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Preclinical Studies on Toxicity, Antitumour Activity and Pharmacokinetics of Cisplatin and Three Recently Developed Derivatives*

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Abstract—*Preclinical studies were performed in mice, rats and dogs of* cis-*diamminedichloroplatinum(II) (CDDP) and its derivatives* cis-1,1-*di(aminomethyl) cyclohexane platinum*(II) *sulphate (TNO*-6), cis-*diammine*-1,1-*cyclobutanedicarboxylate platinum(II) (CBDCA) and* cis-*dichloro,*trans-*dihydroxybis-isopropylamine platinum(IV) (CHIP). In mice toxicity and antitumour activity were determined. All three derivatives were at least as toxic as CDDP for haemopoietic stem cells and were less active than CDDP against the mouse tumours leukaemia L*1210 *and osteosarcoma C22LR. Toxicology studies in rats revealed no renal toxicity after a single dose of TNO*-6. *Fractionated doses of TNO*-6 *and CBDCA did cause renal toxicity but less than CDDP. CHIP produced little or no kidney damage. In dogs, TNO*-6 (1.5 mg/kg) *produced more severe kidney damage—although this was reversible—than CDDP* (2 mg/kg). *Half-lives of distribution were* 4.0–5.1 min *for TNO*-6 *and* 9.7 min *for CDDP, while half-lives of elimination were* 3.6–6.6 days *and* 5.9 days *respectively. Plasma levels, normalized for the dose, were at least two times higher after TNO*-6 *than after CDDP. Twelve weeks after drug administration, plasma levels were undetectable, while tissue concentrations could still be measured. The platinum concentration in kidney cortex was higher after CDDP than after TNO*-6.

INTRODUCTION

IN 1972 *cis*-diamminedichloroplatinum(II) (CDDP) was introduced into clinical trials by the National Cancer Institute. It appeared to be a valuable drug for the treatment of several types of cancer, such as testicular and ovarian carcinomas [1–3]. Major side-effects of the drug are nausea and vomiting, renal toxicity and bone marrow damage [4]. The occurrence of these dose-limiting side-effects led to the search for derivatives with a higher therapeutic index. Many derivatives were developed and tested for activity and/or toxicity [5–7]. From the primary results derivatives were selected for more extensive studies [8]. In this communication results on activity and toxicity are presented for three such selected compounds. The studies were performed in mice, rats and dogs.

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MATERIALS AND METHODS

Drugs

In addition to the reference drug CDDP the following compounds were used.

TNO-6: *cis*-1,1-di(aminomethyl)cyclohexane platinum(II) sulphate; NSC 311056.

TNO-1: *cis*-dichloro-1.1-di(aminomethyl)cyclohexane platinum(II); NSC 269608.

CBDCA: *cis*-diammine-1,1-cyclobutanedicarboxylate platinum(II); NSC 241240; Johnson Matthey No. 8.

CHIP: *cis*-dichloro,*trans*-dihydroxybis-isopropylamine platinum(IV); NSC 256927; Johnson Matthey No. 9.

Both TNO-drugs were provided by Dr E. J. Bulten, Institute for Applied Chemistry TNO, Utrecht, The Netherlands. CBDCA and CHIP were obtained via Dr K. R. Harrap, London, who selected the drugs on the basis of antitumour and toxicity studies in mice and rats [8]. The chemical structures of the compounds are presented in Fig. 1. The drugs were dissolved in saline (CDDP and CBDCA) or 5% glucose (TNO-6 and CHIP) by magnetic stirring for 1–4 hr, and then used immediately.

Mice

The LD_{50} after single i.p. or i.v. administration of the drugs was determined in male (C57BL/Rij × CBA/Rij) F_1 hybrid mice, weighing 25–30 g.

The endpoint for haematotoxicity was survival of haemopoietic stem cells in the femur (CFU), and was determined by the spleen colony assay [9].

Comparative antitumour activity was determined in the leukaemia L1210 and the C22LR osteosarcoma models. In the former, cell survival was determined by colony formation *in vitro* after treatment *in vivo* [10]; in the latter model tumour growth delay was used as the endpoint for drug activity [11].

Rats

Increases in serum concentration of urea nitrogen (BUN) and creatinine were chosen as parameters indicating renal toxicity [12]. In our hands mice showed a considerable variation in control values. Sprague–Dawley and WAG/Rij rats of both sexes did not and were consequently used as experimental animals. BUN values were determined at 2, 4, 6 and 8 days after drug injection. Blood was taken from the abdominal aorta and serum was prepared for determination of BUN. On the basis of the preliminary results BUN and creatinine concentrations were also determined in rats 4 days after treatment with single i.p. doses of 10 and 6.7 mg/kg CDDP and 26.7 mg/kg TNO-6.

The same method was used in a following experiment in which CDDP, TNO-6, CBDCA and CHIP were compared. Freshly prepared solutions of the compounds were administered four times at intervals of 2 weeks. Each drug was administered at least four dose levels. The highest dose levels were 4.5, 4.5, 152 and 61 mg/kg for CDDP, TNO-6, CBDCA and CHIP respectively; these were expected to be lethal to the rats. Each lower dose was 2/3 of the previous one. Injections were given i.v. in a volume of 0.005 ml/g body wt. Due to its poor solubility, the higher doses of CBDCA were injected in a volume of 0.01 ml/g body wt. Control rats were injected with the solvents. During the whole period of the study the rats were regularly observed for general condition, abnormal behaviour, dysfunctions, etc. The body weight of all animals was recorded. Toxicity was studied at 4 days (early) and at 3 months (late) after the last of the four drug doses. Rats were killed by bleeding from the heart under anaesthesia. For the early toxicity study assays included observations at autopsy, haematology, determination of BUN and creatinine concentrations, and histopathology of the most important organs. For the late toxicity study assays included determination of the number of leukocytes, determination of BUN and creatinine concentrations, and histopathology of the kidneys.

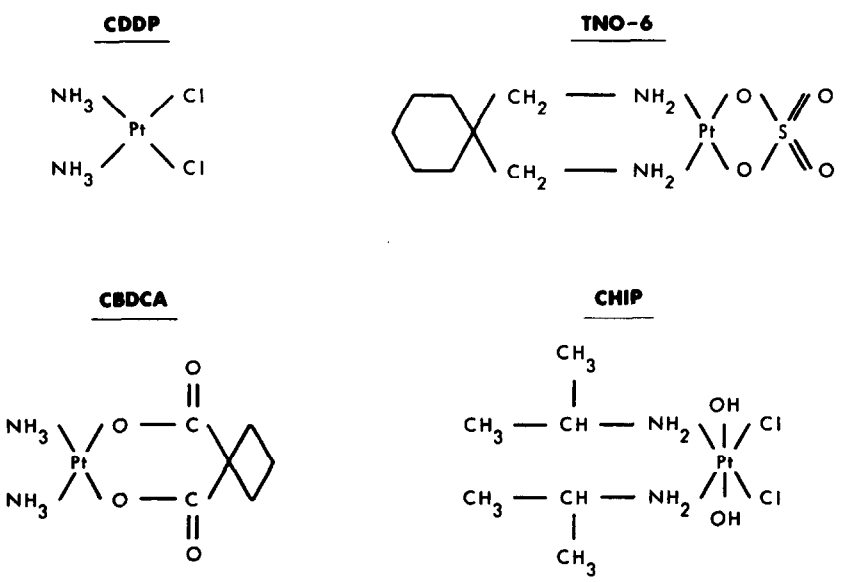

Fig. 1. Chemical structures of CDDP, TNO-6, CBDCA and CHIP.

Dogs

A study in dogs was started in order to confirm the earlier findings of TNO-6 toxicity [D. Barnett, personal communication] and to analyse pharmacokinetics in relation to toxicity. Hybrid dogs were injected (i.v. push) with a single dose of TNO-6 and CDDP. Dog identification and treatment are indicated in Table 1. The animals were observed at least two times per day. At regular time intervals a small amount of blood was drawn for determinations of BUN and creatinine concentrations, determinations of the number of leukocytes and platelets, and for pharmacokinetic studies. For the latter purpose, serum was separated and kept frozen at −30°C until analysis. Twelve weeks after administration of the drugs dogs 136 and 141 were killed. Tissues were removed and kept frozen at −30°C until analysis of the platinum concentration. Platinum concentrations were determined by flameless atomic absorption spectrometry.

