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Preclinical Toxicology of Platinum Analogues in Dogs*

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Abstract—*A toxicology study of* cis-*diamminedichloroplatinum(II)* (*CDDP*), *aqua*(1,1-*bis*-(*aminomethyl*)*cyclohexane*)*sulfatoplatinum(II)* (*TNO*-6), *diammine*(1,1-*cyclobutanedicarboxylato*)*platinum(II)* (*CBDCA*), cis-*dichloro*-trans-*dihydroxo*-cis-*bis*(*isopropylamine*)*platinum-(IV)* (*CHIP*) *and ethylenediaminemalonatoplatinum(II)* (*JM*-40) *was carried out in dogs. The main purpose of the study was to compare the results with those obtained earlier in mice and rats and with the toxicology data in humans. Each platinum compound was tested in three dogs. Each dog received three intravenous bolus injections at intervals of* 3 *weeks. The compounds were administered in dosages of* 1.2, 1.0, 12, 10 (*or* 6) *and* 10 mg/kg, *respectively. Toxic death occurred for two dogs* (*both on day* 54) *from haematotoxicity* (10 mg/kg *CHIP*) *and renal toxicity* (*TNO*-6), *respectively. Serum urea nitrogen and creatinine concentrations were variable after TNO*-6 *and remained within normal values after treatment with the other compounds. Severe proteinuria was observed in all three dogs treated with TNO*-6. *Values returned to normal within* 16 *days. JM*-40 *did not cause significant proteinuria. CDDP, CBDCA and CHIP caused short-lasting and slight proteinuria. CHIP caused a severe reduction in the number of leukocytes and platelets, while the other drugs caused acceptable reductions. Except after the high dose CHIP regimen, haematotoxicity was of a transient nature. Vomiting in order of severity occurred after TNO*-6, *CHIP, CDDP and JM*-40, *while CBDCA did not cause any vomiting. The dogs were sacrificed* 6 *weeks after the last drug dose. Organs were fixed for histopathology to complete and support clinical-toxicological parameters.*

On the basis of the results from the single-dose study in dogs and those obtained earlier in mice and rats it can be concluded that the gain from the use of the dog as a prognosticator for organ toxicity in man was disappointing and limited to the prediction of vomiting.

INTRODUCTION

cis-DIAMMINEDICHLOROPLATINUM(II) (CDDP) is a valuable drug for the treatment of certain types of human cancers [2–4]. In man, its main side-effects are nausea and vomiting, myelosuppression, nephrotoxicity and neurotoxicity [4, 5]. Derivatives of CDDP have been developed in order to improve the therapeutic index. Efforts have been focused on reduction of toxicities on one hand and increase of the antitumour effect on the other. In rodents, less toxicity was observed for, amongst others, TNO-6 [6–8], CBDCA [9, 10], CHIP [11, 12] and JM-40 [13, 14].

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Currently, lethality studies are carried out in mice, mainly to provide safe starting doses for phase I trials in man. Evaluation of haematology and clinical chemistry in untreated control mice showed widely variable results (Atassi, personal communication; [6, 15]). Therefore, these animals could not serve as consistently useful indicators of toxicity in drug-treated mice. At NCI, the rat is being evaluated as a better prognosticator for organ toxicity in man. In the present study dogs were used to investigate the toxicity of CDDP and four analogues. The results were compared with those of clinical trials carried out with the same compounds, as well as with the results from earlier toxicology studies in mice and rats [6].

MATERIALS AND METHODS

Dogs

Male beagle dogs with a body weight of 11.8–15.6 kg were purchased from the Central

Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands. They were housed individually in stainless steel metabolism cages.

Drugs

Besides the parent compound *cis*-diamminedichloroplatinum(II) (cisplatin; NSC 119875; CDDP) the following analogues were used: TNO-6 [aqua(1,1-bis(aminomethyl) cyclohexane)sulfatoplatinum(II), spiroplatin, NSC 311056], CBDCA [diammine(1,1-cyclobutanedicarboxylato)platinum-(II), carboplatin, JM-8, NSC 241240], CHIP (*cis*-dichloro-*trans*-dihydroxo-*cis*-bis(isopropylamine)-platinum(IV), iproplatin, JM-9, NSC 256927], JM-40 [ethylenediaminemalonatoplatinum(II), NSC 146068].

