Br. J. Pharmac. (1981), **73**, 649–654

THE EFFECT OF HEAVY METAL CHELATORS ON THE RENAL ACCUMULATION OF PLATINUM AFTER *cis*-DICHLORODIAMMINEPLATINUM II ADMINISTRATION TO THE RAT

JOSEPH GRAZIANO, BRIAN JONES & PATRICIA PISCIOTTO[1]

Departments of Pediatrics and Pharmacology, Cornell University Medical Center, and Columbia University College of Physicians and Surgeons, New York, New York, U.S.A.

1 Rats received a total of 18 mg/kg *cis*-dichlorodiammineplatinum (II) (CDDP) intravenously and were treated concomitantly with calcium-disodium ethylenediaminetetraacetic acid ($CaNa_2EDTA$), 2,3-dimercaptopropanol (BAL), deferoxamine, 2,3-dimercaptosuccinic acid (DMS) or vehicle. In comparison to controls, renal platinum concentration was significantly reduced in the DMS and deferoxamine-treated groups. However, significant deterioration occurred in the deferoxamine-treated group. The hepatic platinum concentration was unaffected by the chelating agents.

2 Following a dose of 6 mg/kg CDDP intravenously, eight days of treatment with DMS, 50 mg/kg daily, had no effect on renal platinum excretion, while treatment with 100 or 200 mg/kg daily reduced renal platinum concentration by 50%.

3 In order to determine whether DMS could prevent the nephrotoxicity of CDDP, rats were given 6 mg/kg CDDP intravenously, followed by a four day course of DMS treatment at doses of 0, 50, 100 or 200 mg/kg daily begun 3 h after the CDDP dose. DMS failed to prevent renal toxicity as indicated by weight loss, serum creatinine concentration, renal histology, and the urinary excretion of N-acetyl-β-glucosaminidase, a renal tubular enzyme.

Introduction

cis-Diamminedichloroplatinum (II) (CDDP) is one of a number of platinum complexes with antitumour activity (Rosenberg, Van Camp, Trosko & Mansour, 1969). CDDP is one of the most active drugs against testicular tumours (Carter & Wasserman, 1975) and its role in the possible treatment of other types of cancer is under active investigation (Harder & Rosenberg, 1970; Wiltshaw & Kroner, 1976; Yagoda, 1977; Wittes, Cvitkovic, Shah, Gerold & Strong, 1977). Although it appears that CDDP exerts its cytotoxic effect through binding to the nucleotide bases of DNA, like the nitrogen mustards this compound will also readily react with nucleophilic groups of protein, such as R—SH, R—S—CH_3, R—NH_2 and >N (Tomson, 1974).

CDDP is associated with cumulative renal toxicity which is dose-related and often dose-limiting (Kociba & Sleight, 1971; Leonard, Eccleston, Jones, Todd & Walpole, 1971; Schaeppi, Heyman, Fleischman, Rosencrantz, Ilievski, Phelan, Cooney & Davis, 1973). Proximal renal tubular lesions produced by CDDP have been well characterized both in animals and man and appear to be similar to the lesions observed with exposure to heavy metals, particularly those occurring in mercury poisoning (Schaeppi *et al.*, 1973; Madias and Harrington, 1978). Heavy metal nephrotoxicity, presumably due to the inactivation of cysteine-rich enzymes, is usually reversible after treatment with chelating agents to remove the heavy metal (Goodman & Gilman, 1975). For this reason we have examined a series of chelating agents for their ability to remove platinum from the kidneys of animals which received CDDP. One of these chelators, 2,3-dimercaptosuccinic acid (DMS), a new orally effective agent for the treatment of heavy metal poisoning (Friedheim & Corvi, 1975; Friedheim, Corvi & Walker, 1976; Friedheim, Graziano & Kaul, 1978; Graziano, Leong & Friedheim, 1978a; Graziano, Cuccia & Friedheim, 1978b), appears capable of reducing the renal platinum concentration by inducing platinum excretion into the urine. However, in these experiments, in which DMS therapy was started 3 h after CDDP administration, we were unable to prevent renal injury as determined by histology and by urinary elimination of N-acetyl-β-glucosaminidase, an enzyme that has been reported to be a sensitive indicator of

[1]Present address: Department of Laboratory Medicine, University of Connecticut Health Center, Farmington, CT 06032 U.S.A.

