Abstract. Background: Nedaplatin (NDP) was developed as a second-generation platinum complex. The antitumor efficacy of the combination of NDP with docetaxel (TXT) was evaluated against human head and neck carcinoma. The antitumor activity of NDP plus TXT was compared with that of some other platinum compounds, cisplatin (CDDP) and carboplatin (CBDCA) plus TXT. Materials and Methods: Mice implanted with HNC-3 or KB3-1, human head and neck carcinoma were administered i.v. NDP, CDDP or CBDCA plus TXT. Results: The antitumor efficacy was enhanced significantly by the combination of NDP with TXT. Combined NDP plus TXT treatment exerted antitumor efficacy comparable to that of combined CDDP plus TXT treatment. Thrombocytopenia induced by NDP was not enhanced by the combination of NDP and TXT. Conclusion: The results suggest that combined NDP and TXT can alleviate thrombocytopenia caused by NDP and that this combination may have significant potential in clinical use.

Nedaplatin (NDP, Aqupla®, Shionogi & Co., Ltd., Osaka, Japan) was synthesized at our laboratory. It is a second-generation platinum preparation which exerted excellent antitumor activity against various solid cancers in preclinical studies. In phase II clinical studies, this drug was effective against breast cancer (11, 12), non-small cell lung cancer (13, 14), gastric cancer (15), head and neck cancer (16), ovarian cancer (29) and esophageal cancer (18). We previously reported the effects of NDP used in combination with etoposide (19), 5-Fluorouracil (20, 21), cyclophosphamide (22), gemcitabine (23) and paclitaxel (TXL) (24, 25). We previously reported that the administration of TXL prior to NDP had a sparing effect of the thrombocytopenia induced by NDP (26). The present study additionally evaluated the sparing effect of TXT when used in combination with NDP.

Materials and Methods

Animals and tumors. BALB/c nude mice (9-10 weeks old, female; CLEA Japan, Inc.) were used for this study. HNC-3 human head and neck carcinoma was obtained from the Central Laboratories for Experimental Animals. KB3-1 human head and neck cancer was kindly provided by Dr. S. Akiyama (Kagoshima University Medical School, Kagoshima, Japan). These tumors were maintained by serial subcutaneous (s.c.) transplantation as tumor fragments in BALB/c nude mice.

Drugs. NDP was obtained from Shionogi and Co., Ltd. CDDP and CBDCA were purchased from Nippon Kayaku (Tokyo, Japan) and Bristol-Myers Squibb (Tokyo, Japan), respectively. TXT was purchased from Sanofi-Aventis (Tokyo, Japan). Each drug was dissolved in saline immediately before use.

In vivo therapeutic experiments. A tumor fragment of KB3-1 and HNC-3 (approximately 1-2 mm³) was implanted s.c. into the back of mice. The mice implanted with HNC-3 and KB3-1 were grouped so that the mean tumor volume would become about 220 mm³ and 460 mm³, respectively, in each group. Six to 8 mice were allocated to each group. TXT and the platinum compounds were administered by a single i.v. injection. TXT was administered at dose levels of
The doses of platinum compounds used were 20, 30 and 40 mg/kg for NDP, 9 mg/kg for CDDP and 85.5 mg/kg for CBDCA. In animals treated with a combination of TXT and platinum compounds, the latter were administered 8 h after each dose of TXT. All experiments were conducted after approval of the Shionogi Animal Care and Use Committee.

Evaluation of antitumor efficacy. During the experiment, the tumor size and body weight were measured twice a week. The tumor volume was calculated using the method reported elsewhere (27). The growth inhibitory effect was estimated using the treated/control ratio (T/C). The effect of combined drug therapy in enhancing the antitumor activity of individual drugs was evaluated on the basis of the combination ratio (CR) (23). 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 agent), a CR equal to 1 indicated additivity and a CR greater than 1 indicated antagonism.

Evaluation of hematotoxicity. The hematotoxicity of the drugs was evaluated in tumor-free mice to avoid the possible physiological influence of the tumor. Using the same schedule as that employed for the experiment, TXT (80.3 mg/kg) and NDP (30 mg/kg) were administered and blood was sampled every day from the group (3 mice/group). Blood sampled from the abdominal aorta under anesthesia was subjected to white blood cell (WBC), red blood cell (RBC) and platelet counting, using an automated blood cell counter (Sysmex K-4500, Sysmex, Kobe, Japan). Nucleated bone marrow cells (BMC) in the specimen sampled from the right femur were counted with a particle counter (Sysmex CDA-500, Sysmex, Kobe, Japan).

