Antitumor Activity, Induction of Cross-Resistance, and Nephrotoxicity of a New Platinum Analogue, cis-1,1-Diaminomethylcyclohexaneplatinum(II) Sulfate, and of cis-Diaminedichloroplatinum(II) in an Immunocytoma Model in the LOU/M Rat

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ABSTRACT

A newly synthesized platinum analogue, cis-1,1-diaminomethylcyclohexaneplatinum(II) sulfate (TNO-6), was compared with cis-diaminedichloroplatinum(II) (cis-DDP) for antitumor activity and nephrotoxicity. Antitumor activity was determined in an IgM immunocytoma model in the LOU/M rat. Tumor cells were inoculated on the left flank, and therapy was started when a tumor diameter of 10 to 30 mm was reached. At the start of the therapy, the primary tumor had already metastasized to the draining lymph node and liver. Both platinum compounds, dissolved in 5% glucose water, induced an almost complete tumor regression within 10 to 14 days (average, 84% tumor load reduction) and prolonged survival, compared to that of nontreated animals. The antitumor activity induced by repeated i.p. administration of cis-DDP and TNO-6 reached its maximum at a dose of 1.0 mg/kg body weight (twice a week for 7 weeks). This treatment regimen resulted in a higher tolerable dose for cis-DDP of 1.0 mg/kg and for TNO-6 of 2.0 mg/kg. However, when rats were treated with a 2.0-mg/kg dose of TNO-6, no increase in antitumor activity was obtained. For both platinum compounds, tumor recurrence occurred in almost all animals within 2 to 7 days after the maximum tumor load reduction. Tumors that recurred were found to be cross-resistant to both platinum compounds tested but were sensitive to treatment with doxorubicin (Adriamycin). With regard to toxicity, repeated administration of TNO-6 (1.0 mg/kg twice a week for 7 weeks) induced less decrease of body weight than did cis-DDP. For TNO-6, even in the highest dose investigated (2.0 mg/kg twice a week for 7 weeks), no nephrotoxicity was observed on histological examination of kidney and blood urea and creatinine values, whereas for cis-DDP nephrotoxicity was still present in the lowest dose investigated (0.5 mg/kg).

From the comparison of the antitumor activity and nephrotoxicity of TNO-6 and cis-DDP, administered i.p. in 5% glucose solution, it is concluded that both drugs have comparable antitumor activity and potency. In contrast to the effects of cis-DDP, no nephrotoxicity was observed with TNO-6; thus, TNO-6 might be a good alternative to cis-DDP in avoiding nephrotoxicity during platinum therapy.

INTRODUCTION

Since the discovery of its antitumor effect by Rosenberg et al. (20), cis-DDP2 has demonstrated a remarkable chemotherapeutic potential in a variety of experimental tumor models in laboratory animals (21) and in human neoplasms, such as testicular, ovarian, and lung tumors and carcinomas of the head and neck (5, 21). However, an impediment to the use of cis-DDP is its renal toxicity (4, 10, 11, 15, 24). A reduction in nephrotoxicity can be achieved by prehydration and forced diuresis during cis-DDP treatment (7, 12, 25). Also, an increase in the NaCl concentration of the vehicle used for cis-DDP administration diminishes renal toxicity (14).

Reduction in nephrotoxicity can be obtained by modification of the drug itself. Therefore, much effort is directed toward synthesizing new platinum analogues (16, 18, 19, 23). In determining the activity of these new platinum compounds, comparison with the clinically used cis-DDP with regard to the antitumor activity and nephrotoxicity is necessary. In recent years, the L1210 leukemia has been widely used to evaluate the antitumor effect of various platinum analogues (16, 19, 21). cis-DDP-resistant L1210 sublines have also been isolated (6, 19, 26).

From the newly synthesized, more complex organoplatinum compounds, TNO-6 (NSC-311056) was chosen, as this platinum analogue was used in a clinical evaluation under sponsorship of the European Organization for Research on Treatment of Cancer. In this Phase I study, lack of renal toxicity was recently found (17). Moreover, data of Rose et al. (19) showed in mice treated with TNO-6 antitumor activity comparable to that obtained with cis-DDP and lack of elevation of blood urea nitrogen levels, indicative of a lower nephrotoxicity (19).

