Biochemical Pharmacology, Vol. 36, No. 12, pp. 1925–1932, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

THE COMPARATIVE PHARMACOKINETICS OF CARBOPLATIN AND CISPLATIN IN MICE AND RATS

ZAHID H. SIDDIK,* DAVID R. NEWELL, FRANCES E. BOXALL and KENNETH R. HARRAP
Department of Biochemical Pharmacology, Drug Development Section, Institute of Cancer Research, Belmont, Sutton, Surrey SM2 5PX, U.K.

(*Received* 11 *September* 1986; *accepted* 6 *January* 1987)

Abstract—The plasma, urinary and biliary clearances of cisplatin and its non-nephrotoxic analogue, Carboplatin (*cis*-diammine-1,1-cyclobutane dicarboxylate platinum II, CBDCA, JM8) have been determined in mice and rats following intravenous administration of the compounds. The plasma concentration–time curves were biphasic during the time period studied (0–60 min), with $t_{1/2\alpha}$ of 2–3 min for both platinum complexes and $t_{1/2\beta}$ of 10–15 min for cisplatin and 25–26 min for Carboplatin. The kinetic rate constants, k_{12} and k_{21}, were similar for both Carboplatin and cisplatin, indicating that there was no appreciable net accumulation of the compounds in the peripheral tissues. Immediately after administration, Carboplatin became reversibly bound to plasma proteins *in vivo* to the extent of about 20%. Appreciable irreversible binding appeared after the first 60 min and increased steadily, so that by 4 hr only 34% of the compound was present in the plasma as the free drug. In comparison, binding of cisplatin to plasma was exclusively irreversible and, after the first 10 min, free drug disappeared rapidly, such that by 60 min free platinum was not detectable. The plasma clearance of free cisplatin (26.1 ml/min/kg) was significantly greater than that of either Carboplatin (10.3 ml/min/kg) or inulin (10.1 ml/min/kg). The main route of excretion of the two platinum complexes was via the urine, with 80–90% of Carboplatin and 43–48% of cisplatin being excreted within 4 hr. In the rat, the Carboplatin excreted in the urine was predominantly as the unchanged compound. The renal clearance of cisplatin (12.3 ml/min/kg) was significantly greater than that of either Carboplatin (9.3 ml/min/kg) or inulin (9.6 ml/min/kg), suggesting that cisplatin was excreted by an active renal secretory mechanism whilst Carboplatin was eliminated by glomerular filtration alone. Biliary excretion of the two compounds was only 0.4–1.2% of the administered dose in 6 hr, with biliary clearance of cisplatin (0.27 ml/min/kg) being fivefold greater than that of Carboplatin (0.053 ml/min/kg). The results indicate that the major pharmacokinetic differences between Carboplatin and cisplatin relate to their renal handling and their reactivity with macromolecules. These differences may well underline the substantial lack of Carboplatin nephrotoxicity in comparison with cisplatin.

Since its introduction into clinical practice, cisplatin has assumed an important role in cancer chemotherapy, particularly against cancers of the ovary, testes, bladder and the head and neck [1]. However, the use of cisplatin is complicated by a number of side-effects, notably nephrotoxicity, nausea and vomiting, peripheral neuropathy, myelotoxicity and ototoxicity [1]. Of these, irreversible renal damage is often dose-limiting despite the inclusion of forced diuresis in the treatment protocol [2]. In recognition of the limited clinical utility of cisplatin, a number of analogues have been developed, some of which have received clinical trials [3].

Our analogue development programme at the Institute of Cancer Research has been successful in identifying Carboplatin (*cis*-diammine-1,1-cyclobutane dicarboxylate platinum II, CBDCA, JM8; Fig. 1) as a viable alternative to cisplatin [4]. The analogue not only maintains the useful antitumour properties of the parent compound but is generally very much less toxic in both animals [4] and patients [5]. Nephrotoxicity, in particular, is not of clinical concern with Carboplatin [5], and indeed it has been difficult to demonstrate this toxic side effect in rodents even at lethal doses of the analogue [6]. Interestingly, the difference in the nephrotoxic activity in rodents between Carboplatin and cisplatin does not appear to be related to renal platinum levels, which are similar during the protracted elimination phase following maximally tolerated doses of the two platinum complexes [7].

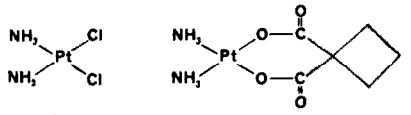
The work described in this paper examines the plasma, renal and biliary clearances of Carboplatin and cisplatin in rodents to help explain the selective absence of nephrotoxicity in animals receiving the analogue. From a comparison of these data, pharmacokinetic differences have been identified which may explain why Carboplatin is not nephrotoxic.