0007-1188/81/070649-06 $01.00

renal damage due to CDDP (Jones, Bhalla, Mladek, Kaleya, Gralla, Alcock, Schwartz, Young & Reidenberg, 1980).

Methods

Drug treatment and tissue sampling

Evaluation of chelating agents Thirty male albino Sprague-Dawley rats (175–225 g; Charles Rivers Lab., Wilmington, Mass, U.S.A.) received *cis*-diamminedichloroplatinum II; (Ben-Venue Lab. Inc., Bedford, Ohio, U.S.A.), 9 mg/kg intravenously. Subgroups of six rats received calcium-disodium ethylenediaminetetraacetic acid ($CaNa_2EDTA$, Riker Lab. Inc., Northridge, Ca., U.S.A.), 2,3,dimercaptopropanol (BAL, Aldrich Chemical Co. Inc., Milwaukee, Wisc., U.S.A.), deferoxamine (CIBA Pharmaceutical Co., Summit, N.J., U.S.A.), or 2,3-dimercaptosuccinic acid (DMS; Eastern Chemical, Hauppauge, N.Y., U.S.A.) at a dose of 30 mg/kg intraperitoneally 3 h after CDDP administration and 15 mg/kg intraperitoneally twice daily for the following four days. EDTA and deferoxamine were administered in saline (0.9% w/v NaCl solution), BAL in corn oil and DMS in 5% sodium bicarbonate; all drugs were prepared at a concentration of 10 mg/ml. A control group of six rats received only saline. A second dose of CDDP (9 mg/kg i.v.) was administered on day eight followed by chelation therapy as above, at a dose of 30 mg/kg (i.p.) at 3 h, and 15 mg/kg (i.p.) twice daily for the following two days. Due to obvious deterioration and weight loss in all treatment groups, rats were killed on day eleven by overdosing with ether. Their kidneys and livers were removed, rinsed with physiological saline, weighed and immediately analysed for platinum content.

All animals were weighed before treatment and when they were killed. Blood was obtained from the tail before administration of CDDP and on day eight for the determination of serum creatinine concentration (Beckman Creatinine Analyzer 2, Anaheim, Ca., U.S.A.).

Tissue platinum as a function of DMS dose Twenty-four male albino Sprague-Dawley rats (150–175 g) received CDDP, 6 mg/kg (i.v.). Subgroups of 6 rats received DMS at doses of 0, 50, 100 or 200 mg/kg daily (i.p.). A single dose was administered on day 1, 3 h after CDDP administration, then two divided doses daily from day 2 to day 8. DMS was administered in 5% sodium bicarbonate, at a concentration of 20 mg/ml (pH 7.2). The control group of rats received the vehicle alone, adjusted to pH 7.2. With each administration of DMS, rats also received 3 ml of 0.45% NaCl (i.p.) to promote diuresis and CDDP excretion. All rats were killed on day 9 by overdosing with ether. Kidneys and livers were removed for platinum analysis as described above.

Effect of DMS on urinary platinum excretion and enzymuria Twenty male albino Sprague-Dawley rats (135–155 g) received CDDP 6 mg/kg (i.v.). Subgroups of 5 rats received DMS at doses of 0, 50, 100 or 200 mg/kg daily (i.p.) as previously described. A single dose was administered on day 1, 3 h after CDDP administration, then two divided doses daily from day 2 to day 4. A control group received the vehicle. A fifth group of four rats received only DMS 200 mg/kg (i.p.) at the same dose schedule.

Throughout the experiment, rats were kept in metabolic cages and urine was collected for N-acetyl-β-glucosaminidase (NAG) and platinum determinations. Urinary platinum was determined on day 1, while urinary NAG was determined on days 1–4. Rats were weighed on days 1, 3 and 5. All rats were killed on day 5 and kidney and intestinal specimens prepared for histology. All histological specimens were examined to determine whether DMS had a protective effect against CDDP toxicity. Blood was obtained for serum creatinine concentration before the administration of CDDP and at the time of death.

N-acetyl-β-glucosaminidase determination

For the NAG assay, modified from Leabach & Walker (1961), all substrates, standards, and the lyophilized bovine serum albumin were obtained from Sigma Chemical (St Louis, Missouri, U.S.A.). All other reagents were purchased from Fisher Scientific (Pittsburgh, Pennsylvania, U.S.A.).