We compared the antitumor activity and the nephrotoxicity of TNO-6 with those of cis-DDP; both drugs were dissolved in a 5% glucose solution, and an IgM immunocytoma model in the rat was used. The results show the IgM immunocytoma model to be well suited for determining the antitumor potency and activity of platinum compounds. Both platinum compounds investigated, cis-DDP and TNO-6, showed similar antitumor activity in this model on a mg/kg basis. However, the recurrence rate was almost 100%. All sublines isolated from recurrences were resistant to continued platinum therapy. Recurrences showed cross-resistance to both platinum compounds investigated but regressed completely during doxorubicin (Adriamycin) treatment.

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2 The abbreviations used are: cis-DDP, cis-diaminedichloroplatinum(II); TNO-6, cis-1,1-diaminomethylcyclohexaneplatinum(II) sulfate.
The lowest dose of cis-DDP (0.5 mg/kg) that showed tumor regression induced lesions in the kidney, whereas even the highest dose investigated for TNO-6 (2.0 mg/kg) did not.

**MATERIALS AND METHODS**

**Animals.** Breeding pairs of LOU/M Wsl inbred rats and the IgM immunocytoma of LOU/C Wsl origin were kindly provided by Dr. H. Bazin (Catholic University of Louvain, Louvain, Belgium) (2). Animals were bred at our institute. Female and male rats used for the different experiments were 12 to 16 weeks of age. Animals were kept in plastic cages, fed with commercial diet (Murano, Hope Farms, Woerden, The Netherlands), and given water ad libitum. They were housed under conventional conditions.

**Tumor Model.** In the LOU/C inbred rat strain, a high incidence of immunocytomas that secrete a variety of monoclonal immunoglobulins occurs (2, 3). Because of poor breeding of the LOU/C rat, in which the IgM tumor originally appeared, the histocompatible LOU/M rat was used as recipient for the IgM immunocytomas. Tumor cells, harvested by trypsinization (0.25% trypsin) of solid tumors, were stored in liquid nitrogen. The tumor cells were used for each experiment after one s.c. in vivo passage. In the experimental animals, $1 \times 10^6$ IgM immunocytoma cells in 0.5 ml of plain Roswell Park Memorial Institute Medium 1640 (Grand Island Biological Co., Europe B. V., Hoofddorp, The Netherlands) were inoculated s.c. on the flank. The growth of the tumor was measured twice a week with vernier calipers and expressed as mean value of 3 perpendicular measurements. Prior to therapy, animals with tumors of a diameter of 10 to 20 mm were randomly distributed over the various treatment groups of the experiments. To quantitate the antitumor activity, 3 calculations were made: (a) percentage of maximal tumor load reduction (size of the tumor at the start of therapy minus minimal obtained tumor size, divided by tumor size at the start of therapy x 100); (b) time in days between start of therapy and regrowth of tumor; (c) time between maximal tumor load reduction and regrowth to 10 mm in diameter.

Most animals given injections of $1 \times 10^6$ IgM immunocytoma cells developed a palpable tumor after 17 to 21 days which grew to a diameter of 2 to 3 cm within the subsequent 6 to 7 days. At this time (approximately Day 25) the tumor showed metastases in the draining (auxiliary) lymph node and micrometastases in the liver. In the terminal stage (mean diameter, 4 to 4.5 cm), metastases were also present in distant lymph nodes and spleen. Although tumor cells were found in the alveolar septa, no metastatic growth was observed in the lung.

**Histology.** Tissue samples were fixed for histological evaluation in 4% buffered formaldehyde and were embedded in paraffin; sections were routinely stained with hematoxylin and eosin. For histological examination of the kidney, formaldehyde-fixed tissue was also embedded in glycol-methacrylate and stained with periodic acid-silver methenamine. Kidney lesions, mainly tubular alterations in medulla, were semiquantitatively scored with the following criteria: none, no alterations in tubuli; mild, alterations in less than 25% of the tubuli; moderate, alterations in 25 to 50% of tubuli; marked, alterations in 50 to 75% of the tubuli; severe, alterations in the whole medulla in more than 90% of the tubuli.