The chemical names of the compounds are given according to the International Union of Pure and Applied Chemistry (IUPAC) [16].

CDDP, TNO-6, CBDCA and CHIP were supplied by Bristol-Myers. JM-40 was obtained from the National Cancer Institute, Bethesda, MD. CDDP, CBDCA and JM-40 were dissolved in sterile saline, TNO-6 and CHIP in a sterile solution of 5% glucose.

Treatment

Each platinum compound was tested in three dogs. Each animal received three intravenous (i.v.) bolus injections at intervals of 3 weeks, except for one dog which, due to technical problems, received the third dose of CBDCA at 6 weeks after the second dose. The compounds were administered at the following doses: CDDP, 1.2 mg/kg (28.8 mg/m^2); TNO-6, 1.0 mg/kg (24 mg/m^2); CBDCA, 12 mg/kg (288 mg/m^2); CHIP (one dog), 10 mg/kg (240 mg/m^2); because of unexpected high toxicity the two other dogs were treated with 6 mg/kg (144 mg/m^2); JM-40, 10 mg/kg (240 mg/m^2). In general, these drug doses were based on extrapolations from the 50% lethal doses (LD_{50}) in mice [6] and the maximum tolerated doses as determined in phase I clinical trials [7, 17, 18]. Each dose per m^2 to the dogs was 60% of the mouse LD_{50}. Conversions of the doses per kg body wt to doses per m^2 body surface were made according to Freireich *et al.* [19]. At the start of the study no clinical data were available for JM-40. For this drug, the dose given to the dogs was extrapolated from the mouse LD_{50} (i.e. 113 mg/kg or 379 mg/m^2 i.v., unpublished observation).

Assessment of toxicity

Biological parameters. At the day of the first drug treatment, the dogs were continuously observed for vomiting during the first 10 h after administration. During the following 14 days they were observed at least twice daily and at least once daily thereafter. At the days of the second and third drug administration the dogs were observed at least once per hour during 8 h. The occurrence of vomiting was recorded. The body weights of the dogs were recorded on the day of the first drug injection and, with some exceptions, daily during the following 10 days, twice a week until day 42, and weekly for the remainder of the study.

Laboratory parameters. Blood samples were collected for haematology and serum chemistry three times per week during the first 2 weeks and at several occasions thereafter. Erythrocytes, leukocytes and platelets were determined with an Electrozone/Celloscope (Particle Data, Inc., Elmhurst, IL). Haematocrits were determined using a micro-haematocrit centrifuge. A decrease in the total leukocyte and platelet count to less than 2×10^9/l and 40×10^9/l was considered indicative of drug-induced leukocytopenia and thrombocytopenia, respectively. Serum chemistry was performed to follow the functions of liver, heart, kidney, etc. For this purpose standard clinical chemistry laboratory methods were used in the determination of the following serum values: Na, K, Cl, CO_2, urea, creatinine, alkaline phosphatase, SGOT, SGPT, LDH, total bilirubin, total protein, albumin, Ca, P, uric acid, glucose, cholesterol, γ-glutamyl transferase, Fe, Mg, diastase and creatine phosphokinase. Serum urea concentrations over twice the control value were considered abnormal.

As an additional parameter for renal toxicity, we assessed the protein content of urine collected over 24 h using the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA). After the first drug dose, urine was collected daily for 14 days. Supplementary collections were made on several occasions.

Pathological parameters. Six weeks after the last drug dose the dogs were sacrificed. A complete autopsy was performed. Organs were fixed in buffered 4% formaldehyde. The fixed material was embedded in paraplast in the routine manner. Sections of 4 μm were cut, stained with hematoxylin and eosin, and examined microscopically.

RESULTS

Toxic deaths

Two dogs died from drug toxicity. Dog 300, treated with TNO-6, did not eat but drank about 3 l. of water daily in the week before its death on day 54. Its body weight decreased from 12.2 kg on day 42 to 8.7 kg on day 50. During this period, the serum values for urea and creatinine rose to 15.3 mmol/l and 135 μmol/l on day 47, respectively. The average pretreatment urea concentration