The reaction mix contained 10 to 25 μl of a 1:20 dilution of urine in distilled water, 0.264 mM 4-methylumbelliferyl *N*-acetyl glucosamine, and 0.01% (w/v) bovine serum albumin in 0.05 M sodium citrate buffer (pH 5.0). The total reaction volume was 1 ml. The reaction was carried out at 37°C for 30 min under constant agitation; 3 ml 0.2 M sodium glycinate buffer (pH 10.65) was then added to stop the reaction. Reaction controls for each urine sample (where urine is added immediately prior to quenching) were run in an identical manner.

The amount of liberated 4-methylumbelliferone was measured as the increase in fluorescence (excitation at 360 nm, emission at 450 nm) on a Farrand Foci spectrofluorimeter using 2 μg/ml quinine sulphate in 0.1 N sulphuric acid as a fluorescence standard. Standard concentration curves were generated from 10^{-5} to 10^{-9} M concentrations of 4-methylumbelliferone in 0.15 M sodium glycinate buffer (pH 10.3). All determinations were performed in duplicate. Urinary NAG concentrations were ex-

pressed as μmol product formed (4-methylumbelliferone) per h of incubation and per mg urinary creatinine.

Urinary creatinine levels were determined with the Gemini Creatinine Reagent Kit from Electronucleonics (Fairfield, New Jersey, U.S.A.).

Tissue and urine platinum determination

Tissues were prepared according to the method of Pera & Harder (1977) with slight modification. A known weight of deionized water (2 ml/g liver, 5 ml/g kidney) was added and the tissue was homogenized with a Virtis Homogenizer (Virtis Corp., Gardiner, N.Y., U.S.A.). An aliquot containing 1 g of tissue was transferred to Pyrex tubes and dried overnight in a Thelco Oven (model 26, Scientific Products, McGaw Park, Illinois, U.S.A.) at 90°C; 1 ml 70% nitric acid was added to the tubes containing dried tissue homogenate. The tubes were capped with marbles and placed in a Tempblock module heater (model H2025; Scientific Products, McGaw Park, Ill.) at 30°C. The samples were allowed to dissolve slowly over 30 min, then the heat was increased to boiling and the samples were refluxed for 24 h. After cooling, the acid phase was transferred to a 10 ml volumetric flask and 1 ml of concentrated nitric acid was added to the lipid phase remaining in the Pyrex tube. The sample was refluxed for another 15 min, and after cooling the second acid phase was transferred to the volumetric flask and then diluted to a total of 10 ml with deionized water.

Urine samples were diluted with 4 volumes of distilled deionized water and analysed directly for platinum as described below.

Tissue and urine samples were analysed by atomic absorption spectrophotometry using a Model 305B Atomic Absorption Spectrophotometer with a platinum hollow cathode lamp, and a Model HGA 2200 Heated Graphite Furnace with a Ramp Programmer and Model 56 Recorder (Perkin Elmer Corp., Norwalk, Ct, U.S.A.). The furnace was programmed to deliver a 25 s drying phase at 100°C, a 20 s ramped heating to 1600°C followed by 30 s of constant temperature charring, then a 10 s atomization at 2700°C. N_2 was used to purge the furnace at a flow rate of 15 ml/min. A platinum standard containing 1000 mg of platinum per litre of 0.5% HCl (Alpha Analytical Lab., Jersey City, N.J., U.S.A.) was diluted to 10 mg/l in 20% nitric acid for use as a stock solution. The stock solution was diluted to prepare standards of 0.5 mg, 1 mg, 2 mg, 3 mg and 4 mg of platinum per litre with 0.5% HNO3. Aliquots of 20 μl were introduced into the graphite furnace using model AS-1 Auto-Sampler (Perkin Elmer Corp., Norwalk, Ct, U.S.A.). All determinations were performed in duplicate.

