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Secondary Screening of Platinum Compounds in Human Ovarian Cancer Xenografts in Nude Mice*

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Abstract—*Five TNO platinum compounds were evaluated for antitumor activities in two human ovarian carcinoma tumor lines grown in nude mice. The most active drug, TNO-38, was investigated in five additional lines with a known range of sensitivity to cisplatin. None of the new compounds showed superior activity to cisplatin. The slightly lower activity of TNO-38 as compared to the parent compound was reproducible in all tumor lines. Besides the similarity in the antitumor activity, a remarkable correspondence in platinum distribution and retention at 24 hr of TNO-38 and cisplatin could be observed. Chromatographic analysis of the compounds in their injection fluids showed single peaks for TNO-26 and TNO-38. The degradation products of the latter drugs may have affected their activity and toxicity. These human ovarian cancer xenografts may offer a reliable screening model for selection of a cisplatin analog with a higher therapeutic index or without cross-resistance for treatment in ovarian cancer.*

INTRODUCTION

NEW ANTICANCER agents usually enter phase I trials, if they have been proven effective in the NCI preclinical screening panel. This panel comprises several well-known murine experimental tumor systems. Recently the NCI incorporated three human xenograft lines derived from lung cancer, breast cancer and colon cancer in an attempt to improve the predictive value of new drugs in the corresponding tumor type [1, 2]. Retrospective analysis of screening and patients' results showed a poor correlation, because of which additional approaches such as the inclusion of *in vitro* assays with human malignant cell lines have recently been discussed [3, 4]. This new approach, which is disease-oriented rather than drug-oriented, has yet to be evaluated for its specificity. Considering the latter aspect, a number of human xenografts derived from the same tumor type growing in athymic nu/nu mice (nude mice) or artificially immune-deprived mice may even be more useful to predict the clinical activity of new anticancer agents in specific tumor types [5–9].

Cisplatin is active in a variety of human cancers including ovarian cancer [10]. A series of analogs have subsequently been synthesized in order to select a compound with a higher therapeutic index or with lack of cross-resistance to cisplatin [11]. In this respect, carboplatin and iproplatin, which have myelosuppression rather than nephrotoxicity as their dose-limiting toxicity, recently entered clinical trials [12–15].

From antitumor activity ranking of new platinum compounds in murine tumors [16, 17] we selected five TNO compounds with overall activity comparable to cisplatin. These analogs were further screened for their activity in a variety of human ovarian carcinoma tumor lines grown in nude mice. Our purpose to find a compound with potentially superior antitumor properties to cisplatin in ovarian cancer also included chromatographic analysis of the experimental drugs in solution and determination of the platinum concentrations in serum and tissues of the most active compound and cisplatin at 24 hr in tumor-bearing mice.

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MATERIALS AND METHODS

Animals and tumor lines

Female B10 LP/cpb athymic nude (nu/nu) mice (TNO, Zeist, The Netherlands) were maintained in cages with paper filter covers. Cages, covers, bedding, food and water were changed and sterilized weekly. Animal handling was done in a laminar down-flow hood.

The following human ovarian carcinoma tumor lines were used: Ov.Pe and Ov.He, which are both moderately differentiated mucinous adenocarcinomas; MRI-H-207, an undifferentiated (adeno)carcinoma; Ov.Gl, a poorly differentiated serous adenocarcinoma; Ov.Ri(C) and FKo, which are both moderately differentiated serous adenocarcinomas; Ov.Me, a carcinosarcoma of the ovary. MRI-H-207 was kindly provided by Dr A. E. Bogden, Mason Research Institute, Worcester, MA, U.S.A. and FKo by Dr W. Kleine, Albert-Ludwigs University, Freiburg, F.R.G.

The tumor lines were grown in nude mice and maintained by serial transplantation. Tumor fragments with a diameter of 2-3 mm were transplanted s.c. into both flanks of 8- to 10-week-old animals. In serial passages, the tumors maintained their histological appearance of origin and kept a consistent growth rate. The pattern of human lactic dehydrogenase isoenzymes could be detected persistently in tumor supernatants.