RESULTS

Mice

LD_{50} values after i.p. and i.v. administration of the drugs are indicated in Table 2. TNO-6 seems more toxic, and CBDCA and CHIP are less toxic than CDDP with respect to mouse survival. All compounds show lower LD_{50} values after i.v. than after i.p. administration, but the differences are not statistically significant. Activity of the four drugs against osteosarcoma C22LR is also shown in Table 2. All drugs were administered i.v. at a dose of 3/4 LD_{50}. CDDP produced an excess tumour growth delay of 7.5 days. Less active were TNO-6 and CBDCA, producing a growth delay of 4.3 and 4.5 days respectively. CHIP was not active against the osteosarcoma.

Survival of haemopoietic bone marrow stem cells (CFU) is presented in Fig. 2. For all drugs the doses are expressed as fraction of the mouse LD_{50}. CDDP and CHIP kill stem cells to the same extent and are clearly less toxic for bone marrow stem cells than TNO-6 and CBDCA. For the former drugs the cause of death may not be bone marrow failure.

The results of treatment of leukaemia L1210 cells are expressed in a similar way (Fig. 2). CDDP and TNO-6 produce ⩾98% kill of leukaemia L1210 cells at mid-lethal doses. CBDCA and CHIP produce a similar cell kill at doses as large as 2–3 times the LD_{50}.

From the studies using normal haemopoietic stem cells, leukaemia L1210 cells and the solid osteosarcoma C22LR it appears that CHIP is not effective or very toxic for normal CFU. CBDCA appears to be rather toxic, but not very effective. TNO-6 seems to be effective and rather toxic. Of all four platinum derivatives, the parent compound CDDP has still the highest therapeutic index.

Rats

Single dose study. The occurrence of kidney damage, as expressed by elevated BUN values, was determined at 2, 4, 6 and 8 days after treatment with CDDP and TNO-1. The results (Fig. 3) show

Table 1. Identification and treatment of hybrid dogs with platinum derivatives

Dog No. and sex	Weight (kg)	Drug	Dose mg/kg	Dose mg/m²	Dose mg Pt/kg	i.v. injection volume (ml)
142 ♀	18.9	TNO-6	2.0	52.5	0.90	18.9
141 ♂	27.2	TNO-6	1.5	44.8	0.68	20.4
138 ♀	19.5	TNO-6	1.5	40.0	0.68	19.5
136 ♀	16.5	CDDP	2.0	50.8	1.30	16.5

Table 2. Toxicity of platinum derivatives in mice and rats, and their effectiveness against a mouse osteosarcoma

Drug	Mice LD_{50} i.p. (mg/kg)	Mice LD_{50} i.v. (mg/kg)	Mice Tumour growth delay (days ± SE)*	Rats LD_{50} i.v. (mg/kg)†
CDDP	14.2	13.0	7.5 ± 0.5	5
TNO-6	13.0	8.9	4.3 ± 0.6	4
CBDCA	150.0	140.0	4.5 ± 0.6	85
CHIP	60.0	45.0	0.5 ± 0.4	30

The differences between i.p. and i.v. LD_{50} values in mice are not statistically significant.

*Growth delay of osteosarcoma C22LR after single i.v. treatment with 3/4 LD_{50}. For each group, 4–5 mice carrying 8–10 tumours were used.

†The indicated doses were administered i.v. four times at intervals of 2 weeks.

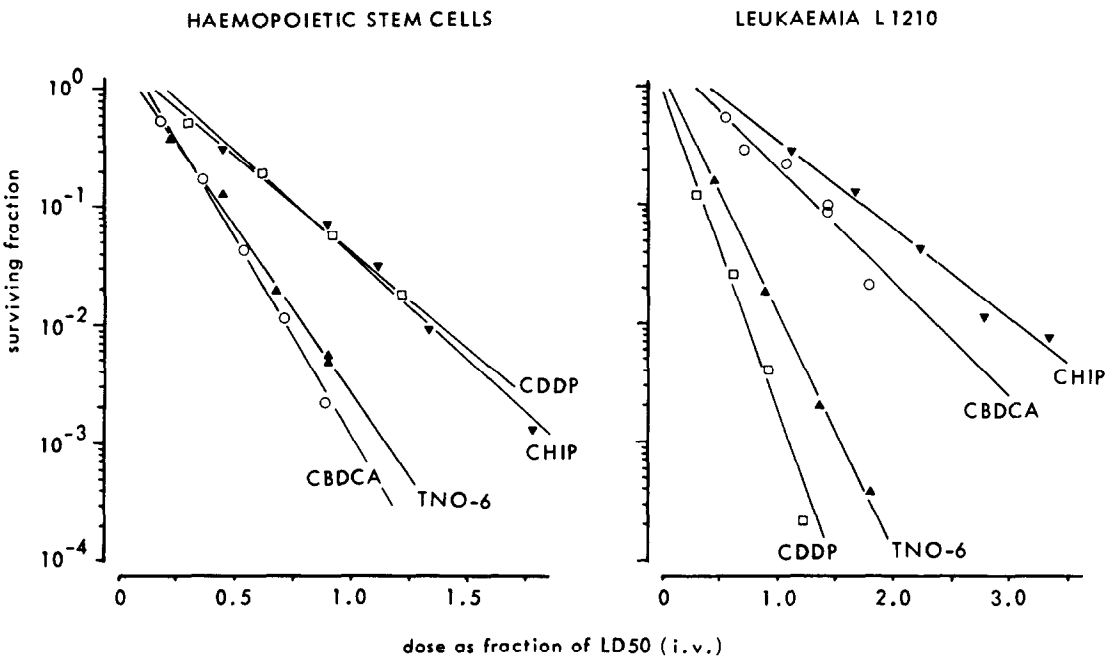

Fig. 2. Survival of haemopoietic stem cells and leukaemia L1210 cells in mice (n = 5) *after treatment with platinum derivatives.*

that, at least for CDDP, the highest BUN-values were measured at day 4.

This was the basis for the use of the 4-day interval in subsequent studies. A single dose of TNO-1 did not cause a significant increase in BUN concentration. Kidneys of the treated rats were removed and histologically examined to see whether changes in BUN reflect kidney pathology. The kidneys of rats treated with an LD_{50} dose of CDDP show clear tubular necrosis with a strong radiomimetic effect on the nuclei of the tubular cells (Fig. 4B). A single LD_{50} dose of TNO-1 causes

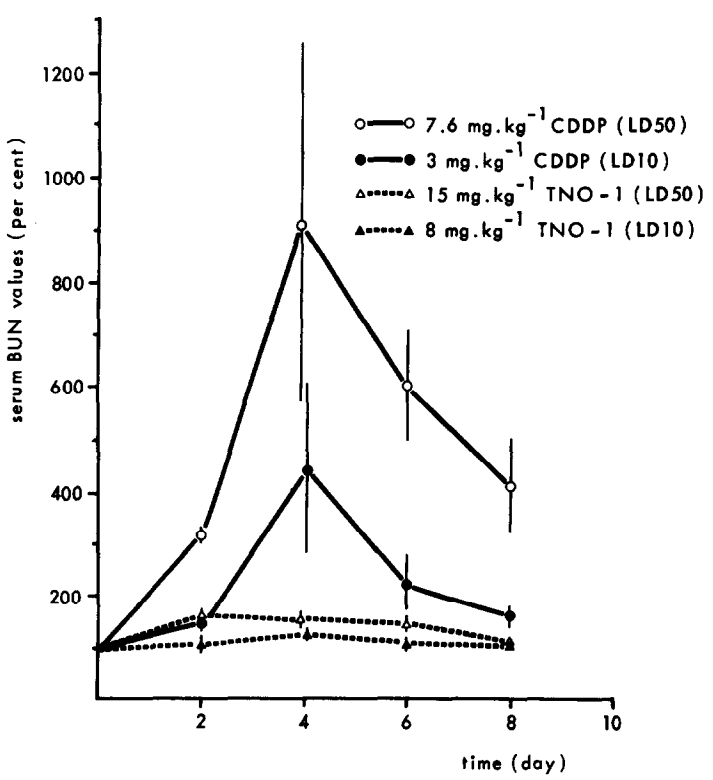

Fig. 3. *Time dependence of blood urea nitrogen (BUN) values* (± 1 S.D.) *in serum of male Sprague-Dawley rats* (n = 3) *treated with single i.p. doses of CDDP and TNO-1. Control rats were injected with saline.*

hardly any change in the tubular and glomerular apparatus, with only little radiomimetic effect on the nuclei of the tubular cells (Fig. 4A).