MATERIALS AND METHODS

Chemicals. Carboplatin and cisplatin were gifts from the Johnson Matthey Research Centre (Sonning Common, Reading, U.K.). These compounds were dissolved in normal saline immediately prior to administration. Inulin-[^{14}C]carboxylic acid (sp. act. 13.5 mCi/mmol) was purchased from Amersham (U.K.).

Animals. Male and female Balb C$^-$ mice (20–25 g) and female Wistar rats (190–220 g) were used

* Present address: M. D. Anderson Hosp., 1515 Holcombe Blvd., Box 52, Houston, TX 77030.



Cisplatin Carboplatin

Fig. 1. Chemical structures of cisplatin and Carboplatin.

throughout. The animals, bred at The Institute of Cancer Research, had free access to food and water. During anaesthesia, the rectal temperatures of the animals were maintained at 37 ± 0.5° with heating lamps. Both cisplatin and Carboplatin were administered at doses equivalent to, or below, the maximum tolerated doses, being respectively 4 and 80 mg/kg for mice and 6.5 and 60 mg/kg for rats.

Urinary excretion studies. Mice were anaesthetised with pentobarbital sodium (100 mg/kg, i.p.; 10 ml/kg). The bladder was exposed through a short abdominal mid-line incision and catheterised with polythene tubing (Portex Ltd., Hythe, Kent, U.K.; i.d. 0.28 mm, e.d. 0.61 mm). The other end of the tubing was attached to a 50 μl graduated micropipette (Corning Glass Works, New York, U.S.A.) into which the urine was collected. After a 30 min stabilisation period, a freshly prepared solution of Carboplatin (5 and 80 mg/kg) or cisplatin (4 mg/kg) was injected via a tail vein. Anaesthesia was maintained throughout the experiment with additional pentobarbital sodium (35 mg/kg, i.p.) as required. Urine was collected every hour for 4 hr, the volume measured and then washed out into polypropylene micro-tubes with 0.25 ml of distilled water. The samples were kept on ice and analysed for total platinum.

Plasma and renal clearance studies. Rats were anaesthetised with pentobarbital sodium (50 mg/kg, i.p.; 2 ml/kg), and the trachea and carotid artery were cannulated using Portex polythene tubing (i.d. 1.40 mm, e.d. 1.90 mm and i.d. 0.50 mm, e.d. 1.00 mm respectively). In addition, the external urethral orifice was ligated to prevent any involuntary urine expulsion. The animals were allowed 30 min to stabilise before administering a freshly prepared solution of the compounds. Carboplatin (20 mg/kg) or cisplatin (2 mg/kg) was co-administered with [^{14}C]inulin (8 μCi/kg) via the femoral vein (2 ml/kg). Blood samples (0.2 ml) were collected from the carotid artery at 2, 4, 6, 8, 10, 15, 20, 30, 45 and 60 min into heparinised polypropylene micro-tubes. The samples were cooled immediately on ice and then centrifuged in a Beckman Microfuge (Model B) to isolate the plasma. Sampled blood was replaced with an equal volume of heparinised saline (10 iu/ml) by injection via the carotid artery cannula. Anaesthesia was maintained by administration of additional pentobarbital sodium (15 mg/kg, i.p.) as necessary. After 4 hr, the experiment was terminated and the urine collected from the bladder. Plasma and urine samples were kept on ice throughout and analysed immediately for [^{14}C], total platinum and unchanged Carboplatin. In addition, aliquots of plasma were added to an equal volume of cold trichloroacetic acid (TCA; 20%, w/v), kept on ice for 10 min and the supernatant, after microfuging, was analysed for platinum.

Biliary clearance studies. A separate group of rats was used for these studies. Animals were anaesthetised as before and the common bile duct cannulated using Portex polythene tubing (i.d. 0.28 mm, e.d. 0.61 mm) as described previously [8]. Carboplatin (2.5 and 20 mg/kg), cisplatin (2 mg/kg) or [^{14}C]inulin (8 μCi/kg) was injected alone (2 ml/kg) via the femoral vein, and bile collected into preweighed polypropylene micro-tubes at 15, 30, 60 min and every hour thereafter for a further 5 hr. The volume of the bile was determined gravimetrically assuming a density of 1 g/ml. The bile was analysed for total platinum or [^{14}C] content.

In vivo *plasma binding studies.* Rats were anaesthetised with pentobarbital sodium as before, and Carboplatin (20 mg/kg) or cisplatin (2 mg/kg) was injected i.v. (femoral vein). At various times after drug administration, blood (5–6 ml) was collected through the abdominal aorta, placed into heparinised tubes and immediately cooled on ice. The blood was then centrifuged at 2000 *g* for 10 min at 4°. The major part of the plasma was ultrafiltrated at 4° using Amicon CF50A ultrafiltration membrane cones as described by Harland *et al.* [9]. An aliquot of the plasma was treated with TCA as described above. The total platinum in the plasma, ultrafiltrate, and the TCA-plasma supernatant were determined. Platinum in the ultrafiltrate represents free (unbound) species, whereas the TCA-soluble platinum represents the sum of free and reversibly bound species.