Treatment and evaluation

Besides cisplatin (Platinol, powder containing 10 mg cisplatin and 90 mg sodium chloride; Bristol Myers, Weesp, The Netherlands), five new platinum compounds (Fig. 1) were studied (Dr H. A. Meinema, Institute of Applied Chemistry, TNO, Utrecht, The Netherlands). All drugs were dissolved in distilled water: cisplatin 1 mg/ml, TNO-25 15.5 mg/ml, TNO-26 5 mg/ml, TNO-27 1 mg/ml, TNO-32 0.83 mg/ml and TNO-38 2 mg/ml. For TNO-25, TNO-26 and TNO-32 a clear solution was only obtained after low-power sonication for 4 hr at room temperature. Single agents were injected i.v. weekly at a dose approximating maximal toleration. At this maximum tolerated dose (MTD) the mice were allowed a weight loss of 10–15% in the first week.

In each experiment, groups of 10–12 mice were randomized into 5–7 animals for treatment and 4–5 animals for control. At the start of treatment all tumors had a volume of 50–150 mm^3. Tumors were measured weekly in three dimensions by the same observer with a slide caliper. The volume was calculated by the equation of length × width × thickness × 0.5, and expressed in mm. Because of the variation in size at the initiation of

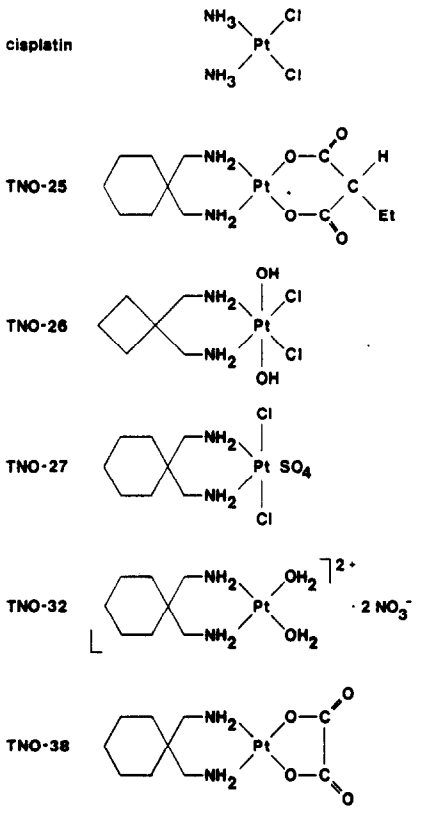

Fig. 1. Chemical structures of cisplatin and platinum compounds used in the experiments; cisplatin, cis-diamminedichloroplatinum(II); TNO-25, (1,1-bis(aminomethyl) cyclohexane)(2-ethylmalonato)platinum(II) TNO-26, (1,1-bis(aminomethyl)cyclobutane) dihydroxydichloroplatinum (IV); TNO-27, (1,1-bis(aminomethyl)cyclohexane)dichlorosulfatoplatinum(IV); TNO-32, diaqua(1,1-bis(aminomethyl) cyclohexane)platinum(II)dinitrate; TNO-38, (1,1-bis(aminomethyl)cyclohexane)oxalatoplatinum(II).

treatment, volumes were converted to the initial tumor volume. The relative tumor volume was expressed by the formula V_t/V_0, where V_t is the volume at any given day and V_0 the volume at the start of treatment. The ratio of the mean relative volume of treated tumors over that of control tumors multiplied by 100, $(T/C)\%$, was calculated at each evaluation. For each experiment the lowest value within 5 weeks after the last drug administration was considered the optimal ratio. Dead animals within 2 weeks after the final injection were considered as toxic deaths and were excluded from the study. Complete remission was defined as the total disappearance of the tumors without regrowth within the next month.

Chromatography

TNO platinum compounds dissolved in their injection fluids at the concentration used for treatment were analyzed by reversed-phase high-performance liquid chromatography (HPLC) as described previously [18, 19]. The platinum complexes were detected by differential pulse amperometry at a hanging mercury drop

electrode at -540 mV vs Ag/AgCl using a pulse of -100 mV every 0.5 sec and a low-pass filter of 0.3 sec.

Platinum analysis

Groups of six mice with a tumor volume of 400–1200 mm^3 in each flank were treated with a single i.v. injection of TNO-38 7 mg/kg. At 24 hr the animals were bled from the axillary vein under ether anesthesia. Thereafter, the liver, kidneys, brain and tumors were removed and weighed. Total platinum concentrations were analyzed after pretreatment of the samples by flameless atomic absorption spectrometry as described earlier [5].