BUN and creatinine concentrations in rats 4 days after treatment with single i.p. doses of CDDP and TNO-6 are shown in Table 3. A supralethal dose (10 mg/kg) of CDDP produced a marked increase in BUN and creatinine concentrations. However, a supralethal dose (26.7 mg/kg) of TNO-6 did not cause an increase of these values. It seems to be justified to conclude that a single dose of TNO-6 did not cause renal toxicity.

Table 3. Blood urea nitrogen (BUN) and creatinine values in serum of rats 4 days *after treatment with single i.p. doses of platinum derivatives*

Drug*	Dose (mg/kg)	BUN (nmol/l ± SE)	Creatinine (μmol/l ± SE)
No treatment		8.6 ± 0.1	58.5 ± 0.6
CDDP	10.0	72.0 ± 13.0	282.0 ± 55.0
CDDP	6.7	55.0 ± 15.0	281.0 ± 88.0
TNO-6	26.7	7.2 ± 0.2	50.3 ± 4.5

*Treatment groups: $n = 3$; control group: $n = 10$.

Fractionated dose study. Results from the fractionated dose schedule were obtained at 4 days and at 3 months after the last of four drug doses, indicating early and late toxicity and/or recovery respectively. During the period of treatment until sacrifice the body weight of the rats was recorded. In neither sex was a reduction in body weight observed. In male rats, however, all drugs caused a dose-dependent lowering of body weight gain compared to control rats. By the end of the 3-month period the body weight had recovered (equal to control rats) for rats treated with TNO-6 and CHIP but not for rats treated with CDDP and CBDCA.

Animal observations during the period of the study revealed toxicity in the highest dose ranges for all four drugs. The rats showed the usual signs of illness: lethargy, erected hairs, arched back, diminished faeces production. The LD_{50} in rats after four i.v. injections is indicated in Table 2. Shortly after treatment with the highest doses of TNO-6 the colour of the sawdust in the cages of the treated rats appeared to be yellow. This lasted until approximately 1 week after treatment. No platinum could be detected in this sawdust using thin-layer chromatography. Rats treated with high doses of CHIP were restless and very sensitive to ether anaesthesia. They were wet around their anus and produced no (solid) faeces for several days.

In most groups bone marrow depression occurred only in the lethal dose range. Sublethal doses of CHIP caused a slightly lower number of leukocytes in female rats. The other drugs caused no important haematological toxicity at sublethal doses. Three months after the last drug dose leukocyte counts of all treated groups were similar to those of the controls.

Urea and creatinine concentrations in serum of rats treated with four doses of platinum derivatives are shown in Fig. 5. CDDP showed an increase in both parameters by a factor of three. No increase was observed for CBDCA. BUN values for CHIP are quite variable. Compared to CDDP, equitoxic doses of CHIP caused one higher, two equal and two lower BUN values. The one rat which received a high dose of CHIP (40.5 mg/kg) showed an equal to control BUN value. Creatinine concentrations were increased after CHIP but much less than after CDDP. Treatment with TNO-6 caused an increase in both parameters at most by a factor of 1.8. This is considerably lower than the increase observed after CDDP. Assuming that increase in serum levels of urea and creatinine reflects renal toxicity [12], all three platinum derivatives showed less or no renal toxicity compared to CDDP.

Histopathology

The main lesions were found in the kidney. Lesions in other organs consist mainly of bone marrow depletion and depletion of paracortical areas in lymph nodes with marked reduction in lymph follicles and follicle centre reactions, reflecting depression of the immune system. For each drug used, 4 days after the last drug dose the kidney demonstrates glomerular damage, leading to protein leakage (Fig. 6). Small casts are formed in Henle's loops, causing dilatation of Bowman's capsule. Proliferative changes of the glomerular tuft leading to an increase in size were also evident. In Fig. 7 these early changes are represented by the trans-sectional area of the Bowman's capsule in juxtamedullary glomeruli measured in the kidneys of rats treated with the highest drug doses. The increase in size of the glomerular tuft is also given (Fig. 7). Compared to controls, significant increases in size of the glomerular tuft, with concomitant hydrops of the Bowman's capsule space, were obtained after treatment with CDDP (Wilcoxon's two-sided rank test, $P < 0.01$) and CBDCA ($P < 0.01$). TNO-6 and CHIP also caused such an increase, but to a much lesser degree; the glomerular tuft in the CHIP group differed only slightly from control material. After 3 months all kidneys demonstrated, to a different extent, cystic changes of the renal cortex with loss of cortical tissue (Fig. 8). The

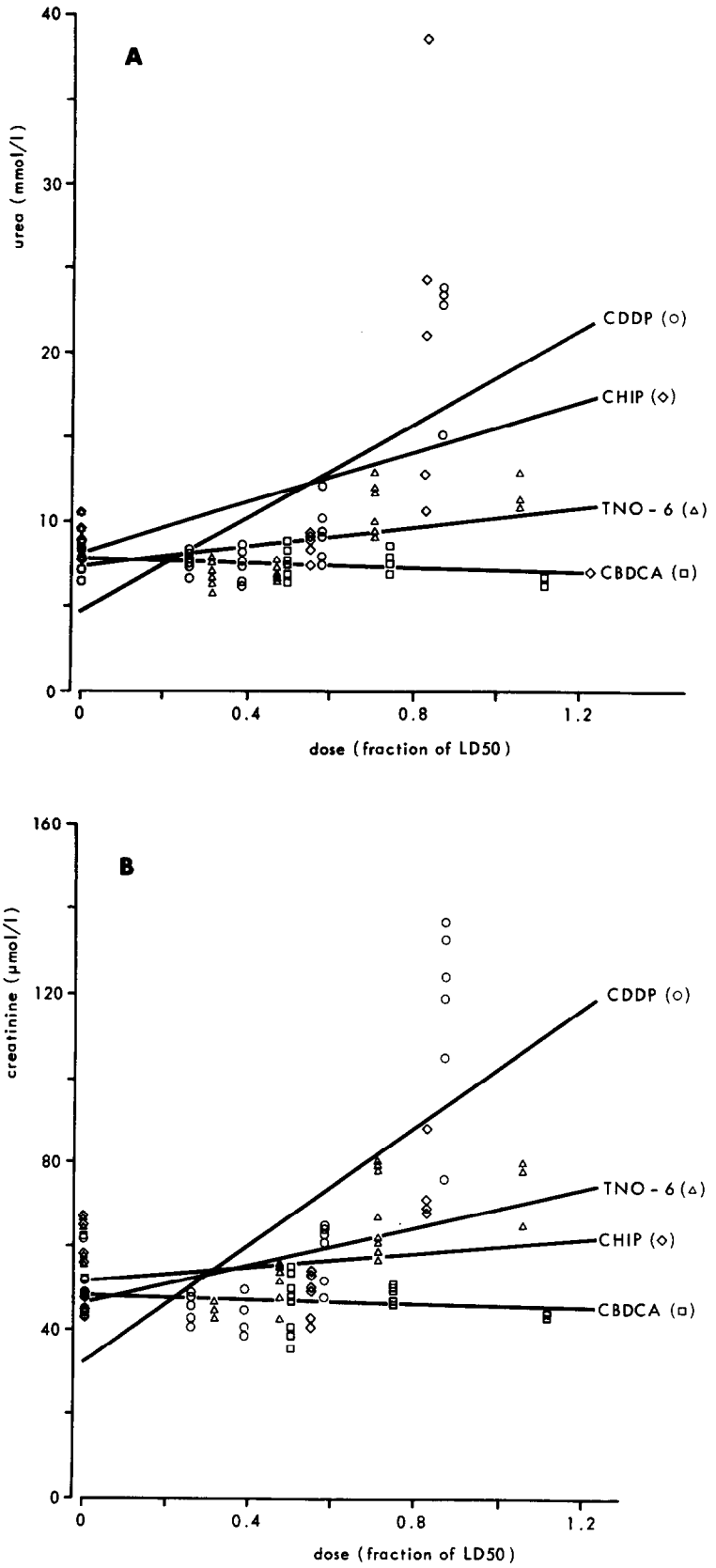

Fig. 5. Urea (A) and creatinine (B) concentrations in serum of rats treated with four doses of platinum derivatives at intervals of 2 weeks. *Blood was collected* 4 days *after the last dose. Each line is a computer-calculated best fit (least squares) of the individual values.*