Sample analyses. The radioactivity from [^{14}C]inulin was determined using standard liquid scintillation counting techniques with an efficiency of 94%.

Unchanged Carboplatin was quantitated by high pressure liquid chromatography as described in detail elsewhere [9]. Urine samples were analysed directly, whereas plasma samples (20 μl) required precipitation of protein with acetonitrile (180 μl) prior to Carboplatin estimation in the resulting supernatant. Quantitation was achieved by comparison of peak heights with standard curves prepared in control plasma and urine.

Samples were analysed for their platinum metal content as described by Leroy *et al.* [10] using an Instrumentation Laboratory Atomic Absorption Spectrophotometer (Model 357) equipped with a Furnace Atomiser (Model 655) and an Auto-Sampler (Model 254). Samples were diluted with 0.1 N HCl and analysed directly.

Pharmacokinetic analyses. The plasma concentration–time data were fitted to a two-compartment open model using an unweighted non-linear least squares computer program [11]. The computer-generated constants were used for further pharmacokinetic analysis using equations described elsewhere [12, 13]. The clearance values were obtained using equations employing area under the concentration–time curves (AUC) for the free drug [12], viz.

$$\text{Plasma clearance} = \frac{\text{Dose}}{\text{AUC}_{0-\infty}}$$

$$\text{Renal clearance} = \frac{\text{Excretion in urine in time } t}{\text{AUC}_{0-t}}$$

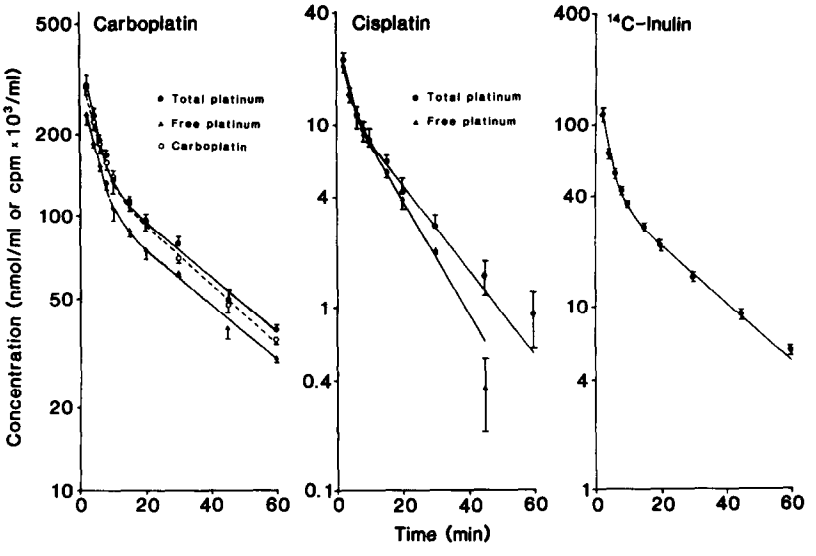


Fig. 2. Plasma levels of total and free drug following intravenous Carboplatin (20 mg/kg), cisplatin (2 mg/kg) and [^{14}C]inulin (8 μCi/kg) administrations to female rats. Each point is a mean ± SD of 3–7 animals.

$$\text{Biliary clearance} = \frac{\text{Excretion in bile in time } t}{AUC_{0-t}}$$

RESULTS

Preliminary experiments in rats indicated that the Carboplatin and cisplatin clearances were not affected by the presence of inulin. Therefore, in the present study, the pharmacokinetics of Carboplatin or cisplatin were determined simultaneously with that of inulin in the same animal. Furthermore, there was no difference between the inulin data from animals receiving either of the two platinum complexes as the second drug, and, consequently, the inulin data have been pooled.

The plasma decay of total and free drug following i.v. administration of Carboplatin (20 mg/kg), cisplatin (2 mg/kg) and [^{14}C]inulin (8 μCi/kg) to rats can be described by biphasic curves over the first 60 min, as shown in Fig. 2. The curves for Carboplatin determined either as total platinum or parent compound are essentially superimposible, indicating that virtually all of the compound in the plasma is present as unchanged Carboplatin. Furthermore, these curves for Carboplatin are parallel to that of the free drug. With cisplatin, however, the free drug during the β-phase decays more rapidly than total cisplatin. For the three compounds, the α-phases are of very short duration with half-lives ($t_{\frac{1}{2}\alpha}$) of the order of 2–3 min (see Table 1). The corresponding β-phase half lives ($t_{\frac{1}{2}\beta}$), however, are much shorter for cisplatin (10–15 min) and inulin (17 min) than for Carboplatin (25–26 min).