Statistics

Antitumor activity of platinum compounds was evaluated by Student's *t* test.

RESULTS

Antitumor activity

TNO platinum compounds were first studied for their activity in Ov.Pe and MRI-H-207, known as tumor lines for their difference in sensitivity to cisplatin [5]. For each drug various doses were applied to determine a weekly i.v. schedule at the MTD. The initial dose was derived from the acute i.p. LD_{50} dose in normal mice. The approximate MTD was measured by determining the toxicity expressed in weight loss in non-tumor-bearing nude mice.

The antitumor effect for the TNO compounds and cisplatin is shown in Table 1. In Ov.Pe the most active drugs were TNO-26 and TNO-27, but their activity was not superior to cisplatin. In MRI-H-207 all platinum compounds demonstrated activity, but only TNO-38 could achieve a

Table 1. *Activity of cisplatin and TNO platinum compounds in tumor lines Ov.Pe and MRI-H-207*

Tumor line	Compound	Dose (mg/kg i.v.)	Days of treatment	Optimum (*T/C*)%*	(day)	Toxic deaths†
Ov.Pe	cisplatin	4	0, 7	45‡	(36)	1/6
	cisplatin	4	0, 7, 14	52‡	(37)	3/6
	TNO-25	80	0, 7, 14	87	(42)	0/7
	TNO-26	20, 10, 10	0, 7, 14	57	(40)	1/6
	TNO-26	20, 10, 10	0, 7, 14	43‡	(34)	1/6
	TNO-27	6	0, 7, 14	49‡	(41)	0/7
	TNO-32	3.5	0, 7, 14	56	(42)	1/7
	TNO-38	9, 7	0, 7	84	(22)	3/6
	TNO-38	7	0, 7, 14	85	(28)	1/6
MRI-H-207	cisplatin	4	0, 7	CR‡	(24)	0/6
	cisplatin	4	0, 7	CR‡	(34)	1/5
	TNO-25	80, 60	0, 7	0.06‡	(29)	1/7
	TNO-25	80, 60, 60	0, 7, 14	0.32‡	(26)	1/7
	TNO-25	100, 60	0, 7	1.06‡	(32)	0/7
	TNO-25	80	0, 7	4.1‡	(20)	0/5
	TNO-26	20, 10	0, 7	0.47‡	(23)	1/6
	TNO-26	20, 10, 10	0, 7, 14	0.005‡	(26)	2/6
	TNO-27	7	0, 7	5.1‡	(17)	2/6
	TNO-27	9	0, 15	5.5‡	(26)	1/7
	TNO-27	6	0, 7	8.8‡	(23)	1/6
	TNO-27	6	0, 7, 14	0.3‡	(20)	2/6
	TNO-32	6	0, 14	0.98‡	(18)	3/7
	TNO-32	5	0, 11	7.6‡	(22)	3/7
	TNO-32	6, 4	0, 14	1.5‡	(20)	2/7
	TNO-32	4	0, 7	1.4‡	(19)	2/6
	TNO-32	4	0, 7, 14	5.1‡	(16)	2/7
	TNO-32	3.5	0, 7	42‡	(13)	2/6
	TNO-38	9, 7	0, 7	CR‡	(27)	1/6
	TNO-38	8	0, 7	0.12‡	(34)	0/5
	TNO-38	7	0, 7	CR‡	(24)	1/6
	TNO-38	7	0, 7	0.59‡	(17)	1/6

*(*T/C*)%, optimal value of the mean of relative volume in treated tumors/mean of relative volume in control tumors × 100, calculated within 5 weeks after completing chemotherapy; CR, complete remission.

†Toxic deaths counted within 2 weeks after completing chemotherapy.

‡Significant difference ($P < 0.02$) between treated and control tumors evaluated by Student's *t* test.

complete remission similarly to cisplatin. The varying schedules for the same compound in MRI-H-207 almost always resulted in a (*T/C*)% related to the amount of drug administered.

Because of the activity of TNO-38 in MRI-H-207, we further investigated this compound in five different ovarian carcinoma tumor lines, in which the effect was compared with that of cisplatin (Table 2 and Fig. 2). Cisplatin was either used at 4 or 5 mg/kg weekly i.v. × 2 or 3 and TNO-38 at 7 or 8 mg/kg weekly i.v. × 2 or 3. From Table 2 it can be concluded that cisplatin reached a *T/C* below 50% in five of seven tumor lines: MRI-H-207, Ov.He, Ov.Gl, Ov.Ri(C) and Ov.Me. TNO-38 reached a *T/C* below 50% in only three of seven tumor lines: MRI-H-207, Ov.Gl and Ov.Ri(C). The overall activity of TNO-38 was lower than that of cisplatin (Fig. 2).