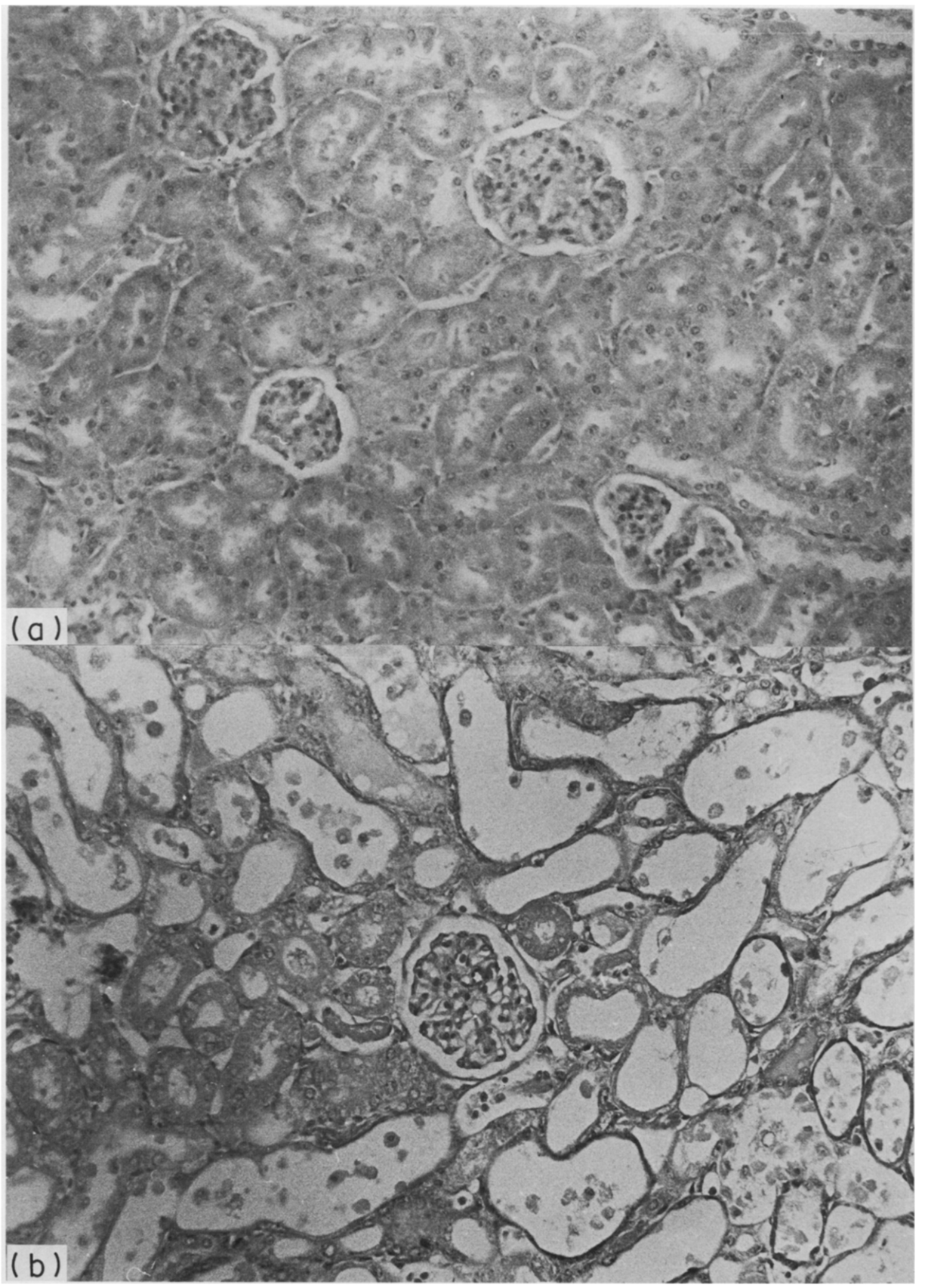

Fig. 4. Histological appearance of renal tubules in male Sprague–Dawley rats on day 6. (a) After one single i.p. injection of 15 mg/kg (LD_{50}) *of TNO-1. No histological evidence of tubular damage could be found following this treatment. (b) After one single i.p. injection of* 7.5 mg/kg (LD_{50}) *of CDDP. Note the pathologic alterations in the tubular epithelium and the presence of hyaline casts in the lumen of the nephron tubules.*

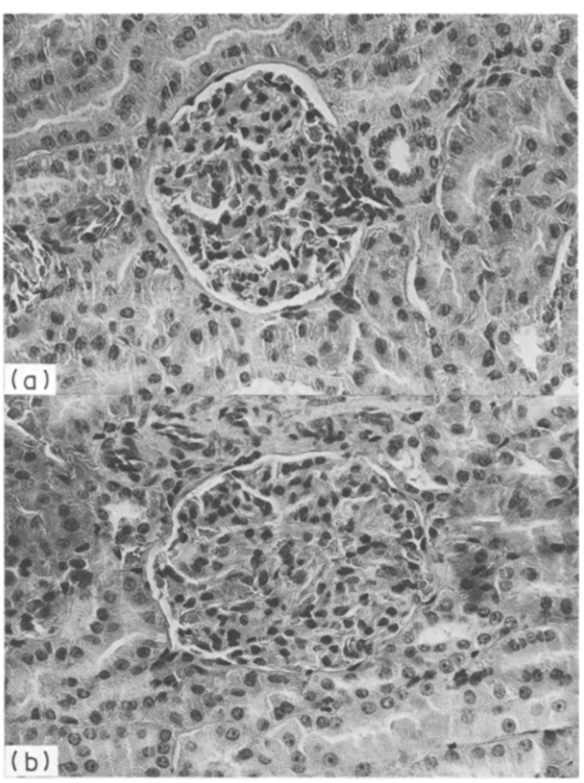

Fig. 6. Histological appearance (×205) of outer cortical glomeruli in a CBDCA-treated (a) and a CDDP treated (b) Sprague–Dawley rat 4 days after the last drug dose. Note enlargement, especially for CDDP, and mixed hypercellularity, most outspoken for CDDP.