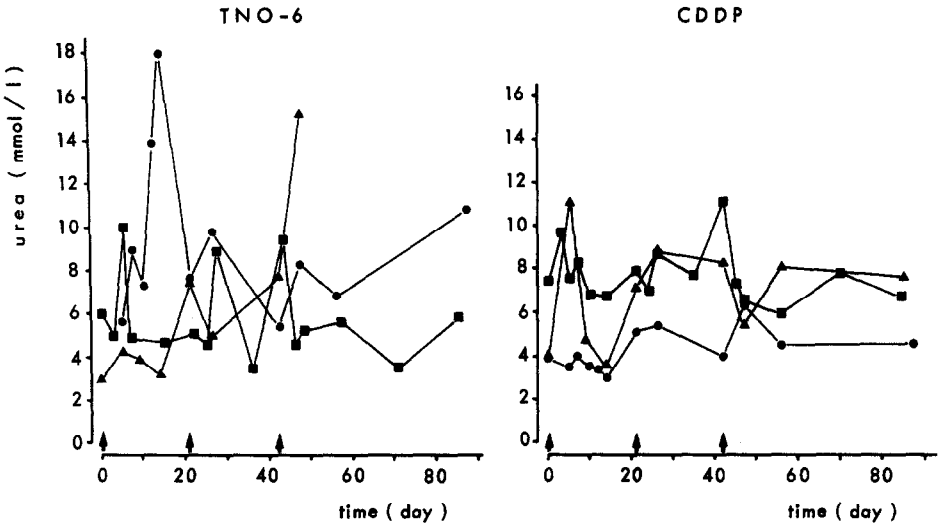

Fig. 1. Urea concentrations in serum of dogs treated with TNO-6 (● dog 932, ▲ dog 300, ■ dog 884) and CDDP (● dog 127, ▲ dog 116, ■ dog 193). The average pretreatment concentration was 4.5 mmol/l (range: 2.7–7.5). Concentrations above twice this value were considered indicative of drug-induced nephrotoxicity. Arrows, days of drug administration.

for the dogs was 4.5 mmol/l (range 2.7–7.5). For creatinine these values were 82 μmol/l (range 65–100). The observed urea and creatinine concentrations were not high enough to conclude that renal failure was the cause of death of dog 300. At autopsy slightly pale kidneys were found. On sectioning, they seemed normal but histopathology showed damage to the entire kidney, consistent with acute renal failure. The damage consisted of irregularity in the basal membranes of glomeruli and widespread cell necrosis in the tubules. Loss of protein was evident by the presence of casts. Dilatation of tubules suggested obstruction.

Dog 237, treated with 10 mg/kg CHIP, also died on day 54. Autopsy and histopathology showed that the cause of death was septicaemia with coliform bacilli. Microorganisms were present in most organs, but there was hardly any inflammatory reaction.

Nephrotoxicity

Urea concentrations in serum of dogs treated with TNO-6 and CDDP are presented in Fig. 1. Concentrations of more than twice the pretreatment value were observed three times for CDDP and seven times for TNO-6 (Fig. 1). The other platinum drugs only rarely caused values above the normal range (data not shown). For TNO-6, most creatinine values fell in the range of 80–110 μmol/l, but repeatedly high values (110–160 μmol/l) were observed (Fig. 2). For CDDP only two measurements were just above 100 μmol/l (Fig. 2). Other values for CDDP as well as for CBDCA, CHIP and JM-40 fell within the normal range. Serum concentrations of electrolytes, liver function enzymes, etc. remained within normal values.

All three dogs treated with TNO-6 exhibited severe proteinurea, with a peak at days 4–5 (Fig. 3). The values returned to normal within three weeks. CDDP produced a mild proteinuria around day 6 (Fig. 3). For both CBDCA and CHIP, only one dog showed mild proteinuria (data no shown). Other dogs as well as those treated with JM-40 did not show significant protein loss by urine. Histopathology of the kidneys showed frank loss of protein after treatment with TNO-6 and CDDP. Some protein deposits were found in Henle's loops after CBDCA. Protein loss was much less or absent for CHIP and JM-40.

Haematotoxicity

After each injection of CHIP leukocyte counts in all three dogs decreased to values below 2×10^9/l. The average pretreatment leukocyte count was 7.2×10^9/l (range 4.7–9.4). The nadir was reached at day 10–11 after drug administration. The other platinum compounds did not cause severe leukocytopenia (data not shown). The average pretreatment platelet count was 119×10^9/l (range 49–193). Platelet counts after treatment with CHIP showed a consistent pattern of decreasing values with a nadir on day 10–15 and recovery thereafter (Fig. 4). The platelets of dog 237 had not yet recovered at the time of the third injection. This may have contributed to its early death. Neither CDDP (Fig. 4) nor the other derivatives (data not shown) caused platelet counts below 40×10^9/l.