**Chemotherapy Regimen.** cis-DDP (NSC-119875) was kindly provided in bulk quantity by Bristol Laboratories (Syracuse, N. Y.), and TNO-6 (NSC-311056) was synthesized at the Institute of Applied Chemistry, TNO, Utrecht, The Netherlands. Rats were anesthetized with ether, weighed, and given i.p. injections of the platinum compounds twice a week. Both drugs were prepared weekly in 5% glucose water and used directly and after storage at +4° for 3 days. By means of thin-layer chromatography, it was determined that TNO-6 in 5% glucose water did not show any trace of decomposition even after storage for 5 days at 45°. Conversely, TLC analysis of cis-DDP in 5% glucose water after storage for 3 days at 4° showed a decrease in intensity of about 15% (differential scanning). Apart from tailing, no well-defined decomposition product could be identified for the main component, cis-DDP. Quantitative analysis by means of atomic absorption spectrometry failed (detection limit).

**Blood Urea and Creatinine Determination.** For assayng nephrotoxicity, blood urea and creatinine levels were investigated. Blood urea levels were determined according to an enzyme kinetic method (Boehringer Monotest Catalogue No. 166421, Boehringer Mannheim, Mannheim, Federal Republic of Germany). Serum creatinine levels were determined according to the modified method of Jaffe (1) with a fixed time measurement. For both measurements, a Corona autoanalyzer (Clinicon AB, Bromma, Sweden) was used. Blood was collected under ether anesthesia by orbital sinus puncture before and during platinum therapy.

**RESULTS**

**Dose-Response Studies with cis-DDP and TNO-6 in the IgM Immunocytoma.** In 2 experiments, tumor-bearing rats (diameter of tumors varying from 1.5 to 3.0 cm) were treated twice weekly, from approximately Day 21 onward, with cis-DDP and TNO-6 administered i.p. In the first study, the antitumor effect was investigated at dose levels ranging from 1.0 to 16 mg/kg. These doses were chosen on the basis of a pilot study. Dosages of 1.0, 2.0, and 4.0 mg/kg for cis-DDP and 1.0, 2.0, 4.0, 8.0, and 16.0 mg/kg for TNO-6 induced a rapid tumor load reduction. However, in most animals treated with a tolerable dose of platinum, the tumor recurred. The effect of both platinum compounds on the tumor size of individual animals for the dosages 1.0, 2.0, and 4.0 mg/kg for TNO-6 and 1.0 and 2.0 mg/kg for cis-DDP is shown in Chart 1. In the high dose levels, animals...
Effect of cis-DDP and TNO-6 on the growth of the IgM immunocytoma in the LOU/M rat

<table>
<thead>
<tr>
<th>Dose (mg/kg body wt.)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cis-DDP</td>
<td>TNO-6</td>
<td>cis-DDP</td>
<td>TNO-6</td>
<td>cis-DDP</td>
</tr>
<tr>
<td>0.25</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>0.5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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</tr>
<tr>
<td>1.0</td>
<td>100 ± 0</td>
<td>90.8 ± 12.7</td>
<td>89.8 ± 14</td>
<td>95.8 ± 9.4</td>
<td>35.0 ± 7.7</td>
</tr>
<tr>
<td>2.0</td>
<td>78.5 ± 16.7</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>26.1 ± 7.1</td>
</tr>
</tbody>
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Table 1: Comparison of cis-DDP and TNO-6 in a Rat Immunocytoma Model

<table>
<thead>
<tr>
<th>% of maximal tumor load reduction</th>
<th>Time between start of therapy and regrowth of tumor (days)</th>
<th>Time (days) from maximum tumor load reduction to regrowth of tumor to diameter of 10 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment 1</td>
<td>Experiment 2</td>
</tr>
<tr>
<td>cis-DDP</td>
<td>cis-DDP</td>
<td>TNO-6</td>
</tr>
<tr>
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</tr>
<tr>
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<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1.0</td>
<td>100 ± 0</td>
<td>90.8 ± 12.7</td>
</tr>
<tr>
<td>2.0</td>
<td>78.5 ± 16.7</td>
<td>ND</td>
</tr>
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</table>