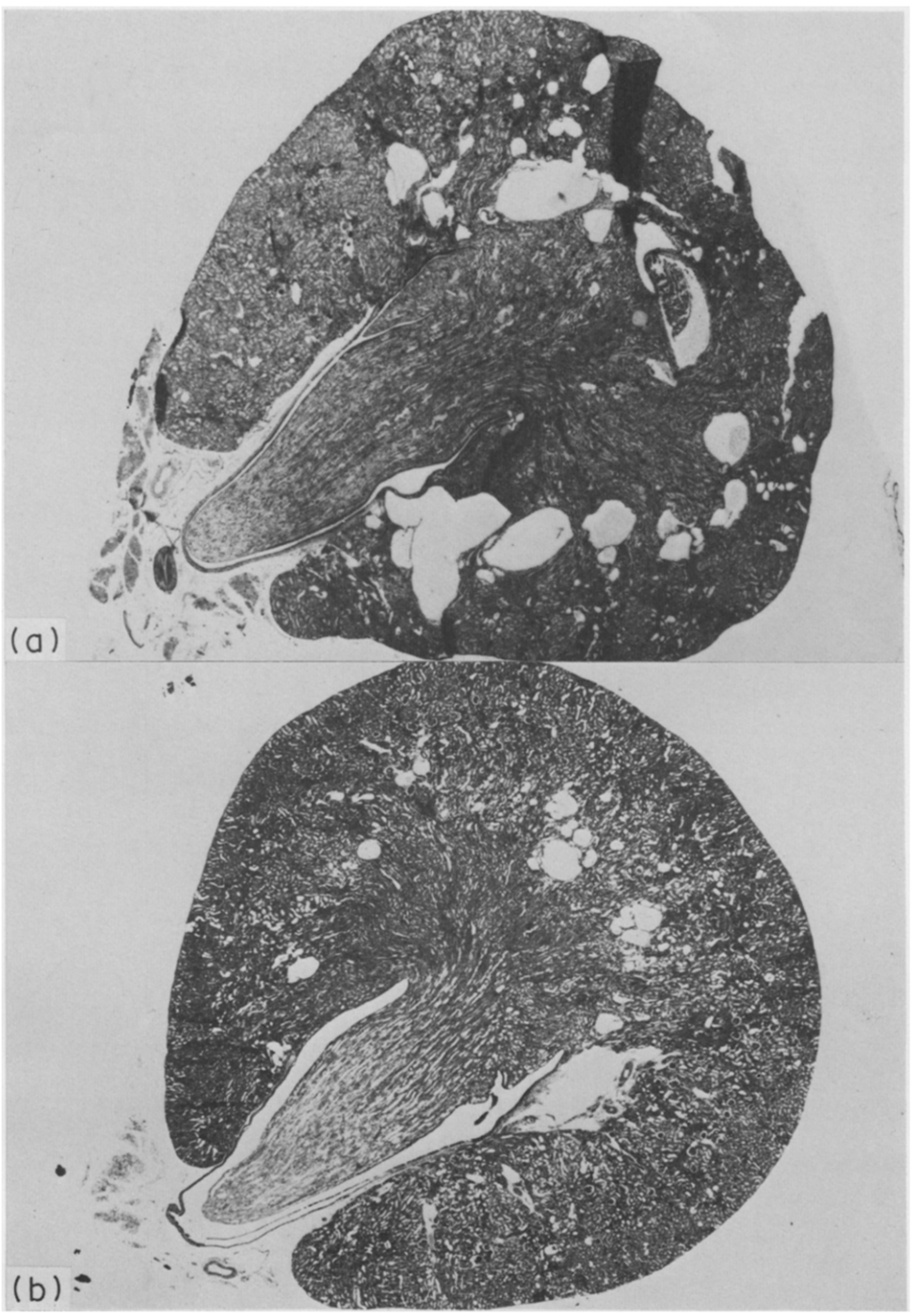

Fig. 8. (*a*) *Obstructive nephropathy in rats* 3 months *after treatment with CDDP. Severe loss of cortical tissue is observed. Images of TNO-6 and CBDCA-treated animals are comparable.* (*b*) *Obstructive nephropathy in rats* 3 months *after treatment with CHIP. There is little loss of cortical tissue.*

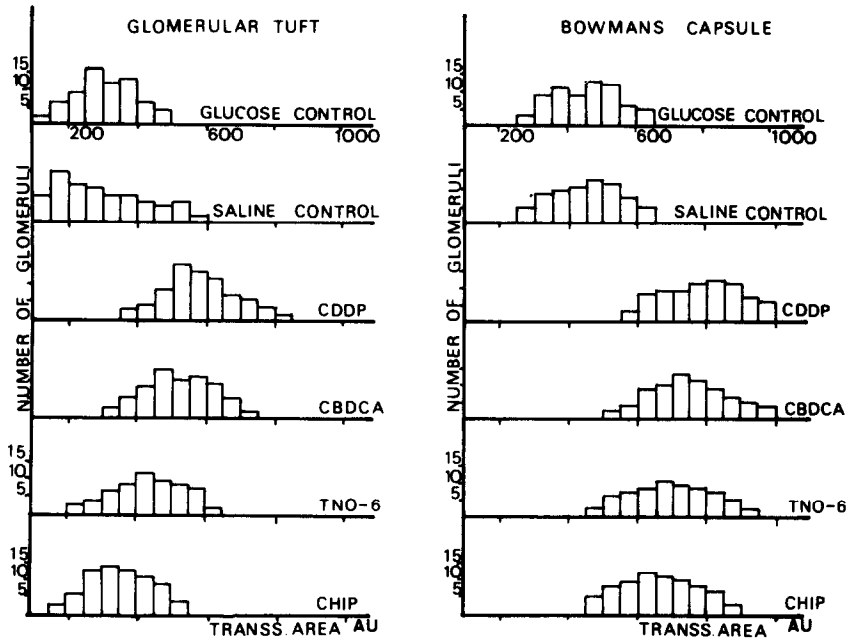

Fig. 7. Trans-sectional areas in arbitrary units (AU) *of Bowman's capsule and glomerular tuft.*

suspected obstructive mechanism probably changed glomerular tuft volume, which made an interpretation of the changes in this parameter very difficult. Therefore these measurements were not repeated after 3 months. At that time the cortical trans-sectional area, representing the amount of cortical tissue, was very much decreased in rats treated with CDDP (Fig. 9). The results for control rats from the early- and the 3-month lesions groups as well as from the saline- and glucose-treated groups were pooled since there was no significant difference between these groups. For TNO-6 and CBDCA the decrease was less, whereas the cortical trans-sectional area in CHIP-treated animals showed the least decrease (Fig. 9). Statistical analysis of the point scores of the cortical trans-sectional area with Wilcoxon's two-sided rank test substantiated these differences (Table 4).

It is clear that CHIP had the least effect on the kidney parenchyma. However, high doses could not be studied because of severe gastrointestinal damage, causing dehydration and death of the animals. Tissue repair capacity, also determining the outcome of obstructive nephropathy, may be suspected to be decreased by platinum-induced DNA damage. The appearance of large bizarre nuclei, considered representative of this mechanism, was studied. Histograms of nuclear size were made of nuclei on a radial line from the cortico-medullary junction to the outer cortex along a medullary ray (Fig. 10). It is evident that, in the early lesions groups, large nuclei appeared. This change was most severe for CDDP. It was less and almost comparable for TNO-6, CBDCA and CHIP. After 3 months the pattern indicated only a slight recovery of the kidneys after CDDP, and an

Table 4. Statistical analysis of values for cortical area point score in renal trans-sections in both early lesions and 3-month repair groups (Fig. 9)

Treatment group	Mean cortical area ± S.D. (point score) Early lesions	*P* value	3-month repair lesions	*P* value
CHIP	160.9 ± 2.5	N.S.	148.5 ± 3.3	<0.05
TNO-6	154.5 ± 2.1	<0.05	141.4 ± 3.7	<0.01
CBDCA	151.8 ± 2.8	<0.05	141.4 ± 4.3	<0.01
CDDP	148.8 ± 3.2	<0.01	119.3 ± 2.2	<0.002

Mean control value ± S.D.: 167.3 ± 2.4 ($n = 16$). All treatment groups (eight rats per group) were statistically tested against the control group (Wilcoxon's two-sided rank test). *P* values are indicated. TNO-6 and CBDCA differ significantly from CHIP and CDDP in both early and 3-month repair lesions ($P < 0.01$). All 3-month repair lesions differ significantly from early lesions ($P < 0.01$).

improvement after TNO-6 and CBDCA, which was more pronounced after CHIP.

It can be concluded that CHIP caused the least nephrotoxicity of the four platinum compounds at the doses used. However, high doses do cause serious gastro-intestinal damage. At lower non-lethal doses substantial diarrhoea remains. CBCDA caused significant nephrotoxic changes which are clearly demonstrable by histopathology, although biochemical analysis failed to demonstrate functional loss. CDDP produced the most serious histopathological changes associated with nephrotoxicity, i.e. severe cortical atrophy. These changes could also be demonstrated by biochemical analysis. Histopathological changes after TNO-6 are intermediate between those seen after CHIP and CBDCA.

Dogs

All four dogs started vomiting 1–2 hr after drug injection. For both dogs treated with 1.5 mg/kg TNO-6 (dogs 138 and 141) this lasted not longer than 2 hr; for both other dogs (CDDP, dog 136; high-dose TNO-6, dog 142) this lasted 1 day. Dogs 142 (2 mg/kg TNO-6) and 138 (1.5 mg/kg TNO-6) produced diarrhoea on days 5 and 6 after treatment respectively. Dog 142 was found dead on day 6 after injection. Histopathology emphasized severe kidney damage and fatty necrosis around the pancreas. There were no signs of haemorrhage or infection. Dog 138 was killed on day 11 in a poor condition. Its mouth was inflamed and haemorrhagic. At autopsy extensive gastro-intestinal haemorrhage was noted and from the blood large numbers of *Proteus* spp. were cultured. Dogs 141 and 136 appeared healthy and normal until termination of day 84.

