup>	0.25±0.10		14

a) i.v.×1 (day 13).

b) i.v.×2 (days 13 and 17).

c) Relative tumor volume on day 18.

d) Treated/control.

e) % of initial.

f, g)  $P < 0.05$ ,  $0.01$  for untreated control by Welch's test.

h, i)  $P < 0.05$ ,  $0.01$  for GEM only by Dunnett's test.

j)  $P < 0.01$  for NDP 20 mg/kg by Dunnett's test.

k)  $P < 0.01$  for CDDP 3 mg/kg by Dunnett's test.

l)  $P < 0.01$  for CBDCA 57 mg/kg by Dunnett's test.

m)  $P < 0.01$  for CBDCA 29 mg/kg by Dunnett's test.

Therapy was started when tumor volume reached approximately 600 mm<sup>3</sup>.

In conclusion, the present results demonstrated that combined chemotherapy of NDP with GEM exerted potent antitumor efficacy against GEM-sensitive as well as -refractory human lung cancer.

## DISCUSSION

In the present study, we focused on the antitumor efficacy of NDP plus GEM against human NSCLC. A synergistic enhancement of growth inhibition (Tables II to V) in the NDP plus GEM therapy was reproducibly demonstrated against the two human lung cancer models tested, including the GEM-sensitive tumor Ma44 and the GEM-refractory tumor NCI-H460. In the Ma44 model, the combination of NDP with GEM showed higher tumor growth inhibition of Ma44 cells than that of NDP or GEM monotherapy (Table II). The T/C value indicated that the combination was more effective, though not significantly different, when compared with the maximum activity of GEM alone (Table I). In clinical application, superior efficacy of the combination to either monotherapy would be

required. Therefore, we tested the efficacy of NDP plus GEM in the advanced Ma44 model in which the therapy was started late. This experimental condition makes Ma44 tumor cells less sensitive to GEM treatment, and is thought to be closer to the clinical situation. The combination of NDP with GEM again resulted in enhanced antitumor activity and it was found to be significantly ( $P < 0.05$ ,  $0.01$ ) more effective than GEM or NDP alone at the MTD (Table II). It is also noteworthy that tumor regression was observed in three of five mice given 20 mg/kg of NDP plus GEM and in two of five mice given 10 mg/kg of NDP plus GEM (Table II). No tumor regression was found in mice given either NDP or GEM alone. These results indicate that combination of NDP with GEM is more effective in terms of response rate. Synergistic enhancement of growth inhibition in NDP plus GEM was also demonstrated against the GEM-refractory tumor NCI-H460 (Table III). Similar results were obtained using GEM-resistant Ma44 cells (unpublished results).

We also compared the antitumor activity of NDP plus GEM with that of CDDP plus GEM or CBDCA plus GEM