Several mice were killed for histological examination of the liver, kidneys and lungs after treatment with TNO platinum compounds (6–13 mice for each drug). Previous studies in mice treated with cisplatin at MTDs had not shown specific histological changes. No toxic effects could be detected in TNO-25 and TNO-38 treated mice. However, various animals treated with TNO-26 or TNO-27 had necrotic areas throughout the liver without mononuclear or polymorphonuclear infiltration, and in several cases kidneys showed concomitant necrosis of tubuli with the presence of protein casts. In mice treated with TNO-32 liver toxicity was not detected, but in one mouse kidneys contained protein casts without cellular infiltration. Because not all mice were examined and the time of autopsy varied with regard to the final injection, no conclusions may be drawn on the frequency and the intensity of the histological changes and on the relation of changes to the dose and schedule of treatment.

Chromatography

In order to gain insight in the presence of degradation products, TNO platinum compounds in their injection fluids were subjected to HPLC analysis (Fig. 3). The respective chromatograms show a single peak for TNO-26 and TNO-38. Several peaks are found for TNO-25, TNO-27 and TNO-32 in aqueous solution. The single peak of cisplatin obtained by HPLC with u.v.-detection is included for reasons of comparison. The peak of TNO-38 contained 100% of the injected amount of platinum, as measured by atomic absorption spectrometry of the fractionated eluate. Because the other TNO compounds were discarded from further studies for potential clinical use, no attempts were made to measure the platinum concentration in the fractionated eluate or to identify the various chromatographic peaks.

Platinum distribution and retention

To obtain further information on the pharmacologic relationship of TNO-38 in comparison with cisplatin, platinum distribution was measured in Ov.Pe and MRI-H-207 tumor-bearing mice. Table 3 summarizes platinum concentrations in serum and tissues at 24 hr after a single i.v. MTD injection of TNO-38 in this study and those obtained at 24 hr after a single i.v. MTD dose of cisplatin from a previous study [5]. Platinum distribution for both drugs was remarkably similar, with higher concentrations in the liver and kidneys than in tumors. Tumor platinum concentrations did not predict drug activity, because the effects of cisplatin and TNO-38 were remarkably lower in Ov.Pe tumors than in MRI-H-207 tumors. Retention of platinum per complete organ was calculated as the percentage of the administered dose (Table 4). Platinum

Table 2. Activity of cisplatin and TNO-38 i.v. in seven human ovarian carcinoma tumor lines in nude mice

Tumor line	Cisplatin Dose (mg/kg)	Days of treatment	Optimum (*T/C*)%*	(day)	Toxic deaths†	TNO-38 Dose (mg/kg)	Days of treatment	Optimum (*T/C*)%*	(day)	Toxic deaths†
Ov.Pe	4	0, 7, 14	52‡	(37)	3/6	7	0, 7, 14	85	(28)	1/6
MRI-H-207	4	0, 7	CR‡	(24)	0/6	7	0, 7	CR‡	(24)	1/6
Ov.He	5	0, 7, 14	44‡	(36)	0/7	8	0, 7, 14	53‡	(27)	0/6
Ov.Gl	5	0, 7	23‡	(35)	1/6	8	0, 7, 14	39‡	(46)	0/7
Ov.Ri(C)	5	0, 7	7.7‡	(25)	3/6	7	0, 8, 14	48‡	(29)	0/5
FKo	5	0, 7	86	(27)	2/6	7	0, 8	77	(22)	0/6
Ov.Me	4	0, 7, 14	36‡	(21)	0/6	7	0, 7, 14	63	(21)	2/6

*(*T/C*)%, optimal value of the mean of relative volume in treated tumors/mean of relative volume in control tumors × 100, calculated within 5 weeks after completing chemotherapy; CR, complete remission.

†Toxic deaths counted within 2 weeks after completing chemotherapy.

‡Significant difference ($P < 0.05$) between treated and control tumors evaluated by Student's *t* test.