Serum chemistry

As in the rat study, serum concentrations of urea and creatinine were used as parameters for renal toxicity [12] (Table 5). All four dogs showed a dramatic increase in both values. The most marked increases were found in dog 142 (died on day 6) and dog 138 (killed on day 11). For both survivors the values had not returned to normal at the end of the experiment (day 84).

In addition, dog 142 showed slight increases in alkaline phosphatase, SGOT, SGPT and bilirubin, which are indicative for (marginal) liver cell damage. The single dog treated with CDDP (No. 136) showed elevated values for lactate dehydrogenase and creatinine phosphokinase.

Haematology

Blood cell counts were made from venous blood (Table 6). In comparison to the CDDP-treated dog(No. 136), the depression of leukocyte count is more severe in dogs 138 and 142 and about similar in dog 141. The depression in the number of platelets and the time at which the depression occurred were similar for TNO-6 and CDDP.

Table 5. Effect of CDDP and TNO-6 on BUN and serum creatinine in dogs

	Dog No.	Treatment	Days after treatment 0	3	5	9	21	84
Urea (mmol/l)	142	2.0 mg/kg TNO-6	6	45	111	–	–	–
	141	1.5 mg/kg TNO-6	5	16	18	–	34	36
	138	1.5 mg/kg TNO-6	6	–	50	152	–	–
	136	2.0 mg/kg CDDP	7	–	34	–	–	22
Creatinine (μmol/l)	142	2.0 mg/kg TNO-6	80	667	1405	–	–	–
	141	1.5 mg/kg TNO-6	92	302	520	–	1105	342
	138	1.5 mg/kg TNO-6	88	–	949	2390	–	–
	136	2.0 mg/kg CDDP	96	–	266	–	–	200

Table 6. Effect of CDDP and TNO-6 on leukocyte and platelet counts in dogs

	Dog No.	Treatment	Days after treatment 0	5	7	10	11	84
Leukocytes ($\times 10^9$/l)	142	2.0 mg/kg TNO-6	6	1	–	–	–	–
	141	1.5 mg/kg TNO-6	8	5	–	–	–	8
	138	1.5 mg/kg TNO-6	6	5	3	0.7	1	–
	136	2.0 mg/kg CDDP	8	5	6	–	5	6
Platelets ($\times 10^9$/l)	142	2.0 mg/kg TNO-6	224	508	–	–	–	–
	141	1.5 mg/kg TNO-6	302	474	–	–	–	266
	138	1.5 mg/kg TNO-6	336	256	–	86	86	–
	136	2.0 mg/kg CDDP	282	148	–	–	74	168

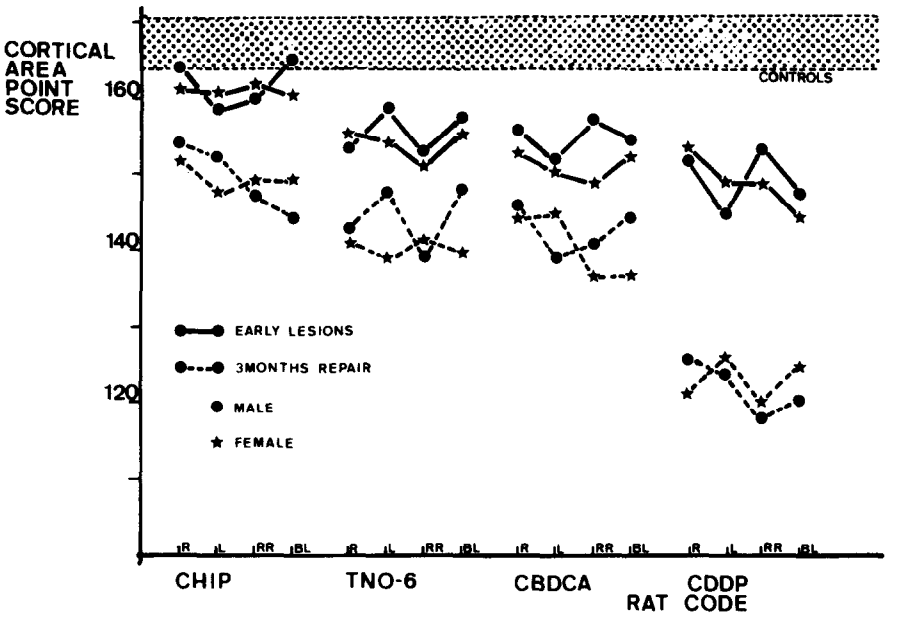

Fig. 9. Renal cortical trans-sectional areas measured by the point score method on photographs of whole kidney sections through papilla. In every treatment group results of four rats (earmarked R, L, RR, BL) receiving the highest drug dose are presented. The range in control rats is represented by the shaded bar in the top of the figure. Within the early lesions groups loss of renal cortical tissue is most outspoken for CDDP (Wilcoxon's two-sided rank test, P <0.01) followed by CBDCA (P <0.05), TNO-6 (P <0.05) and CHIP (not significant). After 3 months *severe cortical loss is evident in rats treated with CDDP (P <0.002) followed by CBDCA (P <0.01), TNO-6 (P <0.01) and CHIP (P <0.05). The results of the statistical analyses are presented in Table* 4.

Pharmacokinetics

The platinum concentrations determined in serum plotted against the time after administration of the drug are shown in Fig. 11. As shown in Table 3 (dose in mg Pt/kg), TNO-6 was administered at a lower molar dose than CDDP. Nevertheless, platinum concentrations were lowest in the dog treated with CDDP. Table 7 summarizes the platinum levels in serum after 24 hr and the same levels after correction for the dose/kg, which, assuming linear kinetics, permits comparison. The best parameter for this comparison is *B* (the intercept of the back-extrapolated elimination line with the ordinate) divided by the dose (*D*) expressed in mg Pt/kg. The *B*/*D* value for CDDP is 0.38; a value of 0.93 could be calculated for both dog 138 and 141 after treatment with TNO-6. Another notable fact is the infinite half-life of elimination in the dog with the highest dose of TNO-6 (2 mg/kg), which developed renal failure.

Table 7. Platinum levels in plasma at 24 hr *after a bolus injection of TNO-6 or CDDP in dogs*

Dog No.	Drug given	μg Pt/ml	μg Pt / ml	kg / mg Pt
142	TNO-6	0.68	0.76	
141	TNO-6	0.52	0.76	
138	TNO-6	0.60	0.88	
136	CDDP	0.45	0.35	

The concentration vs time curves were analysed by a curve-stripping procedure. Calculation of the pharmacokinetic parameters started with the determination of the half-life of elimination during days 1–6. The half-life of distribution was calculated from the concentrations during the first 10 min after subtraction of the elimination phase (curve stripping). The reason for this approach lies in the appearance of the small peaks between 1 and 4 hr after the administration of either TNO-6 or CDDP, which can be attributed to an entero-hepatic circulation of drug species [13]. The calculated pharmacokinetic parameters are summarized in Table 8. These calculations could not be performed for dog 142 because this dog developed a poor renal function after the second day, resulting in an infinite half-life which is not representative for the first day. A mean half-life of disposition of 4.6 ± 0.8 min and a mean half-life of elimination of 5.1 ± 2.1 days were obtained for TNO-6 in the evaluable dogs. Compared to CDDP, the half-life of distribution of TNO-6 was smaller, while no essential difference between the half-lives of elimination could be observed. The overall elimination rate constant for TNO-6 appeared to be smaller than the corresponding value for CDDP, while the distribution rate constants between the central