The high dose (10 mg/kg) of CHIP in dog 237 caused a decrease in erythrocyte count and haematocrit. From day 10 after the first dose until death both values remained around 65% of their pretreatment values. For the other dogs treated with CHIP

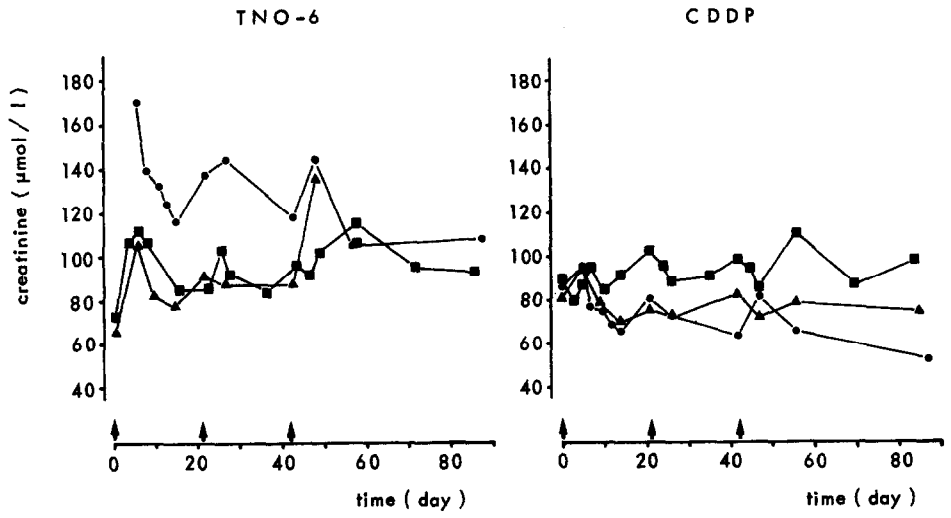

Fig. 2. Creatinine concentrations in serum of dogs treated with TNO-6 (● dog 932, ▲ dog 300, ■ dog 884) and CDDP (● dog 127, ▲ dog 116, ■ dog 193). The pretreatment values fell within a range of 65–100 µmol/l. For TNO-6-treated dog 932 no pretreatment value was obtained. Arrows, days of drug administration.

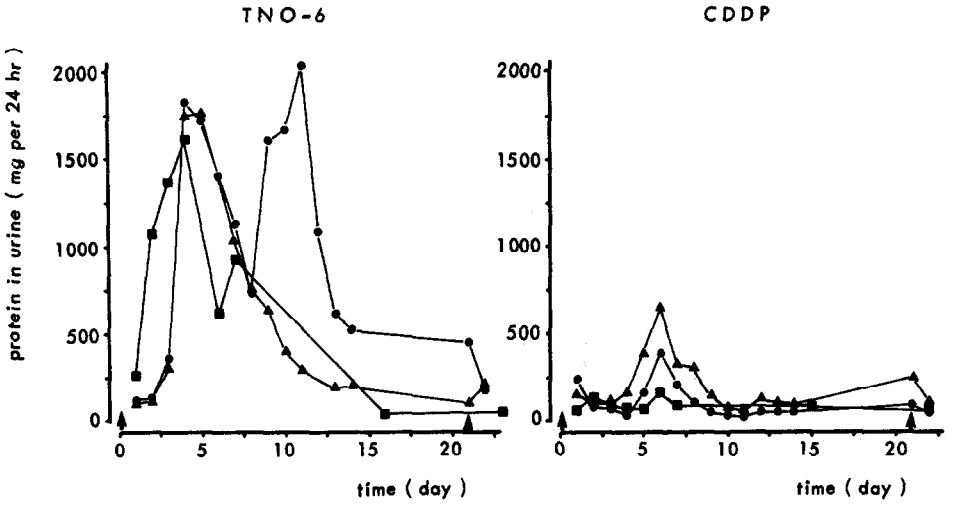

Fig. 3. Protein in urine of dogs treated with TNO-6 (● dog 932, ▲ dog 300, ■ dog 884) and CDDP (● dog 127, ▲ dog 116, ■ dog 193). Arrows, days of drug administration.