Statistics. The statistical significance of differences between the treated and the untreated groups or the treated groups was evaluated using Welch’s test and Dunnett’s test, respectively.

Results

Combination therapy against KB3-1 human head and neck cancer. The antitumor efficacy of NDP and TXT used separately or in combination against human head/neck cancer KB3-1 is shown in Table I. When the antitumor efficacy of

Table I. Antitumor efficacy in combination chemotherapy of docetaxel (TXT) with nedaplatin (NDP) against KB3-1 head and neck cancera.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>No. of mice</th>
<th>RV (mean±SD)</th>
<th>T/C (mean±SD)</th>
<th>CR</th>
<th>Maximum body weight loss(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>4.50±1.39</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>TXT only</td>
<td>14.4</td>
<td>0</td>
<td>8</td>
<td>2.69±0.99b</td>
<td>0.60±0.22</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>28.8</td>
<td>0</td>
<td>8</td>
<td>2.02±0.96c</td>
<td>0.45±0.21</td>
<td>0.2</td>
</tr>
<tr>
<td>NDP only</td>
<td>0</td>
<td>20</td>
<td>8</td>
<td>3.14±1.27b</td>
<td>0.70±0.28</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>30</td>
<td>8</td>
<td>2.71±0.59c</td>
<td>0.60±0.13</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>40</td>
<td>8</td>
<td>2.50±0.87c</td>
<td>0.56±0.19</td>
<td>9.4</td>
</tr>
<tr>
<td>Combination</td>
<td>14.4</td>
<td>20</td>
<td>8</td>
<td>1.88±0.26c,d</td>
<td>0.42±0.06</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>14.4</td>
<td>30</td>
<td>8</td>
<td>1.29±0.44c,e,f</td>
<td>0.29±0.10</td>
<td>0.81</td>
</tr>
</tbody>
</table>

a HNC-3 head and neck cancer fragment was implanted s.c. into the nude mice. TXT and NDP were injected i.v. on day 17. Tumor volumes are shown on day 27. RV, relative tumor volume; T/C, treated/control; CR, combination ratio; Maximum body weight losses are shown as percent of the initial weight.

b $p<0.05$ for untreated control by Dunnett’s test.

c $p<0.01$ for untreated control by Dunnett’s test.

d $p<0.01$ for NDP 20 mg/kg by Dunnett’s test.

e $p<0.05$ for NDP 30 mg/kg by Dunnett’s test.

f $p<0.05$ for TXT 14.4 mg/kg by Dunnett’s test.

14.4 and 28.8 mg/kg. The doses of platinum compounds used were 20, 30 and 40 mg/kg for NDP, 9 mg/kg for CDDP and 85.5 mg/kg for CBDCA. In animals treated with a combination of TXT and platinum compounds, the latter were administered 8 h after each dose of TXT. All experiments were conducted after approval of the Shionogi Animal Care and Use Committee.

Figure 1. Antitumor efficacy of the combination of TXT with NDP. The doses of docetaxel (TXT) and nedaplatin (NDP) were 14.4 mg/kg and 30 mg/kg, respectively. Mice bearing HNC-3 were treated with saline (○), TXT (●), NDP (▲) and combination (●). Relative tumor volumes ± SD are shown.

Figure 2. Evaluation of hematotoxicity. The hematotoxicity of the drugs was evaluated in tumor-free mice to avoid the possible physiological influence of the tumor. Using the same schedule as that employed for the experiment, TXT (80.3 mg/kg) and NDP (30 mg/kg) were administered and blood was sampled every day from the group (3 mice/group). Blood sampled from the abdominal aorta under anesthesia was subjected to white blood cell (WBC), red blood cell (RBC) and platelet counting, using an automated blood cell counter (Sysmex K-4500, Sysmex, Kobe, Japan). Nucleated bone marrow cells (BMC) in the specimen sampled from the right femur were counted with a particle counter (Sysmex CDA-500, Sysmex, Kobe, Japan).
each drug administered independently was evaluated, the T/C was 0.60 for TXT at a dose of 14.4 mg/kg and was 0.45 at a dose of 28.8 mg/kg, respectively. When NDP was administered at the maximum tolerated dose (MTD), 40 mg/kg, the T/C was 0.56. Thus, NDP monotherapy was not effective against KB3-1. When TXT at 14.4 mg/kg and NDP at 20 or 30 mg/kg, which had not shown effectiveness, were combined, the T/C was 0.42 and 0.29, respectively. It was indicated that the antitumor efficacy of the combination of TXT with NDP was enhanced by the activity of these 2 drugs.