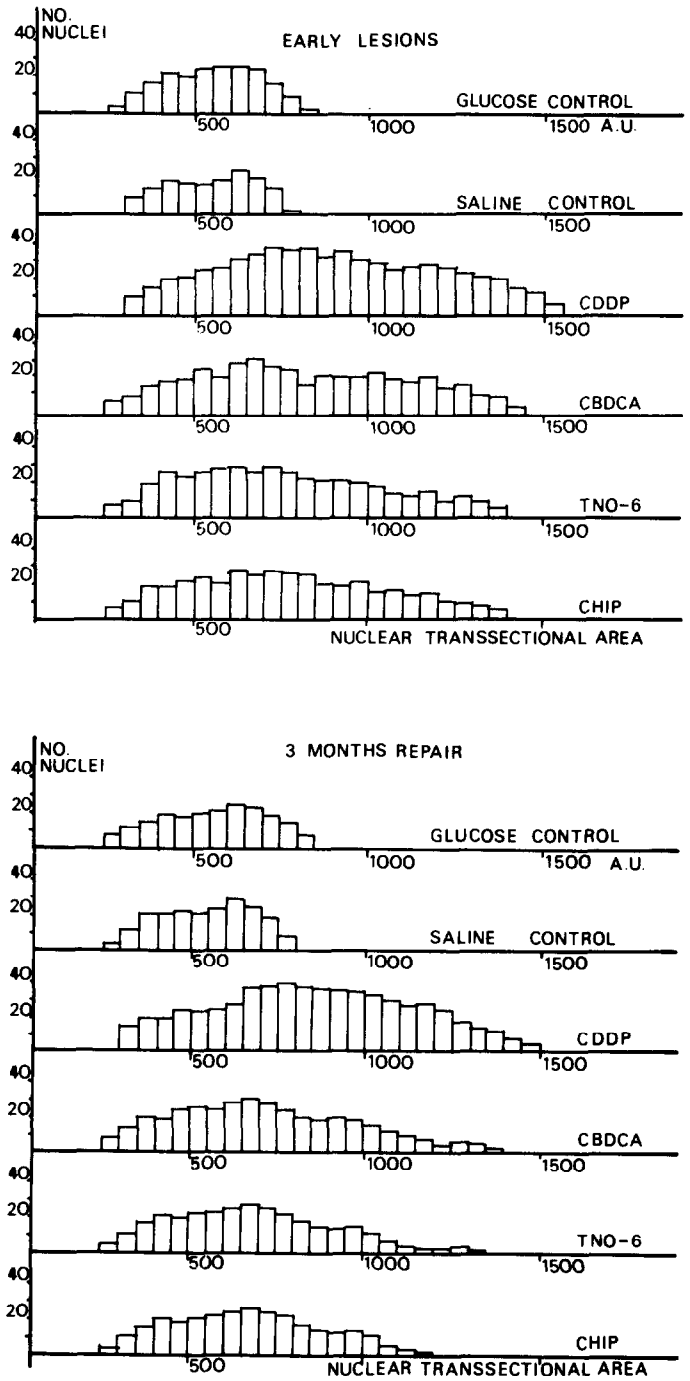

Fig. 10. Histograms of nuclear size on a radius from the juxtamedullary zone to the outer cortex. Nuclear trans-sectional area in arbitrary units (AU). Per drug, four kidneys (from four rats) were analysed, measuring all cells on a line in one complete medullary ray.

and peripheral compartments were higher for TNO-6 compared to CDDP. The peripheral volume of distribution calculated for TNO-6 was about half that of CDDP (19.6 vs 39.6 l). The differences in AUC^{∞}'s reflect the differences in serum platinum levels as already mentioned.

An estimation of the enterohepatic circulation could be made by the procedure described earlier [13]. Figure 12 shows the contribution of the enterohepatic circulation to the platinum levels in serum. The ratio of AUC-EC and AUC^{∞} quantitates this process. In the two dogs treated with TNO-6, 1.8 and 2.8% of the dose re-entered the plasma compartment, while 3.0% of the dose recycled in the dog after CDDP.

Twelve weeks after administration of the drugs, dogs 136 and 141 were killed. Platinum concentrations were determined in several tissues. The results are shown in Table 9. High levels of platinum were still encountered in liver (1.33 and

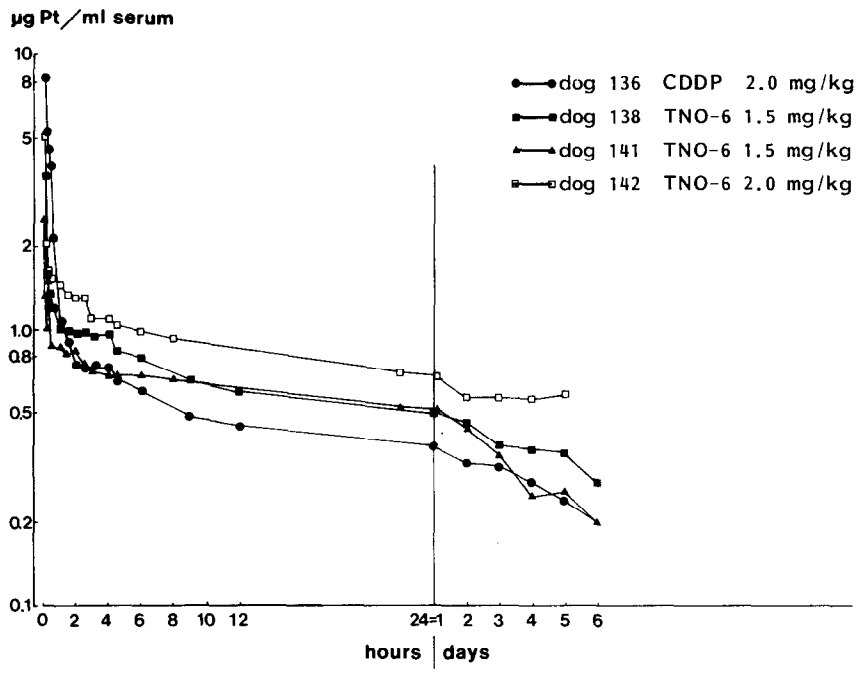

Fig. 11. Platinum concentrations in serum of dogs after an i.v. bolus injection of CCDP and TNO-6 respectively.

0.83 µg Pt/g wet wt for CDDP and TNO-6, respectively) and kidney cortex (1.09 µg Pt/g wet wt for CDDP), while in other tissues lower but still higher than negligible levels were detectable.

DISCUSSION

Studies on toxicity and antitumour effectiveness of CDDP, TNO-6, CBDCA and CHIP were performed in different animal species. Survival of haemopoietic stem cells and of leukaemia L1210 cells after treatment with the platinum derivatives was determined in mice. The parent compound CDDP appeared to be the most effective drug against L1210, while it was least toxic for normal haemopoietic stem cells (Fig. 2). In the primary screening of the EORTC Screening and Pharmacology Group, TNO-6 showed activity against leukaemia L1210 and melanoma B16 similar to that of CDDP. Bradner *et al.* [14] tested a large series of derivatives of CDDP, including CBDCA, CHIP and TNO-1, against leukaemia L1210, Lewis lung carcinoma and B16 melanoma, all in mice. None of these derivatives was superior to the parent compound CDDP. Wolpert-DeFillipes [15] found that CBDCA and CHIP were less active than CDDP against leukaemia L1210, Lewis lung carcinoma, colon tumour 38 and CD8F1 mammary tumour; similar activity was found against B16 melanoma. Despite the fact that the drugs tested are frequently less active than CDDP, they might have a therapeutic gain when their effectiveness is still lower on white blood cells, BUN or emesis induction. We studied renal toxicity in rats and used BUN, creatinine and histopathology as parameters for renal toxicity. Our finding that day 4 after treatment of rats with CDDP is the optimal time interval to determine BUN values (Fig. 3) is in full agreement with

Table 8. Values of pharmacokinetic parameters

Parameter	Dog 141 1.5 mg/kg TNO-6	Dog 138 1.5 mg/kg TNO-6	Dog 136 2 mg/kg CDDP	Units
$t_{½\alpha}$(0–10 min)	4.0(–0.98)	5.1(–0.88)	9.7(–0.95)	min
$t_{½\beta}$	3.6(–0.98)	6.6(–0.96)	5.9(–0.99)	days
D	18.4	13.2	21.5	mg Pt
k_{13}	0.6×10^{-3}	0.4×10^{-3}	1.4×10^{-3}	min^{-1}
k_{12}	130.0×10^{-3}	110.0×10^{-3}	65.0×10^{-3}	min^{-1}
k_{21}	41.0×10^{-3}	25.0×10^{-3}	4.2×10^{-3}	min^{-1}
V_c	7.0	3.9	2.6	l
V_p	22.3	17.0	39.6	l
*AUC$^{\infty}$	4690	8719	6168	µg.min/ml
AUC-EC	86	242	188	µg.min/ml

*AUC$^{\infty}$ = area under the curve after extrapolation of the β-phase to infinity ($= A/\alpha + B/\beta$).