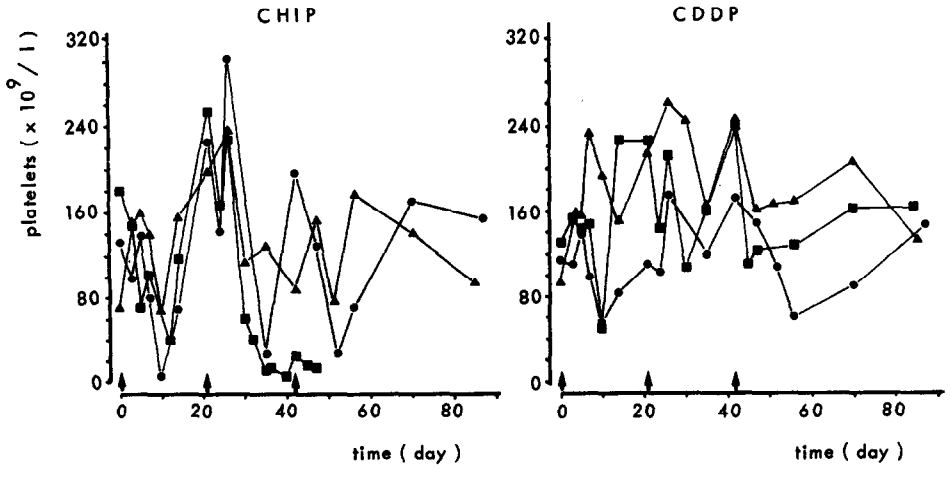

Fig. 4. Platelet counts in dogs treated with CHIP (● dog 005, ▲ dog 075, ■ dog 237) and CDDP (● dog 127, ▲ dog 116, ■ dog 193). Thrombocytopenia was arbitrarily considered to occur at platelet counts below 40 × 10^9/l. Arrows, days of drug administration.

Table 1. *Vomiting by dogs treated with platinum derivatives*

Drug	Dose (mg/kg i.v.)	Dog number	Severity of vomiting after drug injection 1st inj.	2nd inj.	3rd inj.
CDDP	1.2	116	++	++	+
	1.2	127	++	+	++
	1.2	193	++	++	+
TNO-6	1.0	300	++	++	+++
	1.0	884	+++	++	++
	1.0	932	+++	+++	+++
CBDCA	12.0	020	0	0	0
	12.0	021	0	0	0
	12.0	090	0	0	0
CHIP	10.0	237	++	++	++
	6.0	005	+	0	++
	6.0	075	+++	++	+++
JM-40	10.0	121	++	0	+
	10.0	163	0	+	0
	10.0	528	0	0	0

0 No vomiting; + one single vomit; ++ 2–4 vomits; +++ frequent vomiting.

and all dogs treated with the other platinum derivatives erythrocyte counts and haematocrit remained within the normal range.

Post mortem histopathology showed that CDDP caused a marked depletion of red cells, hyperplasia of myelocytes, but no significant effect on megakaryocytes. A greater effect on the bone marrow, especially megakaryosis, was produced by TNO-6 and the high dose (10 mg kg) of CHIP. CBDCA and JM-40 caused a slightly reactive bone marrow, but the number and size of megakaryocytes was normal. Lower doses of CHIP caused loss of follicles and white pulpa in the spleen, which effect was comparable to that of CDDP.

Vomiting

Vomiting, in decreasing order of severity, occurred after treatment with TNO-6, CHIP, CDDP and JM-40, while CBDCA did not lead to vomiting (Table 1). Histopathology showed that CDDP caused marked epithelial damage to the ileum and chronic inflammatory reaction in the crypts of the colon. TNO-6 caused similar gastrointestinal damage, with slightly more inflammation of the colon. Treatment with CHIP caused cell loss in the ileum and, compared to CDDP, more chronic crypt inflammation in the colon. In the ileum, slight repair phenomena were observed after JM-40 and CBDCA, while the colons appeared normal.

Body weight

The body weights of two dogs (numbers 300 and 932) treated with TNO-6 decreased by 13 and 20%, respectively, within 14 days after the first dose. The body weight of the third TNO-6 dog (number 884) as well as of all other dogs showed fluctuations within a range of 8% around the initial weight. Changes of no more than 8% were observed after the second and third drug doses, except for dog 300 as discussed above.