Results

Evaluation of chelating agents

Five groups of rats received CDDP (i.v.), followed by chelation therapy which began 3 h later. The tissue concentrations of platinum, expressed as μg Pt/g wet wt. tissue, are shown in Table 1. Groups of rats receiving either EDTA or BAL (Table 1) showed no significant difference in kidney concentration of platinum as compared with control values. However, the platinum concentration in the liver was increased slightly but significantly ($P < 0.05$) in both groups as compared to the controls. The kidney concentrations of platinum in rats receiving either DMS or deferoxamine were significantly decreased ($P < 0.001$) as compared to controls. There was no significant difference in platinum concentration in the liver after treatment with either DMS or deferoxamine.

Table 1 Tissue concentration of platinum following chelation therapy

Treatment[1]	*Tissue platinum* (μg/g wet wt.) *Kidney*	*Liver*
Control	3.02 ± 0.09	1.28 ± 0.04
EDTA	2.76 ± 0.15	1.42 ± 0.03*
BAL	3.44 ± 0.18	1.45 ± 0.06*
DMS	2.40 ± 0.06***	1.17 ± 0.05
DF	2.18 ± 0.09***	1.23 ± 0.14

[1]All treatment groups received two doses of 9 mg/kg *cis*-dichlorodiammine platinum (II).
Values are mean ± s.e.mean; $n = 6$. EDTA = calcium disodium ethylenediaminetetraacetic acid; BAL = 2,3-dimercaptopropanol; DMS = 2,3-dimercaptosuccinic acid; DF = deferoxamine.
*$P < 0.05$ compared to control values; ***$P < 0.001$ compared to control values.

Serum creatinine concentrations rose in all treatment groups (Table 2). Both EDTA and BAL, drugs which themselves can produce renal toxicity (Goodman & Gilman, 1975), led to even further increases in serum creatinine concentrations but only with EDTA was this significantly different from the control ($P < 0.05$). Each treatment group lost weight during the experiment (Table 2). Rats treated with EDTA or deferoxamine lost significantly more weight than those which received CDDP alone ($P < 0.001$ and $P < 0.02$, respectively).

Tissue platinum concentration as a function of DMS dose

Four groups of rats received 6 mg/kg CDDP (i.v.) and were subsequently treated with various doses of

Table 2 Serum creatinine concentration (Cr) and body weight before and after chelation therapy

Treatment[1]	*Initial Cr* (mg/dl)	*Final Cr* (mg/dl)	*Initial wt.* (g)	*Final wt.* (expressed as % change)
Control	0.48 ± 0.01	1.48 ± 0.07	186 ± 4	−19.0 ± 2.5
EDTA	0.49 ± 0.03	2.08 ± 0.22*	201 ± 7	−31.5 ± 1.8***
BAL	0.49 ± 0.02	2.35 ± 0.55	205 ± 4	−27.7 ± 2.8
DMS	0.50 ± 0.03	1.63 ± 0.20	200 ± 4	−23.5 ± 2.9
DF	0.49 ± 0.03	1.87 ± 0.48	204 ± 3	−31.4 ± 3.1**

[1]All treatment groups received two doses of 9 mg/kg *cis*-dichlorodiammineplatinum (II).
Values are mean ± s.e.mean; $n = 6$. Abbreviations as in Table 1.
*$P < 0.05$ as compared to control values; **$P < 0.02$ as compared to control values; ***$P < 0.001$ as compared to control values.

DMS for eight days. Again, the first DMS dose was administered 3 h after the CDDP injection (see methods). In groups which received doses of 100 or 200 mg/kg daily of DMS, the renal platinum concentration (Table 3) was reduced to approximately half the control value ($P < 0.02$). The liver platinum concentrations were not significantly different from the controls in any of the DMS groups. However, in all groups the liver platinum concentration was markedly reduced in comparison to the previous experiment (Table 1) in which rats received a total dose of 18 mg/kg CDDP (i.v.).

Urinary platinum and NAG excretion as functions of the DMS dose

Groups of rats were treated with 6 mg/kg CDDP (i.v.), followed by four days of DMS treatment which began 3 h after CDDP administration. The objects of this experiment were to determine if DMS treatment could protect against CDDP renal injury, and to determine if urinary platinum excretion occurs in response to DMS.

Urinary platinum excretion appeared to increase as a function of the DMS dose (Table 4), but only the response to 200 mg/kg DMS was significantly different from the control group (12.98 μg/d vs 5.49 μg/d; $P < 0.05$).