* Both drugs were dissolved in 5% glucose water and administered i.p. twice a week for 7 weeks.
* Determined after 6 i.p. treatments.
* ND, experiment not done; NA, not suitable for analyzing with the parameters used.
* p < 0.05 versus a 0.5-mg/kg dose and p < 0.001 versus a 1.0-mg/kg dose of cis-DDP.
* p < 0.001 versus a 0.5- and 1.0-mg/kg dose of TNO-6 and p < 0.05 versus a 0.25-mg/kg dose of cis-DDP.
* p < 0.05 versus a 1.0-mg/kg dose of cis-DDP.

Died clinically tumor free shortly after the start of therapy (cis-DDP, 2.0 and 4.0 mg/kg; TNO-6, 4.0 and 8.0 mg/kg) probably as a result of the toxicity of the drugs. Regarding the antitumor activity, complete tumor regression occurred after 3 to 5 i.p. injections of cis-DDP or TNO-6. The regression proceeded for 10 to 14 days. This time was not dose related (Chart 1). When the percentage of maximal tumor load reduction was calculated, no statistical differences could be found between rats treated with a 1.0- and a 2.0-mg/kg dose of TNO-6; the percentage of maximal tumor load reduction was 90.8 ± 12.7 (S.D.) and 78.5 ± 16.7, respectively. The maximal tumor load reduction induced by cis-DDP (1.0 mg/kg) did not differ significantly (100 ± 0) from that of animals treated with TNO-6 for the dose of 1.0 and 2.0 mg/kg (Table 1). Furthermore, no significant differences were observed between the 1.0-mg and 2.0-mg TNO-6 groups when the time between start of therapy and regrowth of tumor was calculated (28.0 ± 8.7 and 26.1 ± 7.1 days, respectively). However, the time between start of therapy and regrowth of the tumor was significantly prolonged (p < 0.05) when animals treated with cis-DDP (1.0 mg/kg) did not differ significantly (100 ± 0) from that of animals treated with TNO-6 (2.0 mg/kg). The regression proceeded for 10 to 14 days. This time was not dose related (Chart 1). When the percentage of maximal tumor load reduction was calculated (28.0 ± 8.7 and 26.1 ± 7.1 days, respectively). How ever, the time between start of therapy and regrowth of tumor was significantly prolonged (p < 0.05) when animals treated with cis-DDP (1.0 mg/kg) and TNO-6 (2.0 mg/kg) were compared (35.0 ± 7.7 and 26.1 ± 7.1 days, respectively). Since the 1.0 mg/kg level of both cis-DDP and TNO-6 was still effective in the first study, the minimal effective dose was determined in the second experiment. Rats were treated i.p. with dose levels ranging from 0.015 to 1.0 mg/kg. As shown in Chart 2, the 0.5- and 1.0-mg/kg dosage levels of cis-DDP and of TNO-6 induced an almost complete tumor regression after 2 weeks, as noted in the first experiment. In the 0.25- and 0.125-mg/kg groups, tumor growth retardation was found which appeared to be dose related. No antitumor effect was noted with the lower groups. In the 0.25- and 0.125-mg/kg dose levels ranging from 0.015 to 1.0 mg/kg. As shown in Chart 2, the 0.5- and 1.0-mg/kg dosage levels of cis-DDP and of TNO-6 induced an almost complete tumor regression after 2 weeks, as noted in the first experiment. In the 0.25- and 0.125-mg/kg groups, tumor growth retardation was found which appeared to be dose related. No antitumor effect was noted with the lower doses of cis-DDP and TNO-6. To determine the differences in antitumor activity, statistical analysis (Students t test) was made of the percentage of maximal tumor load reduction after 6 i.p. treatments, of the time between start of therapy and regrowth of tumor, and of the time from the maximal tumor load reduction to the regrowth of the tumor to a diameter of 10 mm. Rats treated with a 0.25-mg/kg dose of cis-DDP or of TNO-6 showed significantly less antitumor activity (p < 0.05 and 0.001, respectively) than that achieved with the 0.5- and 1.0-mg doses of cis-DDP or TNO-6 (Table 1). A tendency toward a dose-response relation was seen for the maximum tumor load reduction for both cis-DDP and TNO-6 in the dosages 0.25, 0.5, and 1.0 mg/kg (Table 1). However, for both platinum compounds using the 3 values of antitumor activity, some of these parameters gave significant differences between doses of
0.5 and 1.0 mg/kg. Besides a significant difference (p < 0.05) in maximal tumor load reduction at a dose of 0.25 mg/kg, no differences in antitumor activity between TNO-6 and cis-DDP were found for the various parameters.

