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Toxicology and Pharmacokinetics of (1,1-Bis(Aminomethyl)Cyclohexane)Oxalatoplatinum(II) (TNO-38)

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Abstract—*Preclinical studies on toxicology and pharmacokinetics were performed for (1,1-bis(aminomethyl)cyclohexane)oxalatoplatinum(II) (TNO-38) in rats and a dog after* LD_{10} *and* LD_{50} *assessment in mice. In drug-treated rats, ura and creatinine concentrations were* 1,4–1.9 *times those in control rats. Histopathology showed necrosis of tubular epithelium of the kidneys, which was comparable to damage observed after treatment with cisplatin (CDDP), and extensive necrosis of crypt epithelium, especially in the ileum.*

Similar to CDDP, TNO-38 was emetic in the dog. Non-specific subacute inflammatory changes were observed in the ileum. Renal damage was much less pronounced.

Half-lives of distribution and elimination were 6.2 min *and* 5.2 days, *respectively. The cumulative excretion of* Pt *in urine over* 1 *and* 7 days *after drug treatment was* 38.3 *and* 49.3% *of the dose, respectively. Twelve weeks after drug administration,* Pt *concentrations were highest in kidneys and liver.*

TNO-38 is adequately water soluble. Its reported antitumour activity is consistently lower than that of CDDP. The drug's toxicity was, in general, comparable to that of CDDP. Its pharmacokinetic profile was very similar to that of CDDP. It is concluded that TNO-38 should probably not be further evaluated in clinical studies.

INTRODUCTION

In the search for derivatives of cisplatin (CDDP) with a higher therapeutic ratio, hundreds of platinum compounds have been tested [1–4]. Among the series of compounds from the TNO Institute for Applied Chemistry, (1-1-bis(aminomethyl) cyclohexane)oxalatoplatinum(II) (TNO-38) has been tested by Rose and Bradner [5]. Its activity against leukaemia L1210, melanoma B16, M109 lung and C26 colon tumour was comparable to that of CDDP. The compound was selected by Boven *et al.* [6] for secondary screening in human ovarian cancer xenografts in nude mice. At its maximum tolerated dose, TNO-38 showed slightly smaller effects than observed with the parent drug in all 7 tumour lines used.

From a large series of platinum compounds with interesting activity and absence of elevated BUN values only TNO-38 had adequate water solubility [5]. For these reasons the drug was selected for preclinical studies on toxicology and pharmacokinetics to investigate whether this compound could be a candidate for phase I clinical trials. The studies were carried out in mice, rats and a dog.

MATERIALS AND METHODS

Drug

TNO-38 was supplied by the Institute for Applied Chemistry TNO, Utrecht, The Netherlands (Dr. H.A. Meinema). Its chemical structure is presented in Fig. 1. The drug was dissolved in 5% glucose by magnetic stirring for 1–2 hr at room temperature. This solution was tested for stability

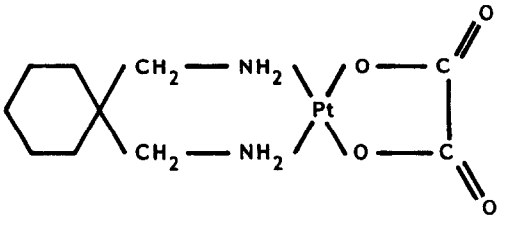

Fig. 1. Chemical structure of TNO-38.

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of TNO-38 by high-performance liquid chromatography [7]. The chromatogram showed only one peak containing 100% of the injected amount of platinum. In our hands, the maximum solubility of the drug was 2.4 mg/ml. The volumes of drug injections were 0.01 ml/g (mice), 0.005–0.01 ml/g (rats) and 1 ml/kg (dog) body wt.

Mice

The LD_{10} and LD_{50} after single i.v. administration of the drug was determined in male (C57BL/Rij × CBA/Rij)F1 hybrid mice, 11–19 weeks of age with body wts of 23–33 g. The dose range of 8–22 mg/kg was covered with dose increments of 1–2 mg/kg using five mice for each dose level. Control mice were injected with 5% glucose. The LD_{10} and LD_{50} values were calculated by probit analysis.

Body wts of the mice were determined 3 times per week for 30 days.

Rats

The LD_{50} after single i.v. administration of the drug was estimated in male (WAG/Rij × BN/Rij)F1 hybrid rats, 40–53 weeks of age with body wts of 326–466 g. The dose range of 4–22 mg/kg was covered with dose increments of 2 mg/kg using at least two rats per dose. Control rats were injected with 5% glucose.