Despite its enhancement of platinum excretion, DMS appeared unable to prevent renal injury. As in the first experiment, in which a higher dose of CDDP was used (Table 2), the administration of DMS at doses of 50, 100, or 200 mg/kg daily failed to prevent the weight loss and elevation of serum creatinine associated with CDDP administration (Table 5). Rats which received 200 mg/kg daily of DMS but no CDDP gained weight and showed no change in serum creatinine during the study period (Table 5). In addition, DMS failed to prevent the urinary excretion of NAG (Table 6). In each of the CDDP-treated groups, urinary NAG rose steadily during the four day study period. In groups of rats which received 50, 100, or 200 mg/kg daily of DMS, urinary NAG was not significantly different from controls which received CDDP alone. Rats which received 200 mg/kg DMS alone showed no change in urinary NAG concentration throughout the study period.

Renal histology showed severe necrosis of the majority of tubules of the inner cortex in all rats in the control group, 2 rats in the groups receiving DMS at a dose of 50 or 200 mg/kg, and 4 rats receiving DMS 100 mg/kg. The remaining rats showed moderate

Table 3 Tissue concentration of platinum as a function of 2,3-dimercaptosuccinic acid (DMS) dose

Drug treatment *CDDP* (i.v.)	*DMS* (i.p.)	*Tissue platinum* (μg/g wet wt.) *Kidney*	*Liver*
6 mg/kg	0 mg/kg	5.03 ± 0.59	0.29 ± 0.17
6 mg/kg	50 mg/kg	5.15 ± 0.35	0.41 ± 0.20
6 mg/kg	100 mg/kg	2.57 ± 0.37**	0.18 ± 0.09
6 mg/kg	200 mg/kg	2.51 ± 0.22*	0.26 ± 0.13

Values are mean ± s.e.mean; $n = 6$.
*$P < 0.01$ compared to control values;
**$P < 0.02$ compared to control values.

Table 4 Urinary platinum excretion as a function of 2,3-dimercaptosuccinic acid (DMS) dose

Drug treatment *CDDP* (i.v.)	*DMS* (i.p.)	*Platinum excretion* (μg/dl)
6 mg/kg	0 mg/kg	5.49 ± 1.69
6 mg/kg	50 mg/kg	9.48 ± 1.78
6 mg/kg	100 mg/kg	9.93 ± 1.72
6 mg/kg	200 mg/kg	12.98 ± 2.27*
0 mg/kg	200 mg/kg	1.36 ± 0.24

Values are mean ± s.e.mean; $n = 5$.
*$P < 0.05$ as compared to control values.

Table 5 Body weight and serum creatinine concentration (Cr) before and after treatment

Drug treatments[1]		*Initial wt.*	*Final wt.*	*Initial Cr*	*Final Cr*
CDDP (i.v.)	*DMS* (i.p.)	(g)	(% change)	(mg/dl)	(mg/dl)
6 mg/kg	0 mg/kg	142 ± 3	−21 ± 3	0.40 ± 0.03	1.96 ± 0.10
6 mg/kg	50 mg/kg	150 ± 4	−21 ± 2	0.50 ± 0.03	1.80 ± 0.21
6 mg/kg	100 mg/kg	144 ± 2	−19 ± 3	0.40 ± 0.03	2.06 ± 0.16
6 mg/kg	200 mg/kg	148 ± 6	−24 ± 3	0.42 ± 0.02	2.12 ± 0.46
0 mg/kg	200 mg/kg	142 ± 7	+23 ± 2	0.38 ± 0.03	0.45 ± 0.03

[1] All treatment groups, except the last, received CDDP 6 mg/kg i.v.
Values are mean ± s.e.mean; $n = 5$.

tubular necrosis except the group receiving DMS alone, where no renal lesions were observed.

Discussion

Nephrotoxicity is a major factor limiting the clinical usefulness of CDDP (Madias & Harrington, 1978). Both animal and clinical studies have described uraemia associated with degeneration and necrosis of the proximal convoluted tubules, distal tubular dilatation and necrosis, interstitial oedema and lymphocytic infiltration in response to CDDP. This nephrotoxic picture is similar to that seen in cases of intoxication with lead and mercury (Schaeppi *et al.*, 1973; Madias & Harrington, 1978), heavy metals whose renal toxicities are reversible in response to effective chelation therapy (Goodman & Gilman, 1975). We therefore set out to determine (a) which chelating agents could reduce the renal platinum concentration, and (b) whether chelation therapy following CDDP administration could ameliorate the nephrotoxicity and, possibly, prevent the cumulative renal toxicity seen clinically in patients treated with repeated doses.