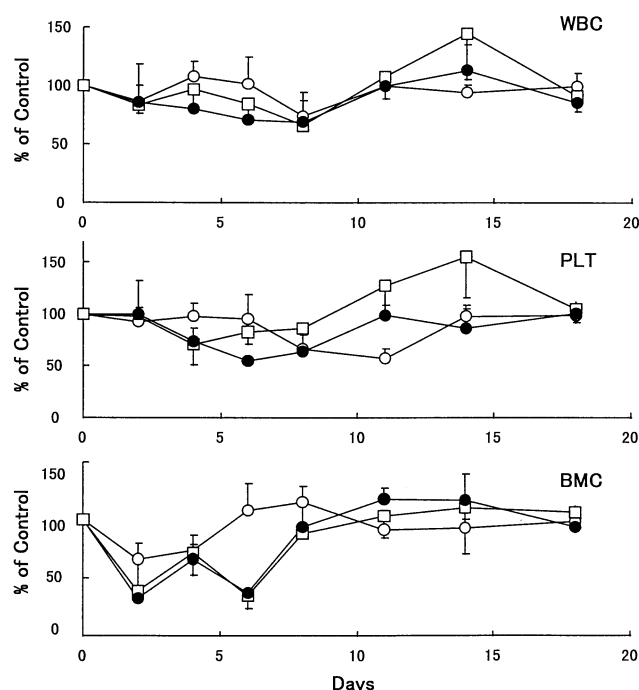


Fig. 1. Hematotoxicity during combined treatment with nedaplatin (NDP) and gemcitabine (GEM). Non-tumor-bearing BDF<sub>1</sub> mice received 20 mg/kg of NDP either alone or with 120×2 mg/kg of GEM. NDP was administered on day 0 and GEM was administered on days 0 and 4. Blood samples and bone marrow cells were collected at each day-point. Numbers of white blood cells (WBC), platelets (PLT) and bone marrow cells (BMC) from mice treated with NDP (○), GEM (□) and the combination (●) were counted as described in "Materials and Methods" and are shown as % of the untreated control±SD.

against NCI-H460 tumor, because both platinum-based combination therapies have been widely applied for clinical use. While administration of the three platinum compounds at 1/2 MTD resulted in similar tumor growth inhibition, NDP plus GEM was found to be the most effective among the three combinations (Table IV). Tumor regression was again observed in five of seven mice given NDP plus GEM and in one of seven mice given CBDCA plus GEM. No tumor regression was found in CDDP plus GEM (Table IV). These results suggested that the efficacy of the combination of NDP with GEM in terms of response rate is superior to that of CDDP with GEM or CBDCA plus GEM. Similar results were also obtained using Ma44 cells (Table V).

Toxicity is another factor which must be considered in combination chemotherapy. We therefore compared the profiles of body weight changes among the therapies. In

most cases, the maximum body weight losses in NDP plus GEM therapy were within the tolerable range (<20% of the initial body weight) for the administration schedule used in this study. We then evaluated the hematotoxicity in the combination therapy at 20 mg/kg of NDP and 120×2 mg/kg of GEM. All the parameters of the untreated mice were within the normal ranges throughout the experiment. The numbers of WBC and PLT decreased with NDP or GEM treatment and the number of BMC mainly decreased with GEM treatment. However, hematotoxicity was not augmented by the combination of the two agents. Thrombocytopenia and myelosuppression, dose-limiting factors of NDP and GEM, respectively, were not enhanced by the combination. This should be beneficial for the combination chemotherapy of NDP with CPM and awaits confirmation in clinical trials.

With regard to the correlation of efficacy or toxicity with the dosing schedule, Braakhuis *et al.* and van Moorsel *et al.* demonstrated that GEM preceding CDDP by 4 h was the best treatment schedule with acceptable toxicity, but when the interval was increased to 24 h, toxicity became unacceptable.<sup>34,35</sup> In the present study, GEM was administered 30 min before the platinum compounds and the toxicity was acceptable in most cases. However, we have found some cases of toxic death in high dose combinations of CDDP or CBDCA with GEM, but not in the combination of NDP with GEM (Table V). We also found that toxicity was not changed when the interval was increased to 4 or 24 h, but simultaneous dosing of NDP and GEM rather caused increased toxicity (unpublished results). Precise determination of the optimum schedule of NDP and GEM is important for appropriate use of these drugs in the clinical context.

The mechanism of the synergistic interaction of GEM and NDP still remains to be resolved. Both compounds affect DNA synthesis by different mechanisms. Therefore, synergistic inhibition of DNA synthesis seems to be one possible mechanism to explain the augmented *in vivo* anti-tumor efficacy of the combination of these compounds. The other possibility is drug-drug interaction, e.g., one compound may increase the exposure to the other compound by competing for the metabolic enzyme(s). It is very important to determine whether pretreatment with gemcitabine changes the area under the curve (AUC) of nedaplatin, because the antitumor activity of nedaplatin is known to be AUC-dependent.

In conclusion, the results presented in this study suggest the potential efficacy of the combination of NDP with GEM for clinical therapy.

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