In particular, the combination of TXT at 14.4 mg/kg with NDP at 30 mg/kg resulted in significant enhancement of the antitumor activity \((p<0.05)\) compared with each drug and a reduction of CR to below 1, indicating synergistic effects of the combined treatment.

### Table II. Antitumor efficacy in combination chemotherapy of docetaxel (TXT) with nedaplatin (NDP) against HNC-3 head and neck cancer.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>No. of mice</th>
<th>RV (mean±SD)</th>
<th>T/C (mean±SD)</th>
<th>CR</th>
<th>Maximum body weight loss(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>2.81±0.43</td>
<td>0</td>
<td>1.3</td>
</tr>
<tr>
<td>TXT only</td>
<td>14.4</td>
<td>0</td>
<td>6</td>
<td>1.38±0.50b</td>
<td>0.49±0.18</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>28.8</td>
<td>0</td>
<td>6</td>
<td>0.62±0.08b</td>
<td>0.22±0.03</td>
<td>2.6</td>
</tr>
<tr>
<td>NDP only</td>
<td>0</td>
<td>20</td>
<td>6</td>
<td>2.44±0.61b</td>
<td>0.87±0.22</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>30</td>
<td>6</td>
<td>2.24±0.62</td>
<td>0.80±0.22</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>40</td>
<td>6</td>
<td>1.40±0.57b</td>
<td>0.50±0.20</td>
<td>4.9</td>
</tr>
<tr>
<td>Combination</td>
<td>14.4</td>
<td>20</td>
<td>6</td>
<td>0.41±0.12b,c,d</td>
<td>0.15±0.05</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>14.4</td>
<td>30</td>
<td>6</td>
<td>0.23±0.11b,c,e</td>
<td>0.08±0.04</td>
<td>0.20</td>
</tr>
</tbody>
</table>

*HNC-3 head and neck cancer fragment was implanted s.c. into the nude mice. TXT and NDP were injected i.v. on day 17. Tumor volumes are shown on day 27. RV, relative tumor volume; T/C, treated/control; CR, combination ratio; Maximum body weight losses are shown as percent of the initial weight.

### Table III. Antitumor efficacy in combination chemotherapy of platinum compounds with docetaxel (TXT) against HNC-3 head and neck cancer.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>No. of mice</th>
<th>RV (mean±SD)</th>
<th>T/C (mean±SD)</th>
<th>CR</th>
<th>Maximum body weight loss(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>6.48±6.63</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>TXT only</td>
<td>14.4</td>
<td>0</td>
<td>7</td>
<td>1.81±0.79c</td>
<td>0.28±0.12</td>
<td>4.0</td>
</tr>
<tr>
<td>NDP only</td>
<td>0</td>
<td>30</td>
<td>7</td>
<td>1.10±0.40c</td>
<td>0.17±0.06</td>
<td>18.6</td>
</tr>
<tr>
<td>TXT+NDP</td>
<td>14.4</td>
<td>30</td>
<td>7</td>
<td>0.20±0.09c,d,e</td>
<td>0.03±0.01</td>
<td>0.63</td>
</tr>
<tr>
<td>CDDP only</td>
<td>0</td>
<td>9</td>
<td>7</td>
<td>2.49±1.38b</td>
<td>0.38±0.21</td>
<td>10.3</td>
</tr>
<tr>
<td>TXT+CDDP</td>
<td>14.4</td>
<td>9</td>
<td>7</td>
<td>0.32±0.22c,d,e</td>
<td>0.05±0.03</td>
<td>0.47</td>
</tr>
<tr>
<td>CBDCA only</td>
<td>0</td>
<td>85.5</td>
<td>7</td>
<td>2.49±1.29b</td>
<td>0.38±0.20</td>
<td>3.0</td>
</tr>
<tr>
<td>TXT+CBDCA</td>
<td>14.4</td>
<td>85.5</td>
<td>7</td>
<td>0.59±0.62c,d,h</td>
<td>0.09±0.10</td>
<td>0.85</td>
</tr>
</tbody>
</table>

*HNC-3 head and neck cancer fragment was implanted s.c. into the nude mice. TXT and platinum compounds were injected i.v. on day 19. Tumor volumes are shown on day 28. RV, relative tumor volume; T/C, treated/control; CR, combination ratio; Maximum body weight losses are shown as percent of the initial weight; NDP, nedaplatin; CDDP, cisplatin; CBDCA, carboplatin.