Of the four chelating agents tested at doses of 30 mg/kg, two, deferoxamine and DMS, significantly reduced the renal platinum concentration (Table 1) in rats which received a total of 18 mg/kg CDDP (i.v.). However, deferoxamine treatment following CDDP administration led to an obvious clinical deterioration, dehydration and a significantly greater weight loss than with CDDP alone or the combination of CDDP plus DMS (Table 2). We therefore focused our efforts on DMS to determine the most effective DMS dose schedule for reducing the renal platinum concentration. Since in the first experiment DMS did not prevent weight loss or the increase in serum creatinine (Table 2), we reduced the dose of CDDP to 6 mg/kg in subsequent experiments and administered fluids parenterally to try to prevent dehydration. Following a dose of 6 mg/kg CDDP (i.v.), eight days of treatment with 50 mg/kg daily of DMS had no effect, while treatment with 100 or 200 mg/kg daily reduced the renal platinum concentration by 50%.

Despite its ability to remove a substantial quantity of platinum from the kidney by promoting urinary platinum excretion (Table 4), DMS was apparently unable to reverse CDDP nephrotoxicity. DMS did not prevent weight loss, nor did it reduce the serum creatinine concentration (Table 5). Rats treated with 6 mg/kg CDDP (i.v.) showed a progressive increase in urinary N-acetylglucosaminidase excretion during the four day study period (Table 6). N-acetylglucosaminidase is primarily a proximal tubular enzyme whose appearance in the urine is a useful clinical indicator of CDDP nephrotoxicity in man (Jones *et al.*, 1980). Various dose regimens of DMS failed to prevent the enzymuria following CDDP treatment (Table 6).

We can only speculate as to why DMS failed to ameliorate CDDP nephrotoxicity. Studies in rats in which blood urea nitrogen was used as an indicator of

Table 6 Urine N-acetyl-β-glucosaminidase (NAG) concentration

Drug treatments		*NAG* (μmol 4-methylumbelliferone h^{-1} mg^{-1} Cr)			
CDDP (i.v.)	*DMS* (i.p.)	*Day 1*	*Day 2*	*Day 3*	*Day 4*
6 mg/kg	0 mg/kg	0.42 ± 0.07	0.85 ± 0.30	0.86 ± 0.33	1.49 ± 0.40
6 mg/kg	50 mg/kg	0.56 ± 0.05	0.67 ± 0.13	1.35 ± 0.31	2.52 ± 0.60
6 mg/kg	100 mg/kg	0.67 ± 0.10	0.59 ± 0.21	1.35 ± 0.11	2.34 ± 0.41
6 mg/kg	200 mg/kg	0.61 ± 0.05	0.72 ± 0.17	1.08 ± 0.27	4.13 ± 1.72
0 mg/kg	200 mg/kg	0.37 ± 0.02	0.48 ± 0.07	0.41 ± 0.06	0.30 ± 0.05

Values are mean ± s.e.mean; $n = 5$.

renal function have shown that blood urea nitrogen concentrations reach a peak four to five days after administration of a single dose of 12.2 mg/kg CDDP (i.p.) and return to near normal by day 14 (Kociba & Sleight, 1971). Perhaps our experiments were too short in duration, and would have shown a more rapid renal recovery if allowed to continue; but the histological appearance of the kidney on day 5 was most discouraging and this seems unlikely. Perhaps those platinum molecules which are not removed by DMS reside covalently bound to a particular enzyme or other macromolecule essential for proximal tubular function.

References

CARTER, S.K. & WASSERMAN, T.H. (1975). The chemotherapy of urologic cancer. *Cancer*, **36**, 729–747.

FRIEDHEIM, E. & CORVI, C. (1975). Meso-dimercaptosuccinic acid: a chelating agent for the treatment of mercury poisoning. *J. Pharm. Pharmac.*, **27**, 624–626.