**Histological Evaluation of Antitumor Activity.** In a separate experiment, animals were autopsied for histological evaluation of the antitumor effect of the treatment at the time that the tumor had completely or almost completely disappeared (the tumor was not measurable but still palpable as a very small nodule). Two groups of 3 animals were treated twice weekly from Day 21 onward with cis-DDP or TNO-6 (1.0 mg/kg).

At histological examination of the inoculation site of the primary tumor, a massive accumulation of macrophages was seen in the subcutis of platinum-treated animals (Fig. 1). In the macrophages, 2 different types of pigment were observed. One pigment consisted of brown granular material which was positive with the Perl's stain and was identified as hemosiderin. The other pigment consisted of yellow granules which showed yellow autofluorescence and were identified as ceroid pigment. Besides small areas with necrotic tumor cells, foci with vital tumor cells were also seen. The livers in all 3 animals treated with cis-DDP and in 2 of 3 TNO-6-treated animals were free of tumor cells.

Animals with tumor recurrence after treatment with cis-DDP or TNO-6 showed metastases similar to those in nontreated tumor-bearing rats, with involvement of the draining (axillary) and distant lymph nodes, liver, and spleen.

**Resistance Studies.** To investigate whether the observed recurrence was caused by alterations in the tumor cell itself, we isolated tumor cells from solid tumor recurrences. These tumor cells, designated sublines IgM/DDP and IgM/TNO-6, were transferred to 3 to 6 recipients (1 x 104 cells s.c. in 0.5 ml of Roswell Park Memorial Institute Medium 1640) to test their sensitivity to platinum therapy. Six sublines isolated from recurrences after treatment with a 1.0- or 2.0-mg/kg dose of cis-DDP and TNO-6 were investigated (3 sublines for each platinum compound). By post inoculation of 1 x 10^4 tumor cells

**Toxicity of cis-DDP and TNO-6.** The mean survival time in days of IgM immunocytoma-bearing rats treated with either cis-DDP or TNO-6 (1.0 mg/kg) until tumor regression was complete. Thereafter, therapy was continued with the homologous platinum compound, whereas the parent immunocytoma regressed almost completely after 4 i.p. treatments. Representative results obtained with 2 of these sublines are shown in Chart 3.

We next studied whether the recurrence could be prevented by switching from one platinum compound to the other at the time of complete tumor regression. Tumor-bearing animals were treated with either cis-DDP or TNO-6 (1.0 mg/kg) until tumor regression was complete. Thereafter, therapy was continued with the same dose of the platinum compound not used before. Control animals were treated during the entire experiment with the same platinum compound. As shown in Chart 4, recurrences could not be prevented by changing the platinum compound.

To investigate whether cross-resistance was limited to both platinum compounds tested, tumor recurrences after platinum therapy were treated with doxorubicin (Adriblastine, Farmitalia, Milan, Italy), a drug also interacting with DNA. Animals were treated with TNO-6 until a tumor recurrence with a diameter of approximately 1.0 cm had developed. At that time, doxorubicin was injected i.v. (1.0 mg/kg) for 5 consecutive days followed by 2 additional injections after another interval of 5 days. As shown in Chart 5, doxorubicin induced complete regression of the TNO-6-resistant tumors.