Body wts of the rats were determined 3 times per week for 30 days, and weekly thereafter until day 60.

Two rats, 15 days after treatment with 20 mg/kg TNO-38, as well as two control rats were bled from the heart under anaesthesia. Serum was prepared and the concentrations of Na, K, urea, creatinine, protein and glucose were determined. Autopsy was performed on these rats as well as on the rats dying from drug toxicity. Organs were fixed in buffered 4% formaldehyde for histopathology. Pancreatic tissue samples for transmission electron microscopy were fixed in 1% glutaraldehyde in 0.1 M cacodylate buffer.

Dog

Toxicology and pharmacokinetics were studied in only one male Beagle dog, 16 months of age with a body wt of 14.1 kg. The dog received three i.v. bolus injections of 1.8 mg/kg (42.9 mg/m^2) TNO-38 at intervals of 3 weeks. Each dose was about 60% of the mouse LD_{50}. Conversion of the dose per kg body wt to dose per m^2 body surface was made according to Freireich *et al.* [8].

The occurence of vomiting was recorded. After the first drug dose, there was a continuous observation for 9 hr. After the second and third dose the dog was observed only once per hour during 6 hr.

The body wt of the dog was recorded 5 days, 2 days and immediately before the first drug injection, daily during the following 10 days, twice a week until day 28 and at least weekly for the remaining part of the study.

Blood samples were collected 5 days, 2 days and immediately before the first drug injection, 3 times per week during the following 2 weeks and at several occasions thereafter for haematology and serum chemistry. Determinations of leukocytes and platelets were performed using an Electrozone/Celloscope (Particle Data, Inc., Elmhurst, IL). In the serum the concentrations of Na, K, urea, creatinine, protein and glucose were determined.

As an additional parameter for renal toxicity, the protein content of urine was assessed (Bradford's method). Urine was collected 5 and 2 days before the first drug injection, on the day of drug treatment and daily thereafter until day 20.

To facilitate comparison with other platinum derivatives studied in the U.S.A. where the NCI demands a dog, treated with the LD_{10} to be sacrificed on day 8 [9], it was decided to take a kidney biopsy 8 days after the first drug dose. The biopsy was processed for histopathology.

Six weeks after the last drug dose the dog was killed. Autopsy was performed. Organs were fixed in buffered 4% formaldehyde for histopathology.

For pharmacokinetic studies, blood samples were collected just before and at regular times (0, 10, 20, 30, 60, 90 min, 2, 2.5, 3, 4, 5, 6, 9, 20, 24 hr, 2, 3, 4, 5, 6, 7, 9, 12, 14 days) after the first administration of the drug. Up to 6 hr all samples were spun down immediately after withdrawal. Plasma was ultrafiltrated. RBCs were washed twice with saline. After 6 hr only plasma and packed RBCs were prepared. After the first injection urine was collected during the first 7 days in 24 hr portions.

At autopsy, blood and urine samples were collected as well as samples from kidney cortex and medulla, testis, liver, skin, lung, muscle, small intestine, pancreas, spleen and brain. All samples were frozen at −30° C until analysis. Platinum concentrations were determined by means of flameless atomic absorption spectrofotometry, as described earlier [10].

The following pharmacokinetic parameters were calculated: half-lives of platinum in plasma and plasma ultrafiltrate, the cumulative urine excretion during 1 week and retention of platinum in tissue samples after 2 months.

RESULTS

Mice

The LD_{50} of a single i.v. injection of TNO-38 in mice was 19.2 mg/kg (i.e., 64 mg/m^2). The LD_{10}

was 17.7 mg/kg (Fig. 2). Body wts of mice treated with 18 mg/kg (approximate LD_{10}) were minimal on day 6 [reduced by 4.8 g (17.8%)] (Fig. 3).

Rats

In rats, low i.v. doses of TNO-38 (4–10 mg/kg) showed no toxicity. Single i.v. doses of 12 and 14 mg/kg caused minor symptoms of illness. Doses of 16 ($n = 4$) and 18 mg/kg ($n = 4$) caused diarrhea from day 4 to 11. Reduction in body wts on day 6–7 for the 18 mg/kg group was 17.2%. The body wts returned to pretreatment values around day 50.