FRIEDHEIM, E., CORVI, C. & WALKER, C.H. (1976). Meso-dimercaptosuccinic acid: A chelating agent for the treatment of mercury and lead poisoning. *J. Pharm. Pharmac.*, **28**, 711–712.

FRIEDHEIM, E., GRAZIANO, J.H. & KAUL, B. (1978). Treatment of lead poisoning by 2,3-dimercaptosuccinic acid. *Lancet*, **ii**, 1234.

GOODMAN, L.S. & GILMAN, A. (1975). *The Pharmacological Basis of Therapeutics*, Sixth edition. pp. 1298–1299. New York: The Macmillan Company.

GRAZIANO, J.H., LEONG, J.K. & FRIEDHEIM, E. (1978a). 2,3-Dimercaptosuccinic acid: a new agent for the treatment of lead poisoning. *J. Pharmac. exp. Ther.*, **206**, 696–700.

GRAZIANO, J.H., CUCCIA, D. & FRIEDHEIM, E. (1978b). Potential usefulness of 2,3-dimercaptouccinic acid for the treatment of arsenic poisoning. *J. Pharmac. exp. Ther.*, **207**, 1051–1055.

HARDER, H.C. & ROSENBERG, B. (1970). Inhibitory effects of anti-tumor platinum compounds on DNA, RNA and protein synthesis in mammalian cells *in vitro*. *Int. J. Cancer*, **6**, 207.

JONES, B.K., BHALLA, R.B., MLADEK, J., KALEYA, B.A., GRALLA, R.J., ALCOCK, N.W., SCHWARTZ, M.K., YOUNG, C.W. & REIDENBERG, M.M. (1980). Comparison of methods of evaluating nephrotoxicity of *cis*-platinum. *Clin. Pharmac. Ther.*, **27**, 557–562.

KOCIBA, R.J. & SLEIGHT, S.D. (1971). Acute toxicologic and pathologic effects of *cis*-diamminedichloroplatinum (NSC-119875) in the male rat. *Cancer Chemother. Rep.*, **55**, 1–8.

LEABACH, D.E. & WALKER, P.G. (1961). The fluorometric assay of N-acetyl-β-glucosaminidase. *Biochem. J.*, **78**, 151–156.

LEONARD, B.J., ECCLESTON, E., JONES, D., TODD, P. & WALPOLE, A. (1971). Antileukemic and nephrotoxic properties of platinum compounds. *Nature*, **234**, 43–45.

MADIAS, N.E. & HARRINGTON, J.T. (1978). Platinum nephrotoxicity. *Am. J. Med.*, **65**, 307–314.

PERA, JR., M.F. & HARDER, H.C. (1977). Analysis for platinum in biological material by flameless atomic absorption spectrometry. *Clin. Chem.*, **23**, 1245–1249.

ROSENBERG, B., VAN CAMP, L., TROSKO, J.E. & MANSOUR, V.H. (1969). Platinum compounds: a new class of potent antitumor agents. *Nature*, **222**, 385–386.

SCHAEPPI, U., HEYMAN, I.A., FLEISCHMAN, R.W., ROSENCRANTZ, H., ILIEVSKI, V., PHELAN, R., COONEY, D.A. & DAVIS, R.D. (1973). *cis*-dichlorodiammine platinum (II) (NSC-119875): Preclinical toxicologic evaluation of intravenous injection in dogs, monkeys and mice. *Toxic. appl. Pharmac.*, **25**, 230–241.

TOMSON, A.J. (1974). The interactions of platinum compounds with biological molecules. *Recent Results Cancer Res.*, **48**, 38.

WILTSHAW, E. & KRONER, T. (1976). Phase II study of *cis*-dichlorodiammine platinum (II) (NSC-119875) in advanced adenocarcinoma of the ovary. *Cancer Treatment Rep.*, **60**, 55.

WITTES, R.E., CVITKOVIC, E., SHAH, J., GEROLD, F.P. & STRONG, E.W. (1977). *cis*-dichlorodiammine platinum (II) in the treatment of epidermoid carcinoma of the head and neck. *Cancer Treatment Rep.*, **61**, 359.

YAGODA, A. (1977). Future implications of phase 2 chemotherapy trials in ninety-five patients with measurable advanced bladder cancer. *Cancer Res.*, **37**, 2775.

(Received November 11, 1980.
Revised March 19, 1981.)