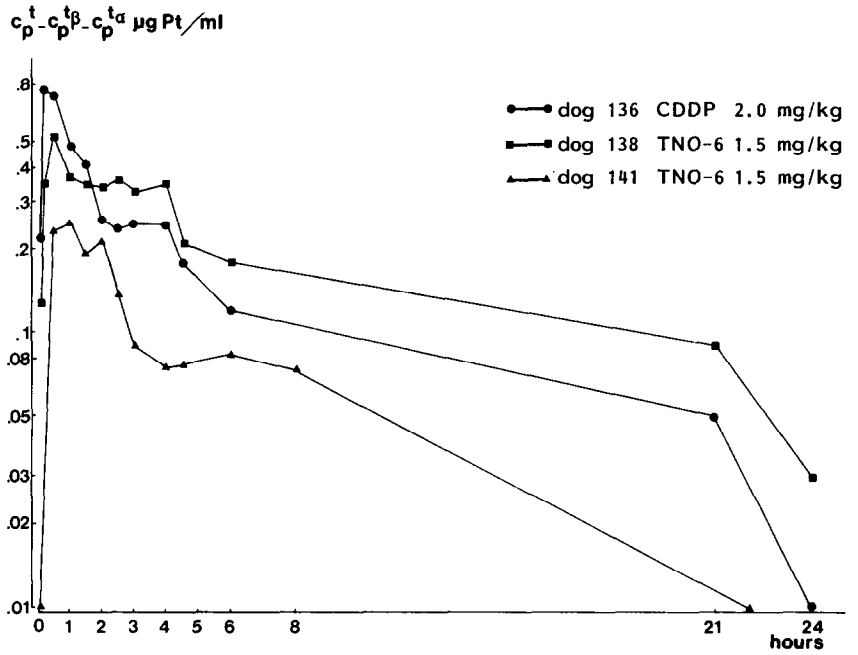

Fig. 12. Contribution of the second influx of platinum after enterohepatic circulation to the platinum concentrations in plasma. C_p^t = measured platinum level in plasma at time t; $C_p^{t\beta}$ = calculated platinum level in plasma at time t by back extrapolation of the elimination line; $C_p^{t\alpha}$ = calculated platinum level in plasma at time t by extrapolating the initial rapid disposition line.

earlier observations by Ward *et al.* [5]. With these methods, CDDP showed severe nephrotoxicity after either single or fractionated doses. A single dose of TNO-6 did not increase BUN and creatinine. For these determinations (Table 3), supralethal doses could be used since the drugs caused death of the rats later than the optimum time interval (i.e. day 4). If no increase in BUN and creatinine concentrations can be observed after these supralethal doses the cause of death apparently is not due to renal toxicity. Repeated doses of TNO-6 did increase BUN and creatinine values, although to a lesser extent than did CDDP. CBDCA caused no increase in these serum values. The increase observed after treatment with repeated doses of CHIP is most likely due to gastrointestinal toxicity, which by producing diarrhoea and dehydration will reduce glomerular filtration rate. TNO-6 and CBDCA approached the same degree of histopathological changes as produced by CDDP.

Table 9. Platinum concentrations in tissues of dogs (µg Pt/g wet wt) 12 weeks after i.v. bolus administration

Tissue	Dog 136 2 mg/kg CDDP (µg Pt/g)	Dog 141 1.5 mg/kg TNO-6 (µg Pt/g)
Liver	1.33	0.83
Kidney cortex	1.09	0.37
Kidney medulla	0.38	0.33
Lung	0.29	0.23
Skin	0.30	0.16
Muscle	0.14	0.05
Plasma	undet.*	undet.*

*undet. = undetectable.

The central mechanism of nephrotoxicity may well be the impaired repair capacity of renal tissue to both obstructive, glomerular and primarily tubulotoxic phenomena (Fig. 10).

Our conclusion is that, of the four platinum derivatives, CHIP causes least glomerular damage and that repair of the damage both in the glomerulus and in the tubular areas is least hampered. Because of this, the obstructive nephropathy in CHIP kidneys is less severe. Moreover, the CHIP kidney is much more capable of repairing the damages caused by the obstruction (Figs 9 and 10; Table 4). Nephrotoxicity of the platinum derivatives under comparison is determined by the balance between initial damage, secondary damage and repair capacity. On the basis of histopathological evaluation the outcome seems to be most favourable for CHIP at the dosages used. Due to gastro-intestinal damage caused by CHIP the overall results are more favourable for TNO-6 and CBDCA.

Mice and rats do not vomit. Consequently, emesis induction could not be studied in these animals.

In other studies in rats CBDCA and CHIP did not cause renal toxicity [8, 16] and were selected for clinical studies in London [17] and Buffalo [18] respectively. From the results of the rat study,

TNO-6 was also expected to cause less nephrotoxicity than CDDP. Therefore it seemed interesting to compare all three derivatives for their clinical effectiveness. Prior to this, the tolerance to TNO-6 had been studied by the Bristol-Myers Company, Syracuse. There, acute death of the dogs was observed after a dose of 2 mg/kg [D. Barnett, personal communication]. This dose is higher than the LD_{50} when extrapolated from the mouse data. It was, however, surprising that the cause of death was renal failure. Our experience with dog 142 confirms their observations. Both dogs treated with a lower dose of TNO-6 showed renal toxicity as expressed by elevated urea and creatinine concentrations, as well as a depression in the number of leukocytes and platelets. CDDP did not produce such a depression in the leukocyte count. All dogs did vomit after drug treatment, although at equitoxic doses TNO-6 seemed to induce less severe vomiting than CDDP.

Pharmacokinetics of CDDP and other platinum compounds in dogs have been described in several studies [19–25]. Our observations for CDDP with regard to the shape of the plasma concentration–time curve and the calculated pharmacokinetic parameters are in agreement with these previous data [21, 22, 24]. Differences with the published values of $t_{½\alpha}$ and $t_{½\beta}$ are mainly caused by the small number of dogs used in the study and the time interval chosen for the calculations. The observation of an infinite half-life of elimination for dog 142 with a deteriorated kidney function indicates that platinum species from TNO-6, just as those from CDDP, are principally excreted by the kidneys.

The entero-hepatic circulation could be estimated from the ratio of AUC^{∞} and AUC-EC [13]. The circulating platinum expressed as a percentage of the dose appears to be the same for CDDP and TNO-6. This observation is complementary to earlier studies in which small [20, 22] and higher [24] amounts of platinum could be detected in the bile of dogs at 4 days after administration of CDDP. Platinum levels after TNO-6 were much higher than after CDDP. The ratios of the B/D values are in agreement with the ratios of the corrected platinum levels in plasma after 24 hours (Table 7). Although transport from the plasma to the peripheral compartments is more rapid for TNO-6 than for CDDP, as reflected by the higher k_{12}- and lower $t_{½\alpha}$ values, it may be explained, at least in part, by a more rapid binding of TNO-6 to plasma proteins as observed *in vitro* (to be published separately). Comparable results were obtained in the rat by Ridgway *et al.* [26]. In that study, platinum levels in blood were much higher after the administration of sulphato-*trans*-1,2-diamminocyclohexane platinum(II) than after the administration of an equivalent dose of CDDP.

Until now, platinum levels in tissues were only measured up to 12 days after administration of CDDP [20, 21]. This study reveals that even 12 weeks after administration of either drug platinum can be detected in various tissues, while it is undetectable in plasma at that time. This means that very prolonged retention of platinum exists in tissues after administration of CDDP and TNO-6, which cannot be gathered from the plasma level. Higher platinum levels after CDDP may be caused by the higher dose of CDDP with respect to the dose of TNO-6. Consequently, more nephrotoxicity was expected for CDDP. However, nephrotoxicity was more severe in dogs receiving TNO-6. This contradiction may be explained by the fact that tissue concentrations were determined 12 weeks after administration and not at the time at which nephrotoxicity is most pronounced.

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