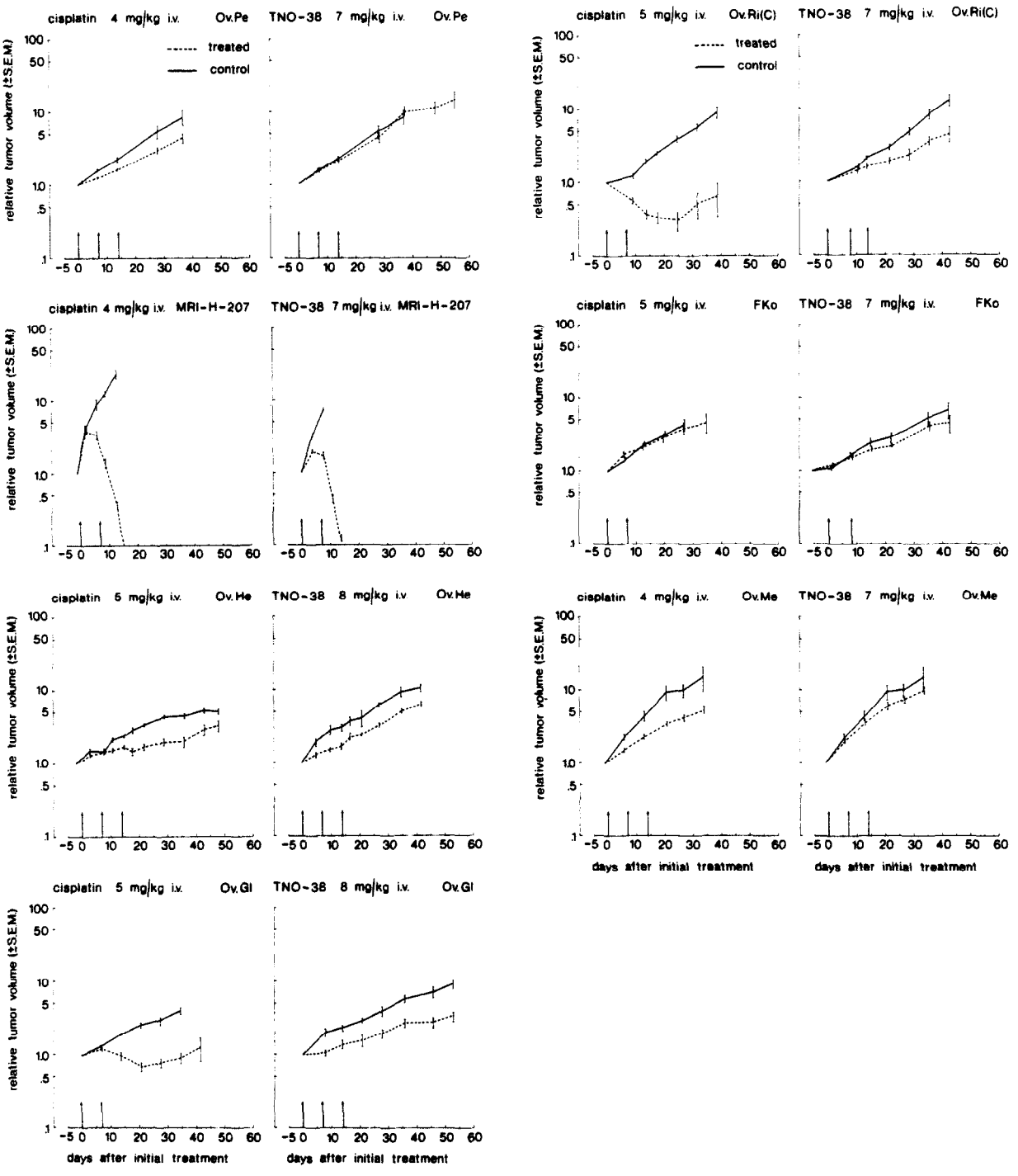

Fig. 2. Treatment results of cisplatin and TNO-38 injected i.v. × 2 or 3 in seven human ovarian carcinoma tumor lines, as compared to controls. The relative tumor volume is the tumor volume at any given day V_t/ the volume at the start of treatment V_0; — — — —, mean of relative volumes of treated tumors; ——, mean of relative volumes of control tumors.

retention after TNO-38 in kidneys and tumors was lower than after cisplatin, but total retention for both drugs was similar. No differences in retention of TNO-38 were observed between mice bearing Ov.Pe or MRI-H-207, as was also shown for cisplatin.