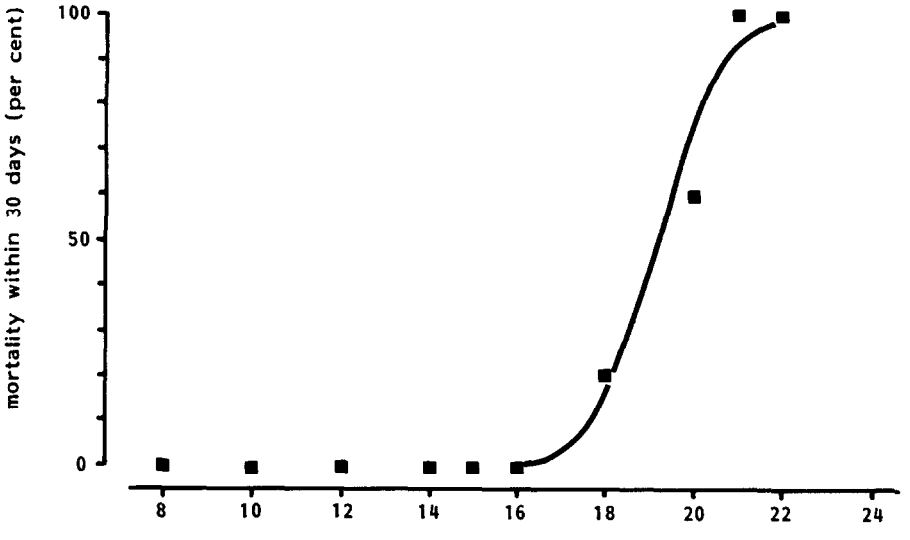

Fig. 2. Probit curve of mortality after treatment with single i.v. bolus injections of TNO-38. For each point five mice were used.

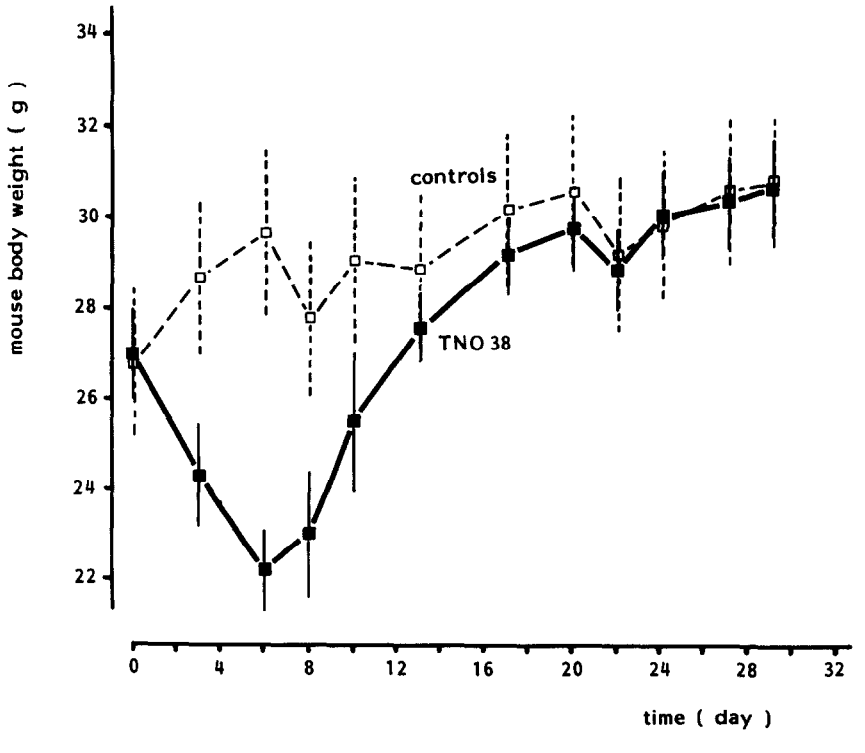

Fig. 3. Body wts of mice after treatment with a single i.v. injection of 18 mg/kg *TNO-38* (■) *and of control mice* (5% *glucose*, □). *For each point, five mice were used. Bars indicate S.D.*

Rats treated with doses of 16 mg/kg and above produced urine with a smell similar to that of diabetic rats and it had a high glucose content (Bili-labstix QLG, Ames).