**Toxicity of cis-DDP and TNO-6.** The mean survival time in days of IgM immunocytoma-bearing rats during repeated administration of cis-DDP and TNO-6 is given in Table 2. Rats treated with cis-DDP (2.0 or 4.0 mg/kg) and TNO-6 (4.0, 8.0, or 16 mg/kg) died due to toxicity of the platinum compounds. Rats treated with lower platinum dosages survived for more than 50 days and were killed because of the size of the recurrent tumor. It is clear from these results that cis-DDP is approximately twice as toxic.
Comparison of cis-DDP and TNO-6 in a Rat Immunocytoma Model

from control values in all TNO-6-treated groups. Only in the high-dose cis-DDP-treated group were increased values found 1 week prior to sacrifice and at time of autopsy. As shown in Table 3, the increase in blood urea values corresponded to the degree of kidney lesions; 2- to 5-fold increases were measured in rats with severe kidney lesions. Only in these latter animals was an increase in serum creatinine levels also noticed (Table 3).

**DISCUSSION**

The aim of the present study was to compare the antitumor activity and nephrotoxicity of TNO-6, a newly synthesized platinum analogue, with those of cis-DDP. For this study, we used as TNO-6. This difference in toxicity is also reflected in the marked loss of body weight in cis-DDP-treated rats when compared to the weights of animals treated with TNO-6 (Chart 6).

Because cis-DDP is known to be nephrotoxic, special attention was given to the kidney. At histological examination of glycolmethacrylate-embedded and periodic acid-silver methenamine-stained kidney sections, marked lesions were observed in rats treated with cis-DDP twice weekly for 7 weeks. These lesions were dose related (Table 3). The most prominent changes were observed in the outer stripe of the outer portion of the renal medulla. In the high-dose cis-DDP group (1.0 mg/kg), lesions extended to the outer cortex. Changes were extensive in the proximal tubules and consisted of tubular dilation with epithelial regeneration, characterized by intermediate to low epithelial cells stained kidney sections, marked lesions were observed in rats treated with cis-DDP twice weekly for 7 weeks. These lesions were dose related (Table 3). The most prominent changes were observed in the outer stripe of the outer portion of the renal medulla. In the high-dose cis-DDP group (1.0 mg/kg), lesions extended to the outer cortex. Changes were extensive in the proximal tubules and consisted of tubular dilation with epithelial regeneration, characterized by intermediate to low epithelial cells that appeared to be devoid of a brush border and had large atypical nuclei (Fig. 2A). Besides tubular expansion, prominent findings were the presence of atrophic tubuli with thickened basal lamina, probably due to tubular collapse, and the presence of interstitial cellular infiltrate (Fig. 2B). Additionally, hyalin casts were identified in nephron segments distal to the proximal tubules. cis-DDP-induced changes were not confined to the renal tubules in that changes were also noticed in the glomeruli, although at a much lower frequency. These changes consisted of glomerular atrophy (Fig. 2B) and hypertrophy of cells of the parietal layer of Bowman’s capsule. As in the controls (Fig. 2C), no kidney lesions were observed in rats treated with TNO-6.

Blood urea values determined at 1-week intervals did not differ...
the IgM immunocytoma model in the LOU/M rat. Results of our experiments show that this tumor model is sensitive to treatment with both platinum compounds, although a high incidence of recurrences was observed. These recurrences had the same growth kinetics as the primary tumor.

Repeated dosages of both cis-DDP and TNO-6 (0.5 and 1.0 mg/kg), dissolved in 5% glucose water, induced a nearly complete clinical tumor regression. Tumor growth retardation was observed at a dosage as low as 0.12 mg/kg for both compounds. By determining the percentage of tumor load reduction, the time between start of therapy and regrowth of tumor, and the time from maximal tumor load reduction to the regrowth of the tumor to a diameter of 10 mm (Table 1), we concluded that maximal antitumor activity was reached at a dose of 0.5 to 1.0 mg of either platinum compound per kg of body weight.