DISCUSSION

Human ovarian cancer xenografts grown in nude mice appear to be useful for the prediction of the potential clinical activity of new platinum compounds as compared to cisplatin in this particular tumor type. Recently, we reported data on the activity of carboplatin, JM-40 (*cis*-ethylenediamino platinum(II)malonate), iproplatin and spiroplatin (TNO-6, aqua-(1,1-bis(aminomethyl)cyclohexane)sulphatoplatinum (II)) in two human ovarian carcinoma tumor lines, which findings suggested a correlation with preliminary clinical results. The additional TNO

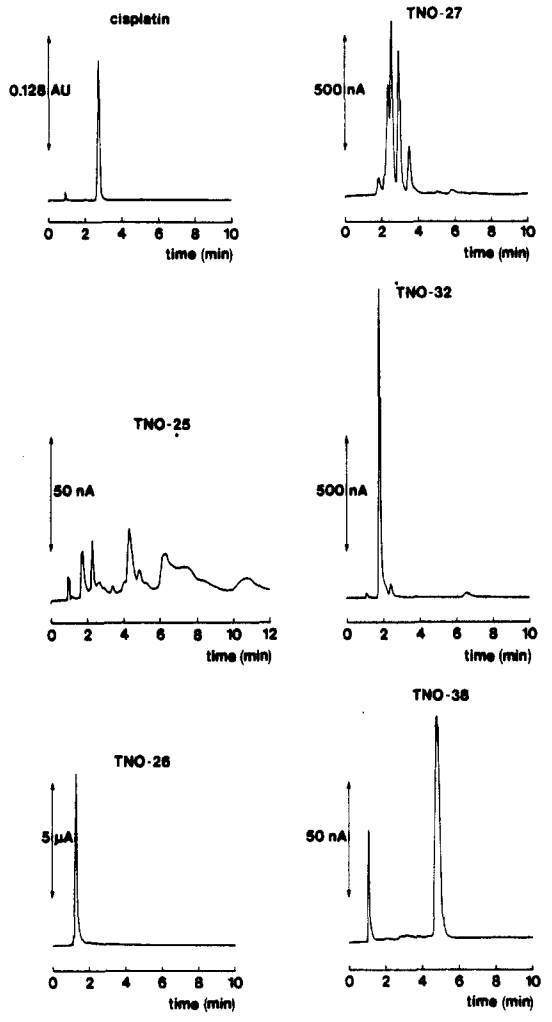

Fig. 3. Chromatograms detected by differential pulse amperometry of TNO platinum compounds dissolved in distilled water at the concentration used for treatment. Peaks at 1 min *represent blank sample. TNO-38 was diluted* 1:20 *to prevent overloading of the HPLC system. For reasons of comparison a chromatogram of cisplatin* (0.03 mg/ml, 214 nm *u.v.-detection) is included; AU, absorption units.*

compounds in the present study were selected because of their high overall activity in experimental murine tumor systems. None proved to be superior in activity or was cross-resistant to cisplatin. For three analogs it was shown on the chromatogram that the aqueous solution contained several peaks, most likely from degradation products. These products may have affected the antitumor activity and the toxicity. Finally, the platinum distribution at 24 hr after administration of TNO-38, which was the most active drug, was remarkably similar to that of cisplatin. The total percentage of platinum retained in complete tissues was similar for both drugs.

In clinical studies carboplatin and iproplatin have been shown to be active in patients with advanced ovarian cancer [12, 15]. We demonstrated that the cisplatin-sensitive MRI-H-207 tumor line was equally sensitive to these analogs [5]. In the present study the TNO platinum compounds also possessed antitumor properties in these xenografts, but with regard to results in Ov.Pe xenografts none appeared to be superior or cross-resistant to cisplatin. The observed antitumor effects appeared highly reliable, because with the same drug reproducible results could be obtained in MRI-H-207. Furthermore, in a variety of human ovarian carcinoma tumor lines with different sensitivity patterns to cisplatin, TNO-38 at MTD always showed smaller effects than observed with the parent drug. The slight distinction in activity between TNO-38 and cisplatin may be partially related to dose-limiting side-effects in the host. The consistency of the data indicates the high standards of these xenografts, which is an essential prerequisite before any conclusion can be drawn on the potential activity of a new analog in ovarian cancer.