Rats treated with 20 mg/kg ($n = 2$) were seriously ill with diarrhea from day 2 to 12. Maximum reduction in body wt was 21.7%. Recovery started on day 12. They were killed on day 15 for serum chemistry and histopathology. At the same time two control rats were sacrificed. Serum concentrations of Na, K, urea, creatinine, protein and glucose are shown in Table 1. For Na, K, protein and glucose, differences between treated and control rats were small or absent. For urea and creatinine, the concentrations in the treated rats were 1.4–1.9 times those in the control rats. At autopsy no evident macroscopic abnormalities were found.

Histopathology of the duodenum showed focal necrosis of Brunner's gland epithelium with some signs of atypical regenerative cells. A slight chronic inflammatory reaction with a focal distribution was seen. Diffuse in the kidneys there was some necrosis of tubular epithelium with atypical regeneration. Some signs of obstruction without conspicuous casts were seen. Focal chronic inflammatory reaction was noted in some of the kidneys.

Rats treated i.v. with 22 mg/kg TNO-38 ($n = 2$) died on day 8 or 9. Autopsy was performed. Their stomachs contained large amounts of food. The mucosa in the small curvature of the stomach and the intestines was haemorrhagic. Upon section, the pelvis of the kidneys contained a greenish mass of fibrin-like material.

Histopathology of the intestinal tract showed extensive necrosis of the (crypt) epithelium, especially in the ileum, and inflammatory changes. Some regeneration was noticeable. In the kidneys extensive tubular necrosis was seen with some early atypical regenerative cells present. Tubular obstructing casts were frequently observed.

At none of the dose levels were significant changes in the pancreas noted. The Langerhans islet cells, up to the level of transmission electron microscopy, were not noticeably altered.

Dog

In the dog, TNO-38 was emetic. The frequency of vomiting was similar to that in dogs treated with a comparable dose of CDDP. The dog's body wt showed only slight fluctuations. A maximum decrease of 8.5% was observed 12 days after the first drug dose. No haematotoxicity was observed: leukocyte and platelet counts remained within normal values.

All serum concentrations of Na, K, urea, creatinine, protein and glucose remained within normal values. From these data there were no indications for specific organ toxicities. The protein content in urine only showed a clear increase compared to the regular concentration 9 days after treatment (Fig. 4).

Histopathology of the kidney biopsy, taken on day 8 after the first drug dose showed some protein-like material in the urinary space of Bowman's capsule. However, no casts or cylinders were found. The tubular epithelium showed no evidence of damage or changes associated with platinum toxicity.

The dog was killed 6 weeks after the last drug dose. At autopsy, the ileum contained many *Ascaris* worms. There were no other macroscopic abnormalities. No evident histopathological change was seen with the exception of some non-specific subacute inflammatory changes found in the ileum.

Total platinum concentrations determined in plasma and plasma ultrafiltrate are shown in Fig. 5. Half-lives, as measured by the curve stripping procedure [11] are given in Table 2. To allow comparison with previously obtained values, calculated from plasma levels of samples collected over 5 days, curve stripping of the plasma concentrations was started from day 5. The half-life between days 5 and 14 ($t_{\frac{1}{2}\gamma}$) was calculated independently and did not differ from $t_{\frac{1}{2}\beta}$. No platinum could be detected in red blood cells at any time. The cumulative urinary excretion, expressed as percentage of the dose, was 38.3, 46.6 and 49.3% over 1, 5 and 7 days after the first i.v. bolus injection, respectively.

Retention of platinum in tissues at 6 weeks after the third dose is indicated in Table 3. To allow comparison with other platinum compounds, the values were also normalized by dividing the con-

Table 1. *Serum chemistry of rats* 15 *days after i.v. treatment with* 20 mg/kg *TNO*-38 (n = 2) *or glucose* (n = 2)

Treatment	Na (mmol/l)	K (mmol/l)	Urea (mmol/l)	Creatinine (μmol/l)	Protein (g/l)	Glucose (mmol/l)
20 mg/kg	146	4.6	15.0	82	54	5.9
TNO-38	143	4.7	10.5	77	57	6.6
5% glucose	143	5.5	7.6	47	64	7.2
	143	5.8	7.6	44	63	6.4