\(b\ p<0.01\) for untreated control by Dunnett’s test.
\(c\ p<0.01\) for TXT 14.4 mg/kg by Dunnett’s test.
\(d\ p<0.01\) for NDP 20 mg/kg by Dunnett’s test.
\(e\ p<0.01\) for NDP 30 mg/kg by Dunnett’s test.

Combination therapy against HNC-3 human head and neck cancer. The combination therapy of TXT with NDP resulted in enhanced antitumor efficacy against human HNC-3 head and neck cancer in comparison with TXT or NDP.
monotherapy (Figure 1, Table II). The sensitivity of HNC-3 to NDP alone was low and the T/C was 0.5 only when the drug was administered at MTD (40 mg/kg). At doses of 20 and 30 mg/kg, NDP did not show antitumor efficacy, with a T/C over 0.5. When TXT was administered separately, the T/C was 0.22 at a dose level of 28.8 mg/kg and 0.49 at 14.4 mg/kg. Thus, TXT at these dose levels resulted in tumor growth suppression. When TXT (14.4 mg/kg) was combined with NDP at dose levels of 20 and 30 mg/kg (at these doses, NDP monotherapy had not shown effectiveness), the T/C was 0.15 and 0.08, respectively. Thus, the antitumor efficacy of combined TXT and NDP treatment at these dose levels was significantly higher than that of TXT or NDP alone (\( p < 0.01 \)). The CR was below 1, following combined treatment with TXT (14.4 mg/kg) and NDP (20 and 30 mg/kg), thus indicating synergistic effects. In each combination, the maximum weight loss was 10% or less, indicating that these regimens of combined TXT and NDP therapy were also acceptable in terms of toxicity.

The antitumor efficacy of TXT and three platinum compounds (NDP, CDDP and CBDCA) used separately or in combination against HNC-3 is presented in Table III. In the combined treatment, TXT (14.4 mg/kg) was combined with each platinum compound at doses equivalent to 75% of the MTD (NDP: 30 mg/kg, CDDP: 9 mg/kg, CBDCA: 85.5 mg/kg). The T/C was 0.03, 0.05 and 0.09 for TXT combined with NDP, CDDP and CBDCA, respectively. Thus, each combination therapy exerted high antitumor efficacy. The CRs for NDP plus TXT, CDDP plus TXT and CBDCA plus TXT were 0.63, 0.47 and 0.85, respectively, indicating that each combination had synergistic effects. The enhancement of antitumor efficacy did not differ significantly among any two combinations with TXT.

**Hematotoxicity.** The hematotoxicity of combined NDP at 30 mg/kg and TXT at 86.3 mg/kg was evaluated. The dosage of the two compounds was 75% MTD, respectively. To avoid a possible physiological influence of the growing tumor on hematological parameters, non-tumor-bearing BALB/c nude mice were used and their peripheral blood cells and marrow cells were measured every other day. The lowest levels of WBC, RBC, platelets and BMC in this study are given in Table IV. Combined dosing resulted in lower WBC and BMC values than single dosing with each drug, but the difference in these parameters between the combined treatment and the TXT single treatment was not statistically significant. No difference in RBC values was noted between the combination therapy and each monotherapy. The change of platelet count throughout this study is indicated in Figure 2. The platelet count in the single TXT treatment and the combination of TXT and NDP showed 40% or more elevation as compared to the untreated control on the fifth day of dosing. From the sixth day on, the platelets decreased over time, reaching the lowest level on the ninth day and rising again thereafter. The percent decrease in platelets was 14%, at maximum, in the single TXT treatment, showing no significant difference from the untreated control. The percent decrease in platelets was greatest in the single NDP treatment, where the platelet count decreased by 50% or more as compared to the control group. The percent decrease in platelet count of the combination therapy was 38%. Thus, thrombocytopenia induced by NDP tended to be alleviated by combined TXT and NDP treatment, although this difference from the NDP single treatment was not statistically significant.