DISCUSSION

The value of the dog model for predicting toxicities of platinum compounds was investigated. Each platinum compound was tested in three dogs to reduce inter-dog variability. Instead of using one dose, a dose–response study would have been more appropriate but this was practically and economically not feasible. Also, at NCI single dose toxicity studies in dogs are usually carried out with the mouse equivalent LD_{10} and 1/10 LD_{10} as calculated on the basis of mg/m^2 body surface area [19, 20]. The LD_{10} is used to make an estimate of the target organ toxicity, while 1/10 LD_{10} is used to assess the safety of this dose. In the present study dogs were treated with single doses of 60% of the mouse equivalent LD_{50} which is about equal to the LD_{10}. At the start of the present study clinical data were already available for CDDP, TNO-6, CBDCA and CHIP, but not for JM-40. These were published during the course of the experiments [21, 22].

Drug toxicity was assessed using biological, laboratory and histopathological parameters. Histopathology 6 weeks after the last drug dose only assessed chronic toxicity. Acute non-lethal toxicity was assessed by haematology and serum chemistry. Attention was focused on the presence or absence of the main side-effects of CDDP: nephrotoxicity,

Table 2. Toxicity correlations between mouse, rat, dog and man

Drug	Vomiting			Haemtotoxicity				Renal toxicity			
	Dog	Man	(Ref.)	Mouse*	Dog	Man	(Ref.)	Rat*	Dog	Man	(Ref.)
CDDP	+	+	[4, 5]	+	−	+	[4, 5]	+	+	+	[4, 5]
TNO-6	+	+	[23,29]	+	−	+	[23,29]	+	+	+	[23,24]
CBDCA	−	+	[17,26]	+	−	+	[17,28]	±	−	±	[25,26]
CHIP	+	+	[18,27]	+	+	+	[18,27]	−	−	−	[18,27]
JM-40	±	+	[21,22]	−	−	−	[21,22]	n.d.	−	+	[21,22]

+ Toxicity present; ± marginal toxicity; − toxicity not observed.
n.d. not determined.
* Results from earlier studies [6].

myelosuppression and vomiting. Other minor observations will not be further discussed.

In contrast to the clinical situation, supporting therapy which could influence the toxicity parameters was usually avoided. It was only given to dog 237 which developed a subcutaneous abscess in its left hind leg on day 10 after treatment with 10 mg/kg CHIP. It was treated with 3 × 1 g ticarcillin (Beecham) daily from day 10 to 16.

Toxic deaths

The occurence of two toxic deaths was unexpected. Based on mouse and human data, the drug doses in the dogs were expected to show signs of toxicity but no death. However, both lethal nephrotoxicity after TNO-6 (dog 300) and lethal haematotoxicity after CHIP (dog 237) were clearly drug related. Septicaemia in the latter dog probably could develop because of drug-induced leukocytopenia.

Nephrotoxicity

Urea concentrations in dogs treated with TNO-6 showed large variations. Moreover, the three dogs did not show the same pattern. After each drug injection, the increase in urea concentration in dog 884 lasted only 1 day and returned to normal values immediately thereafter. Dog 300 died with acute renal failure after a long period of normal urea values.

The use of serum creatinine concentration as a parameter for renal toxicity is less convincing. No values above twice the average control value were observed. As a third parameter for renal toxicity the protein content of urine was determined. TNO-6 caused severe proteinurea which was already obvious during collection of the urine. It foamed and gave an offensive odor. All three parameters for renal toxicity indicated TNO-6 as the most toxic platinum compound. There was, however, variability in response of the three dogs treated with this drug. CDDP was the second most renal-toxic compound. For CBDCA, CHIP and JM-40 less or no renal toxicity or no consistent pattern could be discovered. In general, the severity of histopathological changes in the kidneys parallels the biochemical parameters. In JM-40-treated dogs renal pathology varies. This variation is also found in other parameters for toxicity, such as vomiting. Except for JM-40, these observations in dogs agree with those in man. TNO-6 caused serious and unpredictable nephrotoxicity in 13 out of 292 patients [14]. Therefore, it was discontinued from clinical trials [23, 24]. In comparison with CDDP, renal toxicity in patients was absent or less after treatment with CBDCA [17, 25, 26] and CHIP [18, 27].