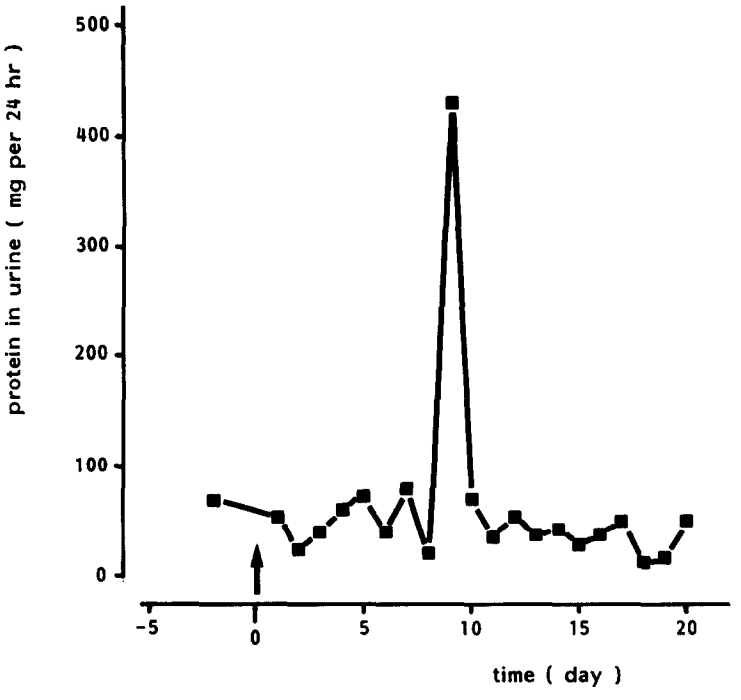

Fig. 4. Protein in urine of a dog after treatment with 1.8 mg/kg *TNO-38 i.v. Arrow indicates day of drug administration. On day 8 a kidney biopsy was taken.*

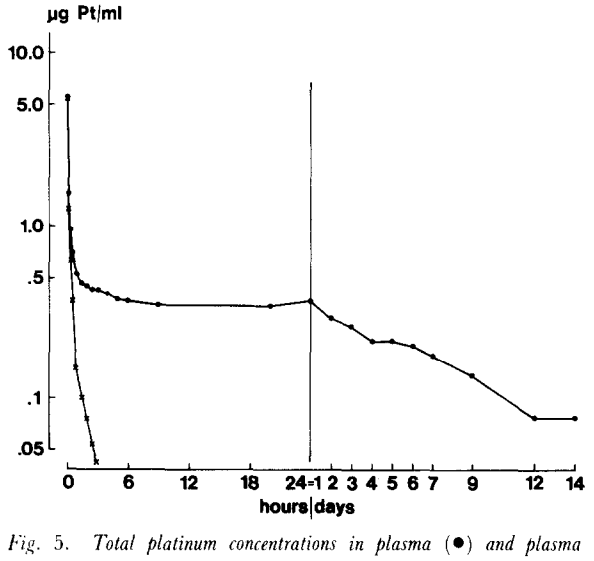

Fig. 5. Total platinum concentrations in plasma (•) and plasma ultrafiltrate (X) in one dog after an i.v.bolus injection of 1.8 mg/kg *TNO-38.*

Table 2. Half-lives of total platinum in plasma and plasma ultrafiltrate in one dog after an i.v. bolus injection of 1.8 mg/kg *TNO-38*

Body fluid	Parameter	Time interval	Half-life
Plasma U.F.	$t_{\frac{1}{2}\alpha}$	0 – 20 min	5.5 min
	$t_{\frac{1}{2}\beta}$	60 –180 min	68.8 min
Plasma	$t_{\frac{1}{2}\alpha}$	0 – 20 min	6.2 min
	$t_{\frac{1}{2}\beta}$	1 – 5 days	5.2 days
	$t_{\frac{1}{2}\gamma}$	5 – 14 days	5.4 days

Table 3. Platinum concentrations in tissues (µg Pt/g wet tissue and normalized by the dose D expressed in mg Pt/kg) *of one dog at 6 weeks after the third i.v. bolus injection of* 1.8 mg/kg *TNO-38*

Tissue	µg Pt/g w.t.	µg Pt/g w.t./D
Kidney cortex	2.25	2.72
medulla	0.75	0.91
Liver	1.21	1.46
Bile	N.D.	N.D.
Testicles	0.19	0.23
Lung	0.56	0.68
Muscle	0.37	0.45
Skin	0.87	1.05
Spleen	0.40	0.49
Pancreas	0.21	0.25
Intestine	0.30	0.37
Brain	N.D.	N.D.
Plasma	N.D.	N.D.