**Table IV. Hematotoxicity in combination chemotherapy of docetaxel (TXT) with nedaplatin (NDP).**

<table>
<thead>
<tr>
<th>Group</th>
<th>Maximum reductionb (day of nadir)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WBC</td>
</tr>
<tr>
<td>Untreated</td>
<td>100±19</td>
</tr>
<tr>
<td>TXT</td>
<td>31±5 (3)d,f</td>
</tr>
<tr>
<td>NDP</td>
<td>61±6 (15)d</td>
</tr>
<tr>
<td>TXT+NDP</td>
<td>27±0 (3)d,f</td>
</tr>
</tbody>
</table>

\( a \) TXT or NDP was injected i.v. on day 0. Doses of TXT and NDP used were 86.3 mg/kg and 30 mg/kg, respectively.
\( b \) percent of untreated control, mean±SD.
\( c \) n=3.
\( d \) \( p<0.05 \) for untreated control by Welch’s test.
\( e \) \( p<0.01 \) for untreated control by Welch’s test.
\( f \) \( p<0.01 \) for NDP by Dunnett’s test.

**Figure 2. The time-course of platelets in the combination of docetaxel (TXT) with nedaplatin (NDP).** Non-tumor-bearing BALB/c nude mice received 75% MTD of TXT and NDP alone or in combination. The number of platelets from mice treated with TXT (□), NDP (△) and the combination (●) were counted and shown as % control (saline-treated)±SD.
Discussion

In preclinical studies using various tumor cell lines, a single dose of NDP suppressed tumor growth markedly and this effect was comparable to, or higher than, that of CDDP or CBDCA (28). In previous studies of the antitumor activity of combined NDP and TXL therapy against lung carcinoma (Lewis lung carcinoma) and ovarian carcinoma (SK-OV-3), the suppression of the growth of each tumor was enhanced by the combination of these two drugs and this effect was higher than that of CDDP plus TXL or CBDCA plus TXL therapy (26, 29). In the present study, we evaluated the usefulness of combined TXT and NDP therapy for two human head and neck cancer cell lines. When the antitumor efficacy of single NDP therapy against KB3-1 and HNC-3 was evaluated, the T/C was 0.56 and 0.50, respectively, when the drug was administered at a dose of 40 mg/kg (equivalent to the MTD of this drug). If a T/C less than 0.5 is deemed effective, this drug was effective only against HNC-3. The antitumor efficacy of NDP monotherapy was thus not potent. This drug was ineffective at a dose of 30 mg/kg (75% MTD) and 20 mg/kg (50% MTD). However, treatment with a combination of NDP and low dose TXT (14.4 mg/kg, 25% MTD) resulted in a T/C below 0.5. Thus, this combination therapy was effective and exerted higher antitumor efficacy than NDP or TXT monotherapy. These results suggest that high antitumor efficacy can be obtained even with low-dose NDP, if the drug is used in combination with TXT. Since the weight loss of the mice was not greater than 20%, this combined therapy seems to be effective and safe.

It has been clinically shown that TXT reduces the thrombocytopenia induced by other platinum compounds (30). In our animal studies, the combined TXL and NDP treatment reduced the thrombocytopenia induced by NDP (unpublished data). The percent decrease in platelet count was 38% following combined TXT and NDP treatment, which was smaller than the decrease (over 50%) observed following NDP treatment, each compared to the untreated control group. Thus, TXT was found to alleviate thrombocytopenia induced by NDP, corroborating the previously reported sparing effect of TXL. This result endorses the clinical report that combined TXT and NDP treatment led to increased antitumor efficacy and reduced adverse reactions (31).

It has also been clinically reported that the use of TXL in combination with other drugs resulted in signs of toxicity, depending on the order of administration of the drugs (32, 33). Regarding the use of TXL in combination with platinum compounds, it has been shown that the incidence of neutropenia is high and that marrow suppression and peripheral neuropathy are reinforced by the sequence of CDDP followed by TXL (34). These signs of toxicity were reproduced in animal studies and the results of our previous study showed that marrow toxicity intensified when TXL was administered after NDP (26). In the combined TXT and NDP treatment as well, the order of administration of the two drugs was important and we observed marked weight loss in animals treated with TXT 2 days after each dose of NDP (data not shown). Furthermore, thrombocytopenia, one of the dose-limiting factors for NDP, was alleviated when TXT was administered prior to NDP. It seems, therefore, advisable to administer TXT before NDP.

Attempts to use TXT in combination with platinum preparations have been clinically reported for patients with non-small cell lung cancer (35), head and neck cancer (36) and ovarian cancer (17). In many of such cases, TXT was combined with CDDP. In the present study, combined TXT and NDP therapy exerted antitumor efficacy comparable to that of combined TXT and CDDP therapy. If combined TXT and NDP therapy is applied clinically, we may expect antitumor efficacy comparable to that of combined TXT and CDDP or TXT and CBDCA therapy, with reduced toxicity of the drugs used in combination with TXT.

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References


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