Besides the antitumor screening data, a second selection criterion for clinical investigation is the stability of a new drug in infusion fluids. Certain platinum analogs tend to be unstable in solution, while their degradation products may influence the antitumor activity and toxicity. For instance, spiroplatin solubilized in iso-osmotic sodium sulphate contained less hydrolysis products than in 5% glucose [18]. Rats treated with the first solution showed less toxicity as measured by proteinuria and body weight than animals treated with spiroplatin in 5% glucose, suggesting that toxicity had been caused by the hydrolysis

Table 3. Platinum concentrations in tissues and serum 24 hr after a single injection of cisplatin or TNO-38 in nude mice bearing Ov.Pe or MRI-H-207*

Tumor line	Compound	Dose (mg/kg i.v.)	*n*	Liver	Kidneys	Brain	Tumors	Serum
Ov.Pe	cisplatin	5	6	2.58 ± 0.24	3.67 ± 0.40	0.13 ± 0.01	0.81 ± 0.15	0.27 ± 0.02
	TNO-38	7	6	2.43 ± 0.31	3.08 ± 0.35	N.D.	0.70 ± 0.19	0.33 ± 0.03
MRI-H-207	cisplatin	5	7	3.29 ± 0.79	5.45 ± 0.95	0.14 ± 0.04	0.68 ± 0.13	0.36 ± 0.06
	TNO-38	7	6	2.24 ± 0.38	2.70 ± 0.36	N.D.	0.34 ± 0.05	0.30 ± 0.05

*Concentrations in tissues in µg/g wet weight and in serum in µg/ml (mean ± S.D.); *n*, No. of animals; N.D., not detectable.

Table 4. Retention of platinum in complete tissues (% of dose) 24 hr after a single i.v. injection of cisplatin or TNO-38*

Tumor line	Compound	Dose (mg/kg)	Dose Pt† (mg/kg)	Liver	Kidneys	Brain	Tumors	Total‡
Ov.Pe	cisplatin	5	3.25	5.11 ± 0.79	1.94 ± 0.20	0.08 ± 0.02	2.03 ± 1.87	9.72 ± 2.40
	TNO-38	7	3.19	5.13 ± 0.42	1.26 ± 0.12	N.D.	0.13 ± 0.03	6.52 ± 0.44
MRI-H-207	cisplatin	5	3.25	5.66 ± 0.82	1.62 ± 0.17	0.08 ± 0.02	0.86 ± 0.76	7.67 ± 1.32
	TNO-38	7	3.19	4.69 ± 0.56	1.13 ± 0.17	N.D.	0.20 ± 0.07	6.06 ± 0.62

*Mean ± S.D.; N.D., not detectable.
†Amount of platinum administered per compound.
‡Total percentage of platinum retained in the liver, kidneys, brain and tumors.

products. For cisplatin, addition of sodium chloride to solutions of cisplatin was shown to reduce hydrolysis of the drug, which resulted in less toxicity in rats [20]. In the case of JM-40, drug solutions appeared to be more stable in 5% glucose than in 0.9% sodium chloride [21]. Of the platinum compounds evaluated in the present study, solubility of TNO-25, TNO-26 and TNO-32 was poor. TNO-25, TNO-27 and TNO-32 showed degradation products in aqueous solution. Obviously, formulation procedures should include chemical characterization of a new platinum compound in various injection fluids prior to clinical investigation.

Calculation of platinum retention in complete tissues in tumor-bearing mice demonstrated a significantly similar organ clearance of platinum at 24 hr after TNO-38 and cisplatin. This may indicate a similar reactivity of both compounds with tissue proteins, because platinum still present at 24 hr after administration may be considered irreversibly bound. The comparable reactivity may reflect comparable antitumor effects. Whether preclinical comparative pharmacokinetic studies of platinum compounds are of importance to gain insight in the antitumor activity and the toxicity to be expected may be an interesting question to be answered in future investigations.

In conclusion, the present study shows that our series of human ovarian carcinoma tumor lines offers a reliable model for screening the activity of new platinum compounds. While TNO-38 appeared to be an active drug, its efficacy was consistently lower than that of cisplatin. Because our previous study using four different analogs demonstrated that the observed activity related well with recent clinical findings, this human tumor model seems to have much value for selecting a cisplatin analog of potential interest in ovarian cancer.

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