N.D.: not detectable

centration, expressed in µg Pt/g wet tissue, by the dose, expressed in mg Pt/kg. Highest retention was found in the kidney and liver, whereas no platinum could be found in bile, brain and plasma.

DISCUSSION

The platinum compound TNO-38 was about as active as CDDP against L1210, B16, M109 and C26 mouse tumours [5]. In the studies of Boven *et al.* [6] TNO-38 was the best of a series of platinum compounds, although its activity was consistently inferior to that of CDDP. In our own hands, TNO-

38 was slightly less effective than CDDP against the C22LR mouse osteosarcoma (unpublished results). According to Cleare [12] this oxalatoplatinum compound should be classified as a relatively unreactive agent with low toxicity. Indeed, Schurig (personal communication) observed no increase in the urea concentration in serum of mice treated with TNO-38. Besides, in contrast to CDDP, TNO-38 did not hydrolyse in aqueous solutions [6].

The acute toxicity in mice was determined since the mouse LD_{10} is used to calculate the safe starting dose in phase I clinical trials. For each dose level only five mice were used. It is questionable if these numbers are sufficient for the definitive establishment of the LD_{10} and LD_{50}. However, if no further clinical studies with TNO-38 are planned, one may wonder whether additional acute toxicity studies are necessary. The LD_{10} after a single i.v. injection of TNO-38 was 17.7 mg/kg. The LD_{50} was 19.2 mg/kg (Fig. 2). This value is higher than the 13 mg/kg i.p. found by Rose and Bradner [5]. The route of drug administration (i.v. vs. i.p.) and the difference in mouse strains may explain the differences.

The decrease in body wt of mice and rats after i.v. treatment with maximum tolerated doses of TNO-38 was 17.8 and 17.2%, respectively. Despite these severe reductions in body wts, the mice and rats recovered completely (Fig. 3).

LD_{50} was converted to rats on the basis of equal drug dose per m^2 body surface [8]. For the rats with an average body wt of 400 g, the expected LD_{50} was 7.8 mg/kg. The actual LD_{50}, however, was between 18 and 22 mg/kg.

The post-mortem finding of large amounts of food in the stomach of rats while the colons did not contain faeces, may be interpreted as paralysis of gastric emptying without loss of appetite as has been observed for CDDP [13]. In rats, the highest doses of TNO-38 caused significant renal toxicity. Biochemistry, confirmed by histopathology, principally indicated tubular damage.

In the dog, treatment with 1.8 mg/kg TNO-38 i.v. caused vomiting with a frequency similar to that of treatment with a comparable dose of CDDP (60% of the mouse LD_{50} in mg/m^2) [14]. Haematotoxicity was not observed. The NCI protocol for single dose toxicity study in dogs demands that a dog treated with the LD_{10} of a drug has to be killed on day 8 for full autopsy and histopathology [9]. To approach this protocol for nephrotoxicity, a kidney biopsy was taken on day 8. Histopathology showed some protein precipitates in Bowman's capsule, but no cylinders or evidence of glomerular damage were observed. Overall, there was no severe tubular damage, but day 8 may be too early to recognize typical pathology in nuclei of tubuli.

The day after taking the kidney biopsy the urine contained a high amount of protein (Fig. 4). This was supposed to be related to the biopsy trauma. This interpretation was confirmed by analysis of urine for the presence of Fe from lysed erythrocytes. On day 7 the urine contained trace amounts of Fe, much Fe on day 9 and very little on day 10. The Fe content of the urine thus parallels the protein content, being normal again from day 10 onwards.

Serum concentrations of urea and creatinine remained within normal values. From the serum chemistry there was no indication of nephrotoxicity which was confirmed by histopathology. The focal changes seen in the ileum are not generally associated with parasitic infection of the type found.

The pharmacokinetic data of TNO-38 obtained in this study were very similar to those for CDDP as found by Van der Vijgh *et al.* [15]. This also corresponds with the similarities found between CDDP and TNO-38 in the observations by Boven *et al.* [6].

In conclusion, TNO-38 is a water-soluble platinum analogue, and is reported to have a lesser antitumor activity than CDDP [5, 6]. In rats, it caused renal toxicity which biochemically and histopathologically resembled changes comparable to CDDP. The drug caused severe gastro-intestinal toxicity in rats. TNO-38 was emetic in the dog, similar to CDDP. Biochemically and histopathologically, renal damage was much less pronounced. It is concluded that TNO-38 is probably not a suitable candidate for clinical evaluation.

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