Results of earlier studies with CDDP, TNO-6, CBDCA and CHIP [6] showed an excellent correlation of the prediction of nephrotoxicity in rats and its occurrence in the clinic (Table 2). These predictions are confirmed by the present dog study. For JM-40, nausea, vomiting and nephrotoxicity were dose-limiting in man [21, 22]. Up to a dose of 1000 mg/m^2 the renal damage, consisting of glomerular and tubular dysfunction, seemed reversible [22]. The maximum tolerated dose in man is 1200 mg/m^2, a dose much higher than that used in the dogs (240 mg/m^2). This could be the explanation for the absence of renal toxicity in the dogs.

Haematotoxicity

CHIP was the only platinum analogue that caused severe haematologic toxicity. Both the high and the low dose of CHIP led to leukocytopenia and thrombocytopenia. These findings were confirmed by changes in the histopathology of the bone marrow. This is in good agreement with clinical observations of thrombocytopenia as dose-limiting toxicity [18]. A decrease in erythrocyte count and haematocrit was seen only after the high dose of CHIP. In contrast to clinical experience no haematotoxicity was seen after CDDP. The absence of thrombocytopenia after TNO-6 and CBDCA in

dogs is in sharp contrast with their dose-limiting effects on platelets in man [17, 25, 28–30] (Table 2). In earlier studies, survival of mouse bone marrow stem cells (colony-forming units spleen, CFU-S) was determined after treatment with the platinum drugs [6]. In Table 2, reductions in CFU-S survival to values below 5% are indicative of toxicity, but a ranking was not made. At equitoxic doses (LD_{50}), TNO-6 and CBDCA reduced CFU-S survival to a much greater extent that did CDDP and CHIP. JM-40 did not cause dose-related myelosuppression in dogs (this study) nor in man [21, 22]. In mice, JM-40 caused hardly any bone marrow toxicity. Overall, the CFU-S system in mice seems a better predictor of haematotoxicity in man than is the dog.

Vomiting

Cancer patients treated with repeated courses of CDDP tend to show an increased severity of vomiting. The dogs in the present study received only three drug courses but no such increase could be observed. Treatment with TNO-6 and CHIP led to more frequent vomiting than CDDP in dogs. TNO-6 caused a mucous vomit, while CHIP caused large volumes of watery vomit. The low frequency of vomiting seen in dogs after JM-40 is in contrast to the severe vomiting in man [21, 22, 31]. However, the drug doses also differ. The dogs were treated with 240 mg/m^2. In man, dose-dependent vomiting became severe above 240 mg/m^2. The mild to moderate vomiting observed in most patients treated with CBDCA [17, 25, 26] was not correctly predicted by dogs, in which we noticed no vomiting at all. At the Bristol laboratories, however, CBDCA did show a moderate emetic potential [32]. For CDDP, TNO-6 and CHIP vomiting in dogs and man was comparable (Table 2).

A new model in emesis research using ferrets was developed by Florczyk *et al.* [33]. In their studies the ferret and the dog appeared to have comparable emetic responses. Originally, low doses of JM-40 predicted a lack of vomiting in ferrets [34]. When the test was repeated with higher doses vomiting was observed in two of the three ferrets tested. The onset of emesis and the number of episodes were generally comparable for JM-40- and CDDP-treated animals. The higher drug doses were lethal to the ferrets. The emetic potential of JM-40 was much less of a problem after non-lethal doses [34].

The occurrence of renal toxicity, haematotoxicity and vomiting after treatment with the platinum compounds of both dogs and man are summarized in Table 2. The results obtained earlier using mice and rats [6] are included. Mice and rats accurately predicted haematotoxicity and nephrotoxicity in man. The dog confirmed the prediction of renal toxicity for all drugs, except JM-40. The difference between the drug doses given to the dogs and man may be the explanation for this incorrect prediction. The clinical haematotoxicity observed after CDDP, TNO-6 and CBDCA was not correctly predicted by the dog. Since mice and rats do not vomit the dog could provide additional information for this type of toxicity. Vomiting in man was correctly predicted for CDDP, TNO-6 and CHIP, but doubtfully for JM-40, and incorrect for CBDCA. It may be concluded that the usefulness of the dog for predicting toxicity of platinum analogues is limited, and hampered by biological variation also known in man.

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