In contrast to the comparable antitumor activity of both compounds, a difference was observed in toxicity between cis-DDP and TNO-6, based on survival and nephrotoxicity. As shown in Table 2, TNO-6 was better tolerated than was cis-DDP, the former compound being less toxic by a factor of 2 after repeated administration. Regarding the nephrotoxicity, the difference between cis-DDP and TNO-6 was even more pronounced. Histologically marked to severe tubular kidney lesions with increased blood urea and creatinine values were observed after repeated 1.0-mg/kg doses of cis-DDP. At the 0.5-mg/kg level, the lowest dose of cis-DDP tested for nephroxicity, mild to moderate kidney lesions were still observed. In contrast, no indication of nephrotoxicity was observed with the methods used with TNO-6 at dose levels up to 2.0 mg/kg, a result which is in agreement with the study of Rose et al. (19) in the mouse; they found higher blood urea nitrogen levels after treatment with cis-DDP but not with TNO-6. Regarding the nephrotoxic effect of cis-DDP, our results largely confirm earlier data (8, 10, 11, 13, 24). However, in the present study, glomerular changes, which consisted of cell hypertrophy of the parietal layer of Bowman’s capsule and glomerular atrophy, were also observed in low frequency. The latter finding, combined with tubular atrophy, indicates nephron atrophy.

Results of the present study, showing in the same strain of rats an equal antitumor activity obtained with TNO-6 on a mg/kg basis with cis-DDP and a lower (nephro)toxicity for TNO-6, indicate that TNO-6 might be a good candidate for avoiding nephrotoxicity during platinum therapy.

Both cis-DDP and TNO-6 dissolved in 5% glucose water induced a rapid tumor load reduction. At the time of maximal regression of the primary tumor, vital tumor cells were still observed upon histological examination. At this time, no tumor cells were seen in the livers of the majority of animals. Thus, metastases observed at the terminal stage of animals with tumor recurrence apparently originated from the tumor recurrence. Tumor recurrence was observed in all experiments. The appearance of recurrences was not dose related; it occurred at high and intermediate dose levels of both cis-DDP and TNO-6.

Tumor recurrences were not only resistant to the homologous platinum compound but also cross-resistant to the other platinum compound. This result is at variance with the results obtained with L1210/DDP-resistant cell lines using TNO-6 (19) or other platinum analogues (6). Doxorubicin, a drug of which the cytotoxicity (like that of cis-DDP) is also based on interaction with DNA, induced regression of tumors that recurred after prolonged (lasting 3 weeks) platinum therapy. The high frequency of recurrences was not caused by alteration of the host, as sublines isolated from these recurrences remained insensitive to platinum treatment in freshly inoculated animals. Thus, the resistance to cis-DDP and TNO-6 is at the level of the tumor cell. Whether this fact is due to a selection of an insensitive tumor cell out of a heterogeneous tumor cell population or to an induced resistance remains to be established. Because our IgM immunocytoma model in the LOU/M rat is sensitive to platinum compounds and because resistant sublines are easily isolated, this model could be a supplement to the L1210 leukemia model in the mouse, a tumor model which is widely accepted for studying antitumor activity and resistance (6, 9, 22).

From the results of the present study, we conclude that the IgM immunocytoma model in the LOU/M rat is a good model in which to study the antitumor activity of platinum compounds. From the comparison of the antitumor activity and nephrotoxicity of TNO-6 and cis-DDP, when both drugs are dissolved in 5% glucose water, we conclude that both drugs have the same antitumor activity. In contrast to the effects of cis-DDP, however, no nephrotoxicity was observed with TNO-6.

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Fig. 1. Inoculation site of primary tumor in animal treated with cis-DDP (1.0 mg/kg) which showed complete tumor regression upon clinical examination. Note the massive accumulation of macrophages in the subcutis and the small area with necrotic tumor cells. H & E, x 200.

Fig. 2. Rat kidney of animal treated with cis-DDP (1.0 mg/kg) twice weekly for 7 weeks (A and B) and of control animal (C). A, extensive dilation with epithelial regeneration of the proximal tubuli. B, atrophic proximal tubuli with thickened basal lamina probably due to tubular collapse and interstitial cellular infiltrate. Note also the glomerular atrophy. C, kidney of control animal. Glycolmethacrylate embedding, periodic acid-silver methenamine, x 200.
Antitumor Activity, Induction of Cross-Resistance, and Nephrotoxicity of a New Platinum Analogue, \textit{cis}\-1,1-Diaminomethylcyclohexaneplatinum(II) Sulfate, and of \textit{cis}\-Diamminedichloroplatinum(II) in an Immunocytoma Model in the LOU/M Rat


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