The pharmacokinetic parameters derived from the computer analyses of the concentration–time data are also shown in Table 1. For Carboplatin the parameters were derived for total and free platinum as well as the parent molecule. However, the kinetic rate constants were similar for the three sets of data for Carboplatin. Only the total and free platinum concentrations in the plasma were used for determination of the cisplatin pharmacokinetic parameters. Quantitation by high pressure liquid chromatography of the parent cisplatin molecule was not possible due to the low plasma concentration of the compound. Table 1 also includes the results for inulin, a marker for the glomerular filtration rate. The values of the rate constants k_{12} and k_{21} for Carboplatin, cisplatin or inulin are similar, indicating that there is no appreciable net accumulation of the compounds in the peripheral tissues over the time period studied. The extrapolated volume of distribution, V_{dext}, for total platinum (363 ml/kg) or the parent compound (322 ml/kg) after Carboplatin is similar to that of inulin (319 ml/kg) but lower than that of total platinum (599 ml/kg) derived from cisplatin. The V_{dext} of free platinum after both Carboplatin (460 ml/kg) and cisplatin (471 ml/kg), although similar, are greater than that of inulin. These data suggest that Carboplatin and cisplatin are not confined to the extracellular inulin space. The volume of the central compartment, V_1, is similarly greater for the platinum complexes than for inulin (125–195 vs 79 ml/kg). This is probably due to the ease of penetration into the extracellular space of some tissues by the relatively smaller molecules, Carboplatin (mol. wt. 371) and cisplatin (mol. wt. 300), than inulin (mol. wt. 5200). The volume of the peripheral compartment, V_2, is similar, however, for inulin and the two platinum complexes, determined as the free drug.

The *in vivo* plasma protein binding of Carboplatin and cisplatin is shown in Table 2. The % free platinum in the plasma decreases much more rapidly with cisplatin than Carboplatin, and by 60 min free

Table 1. Pharmacokinetics of Carboplatin, Cisplatin and inulin in rats

	Compound					
	Carboplatin			Cisplatin		
Parameters*	Parent compound	Total Pt	Free Pt	Total Pt	Free Pt	Inulin
A (cpm/ml or nmol/ml)	268 ± 41†	259 ± 35	205 ± 28	33.2 ± 5.8	20.9 ± 5.7	159 ± 26 × 10^3
B (cpm/ml or nmol/ml)	168 ± 13	148 ± 7	117 ± 6	12.1 ± 3.1	14.6 ± 1.5	51.6 ± 4.6 × 10^3
α (min^{-1})	0.32 ± 0.04	0.24 ± 0.02	0.23 ± 0.02	0.50 ± 0.14	0.47 ± 0.05	0.45 ± 0.07
β (min^{-1})	0.028 ± 0.002	0.027 ± 0.002	0.027 ± 0.002	0.049 ± 0.007	0.067 ± 0.007	0.041 ± 0.003
$t_{1\alpha}$ (min)	2.2 ± 0.3	3.0 ± 0.3	3.0 ± 0.3	1.5 ± 0.5	1.5 ± 0.2	1.6 ± 0.3
$t_{1\beta}$ (min)	25.2 ± 2.5	25.8 ± 1.9	25.8 ± 1.9	14.5 ± 2.1	10.4 ± 1.0	17.1 ± 1.1
k_{12} (min^{-1})	0.14 ± 0.02	0.10 ± 0.01	0.10 ± 0.01	0.24 ± 0.08	0.17 ± 0.05	0.22 ± 0.05
k_{21} (min^{-1})	0.14 ± 0.02	0.10 ± 0.01	0.10 ± 0.01	0.17 ± 0.05	0.23 ± 0.04	0.14 ± 0.02
k_{el} (min^{-1})	0.063 ± 0.008	0.062 ± 0.006	0.062 ± 0.006	0.14 ± 0.02	0.14 ± 0.01	0.13 ± 0.02
V_1 (ml/kg)	125 ± 15	133 ± 13	168 ± 17	156 ± 33	195 ± 28	79.1 ± 11.5
V_2 (ml/kg)	198 ± 15	231 ± 7	292 ± 9	443 ± 146	277 ± 69	240 ± 24
V_{dext} (ml/kg)	322 ± 24	363 ± 18	460 ± 24	599 ± 177	471 ± 42	319 ± 33

* The parameters were derived by fitting the concentration–time data to a two-compartment open model, where A and B are the concentration constants, α and β are the rate constants for the distribution and elimination phase respectively, k_{12} and k_{21} are the rate constants for transfer of drug between compartments 1 and 2, k_{el} is the elimination rate constant, V_1 and V_2 are the volumes of the central and peripheral compartments respectively, and V_{dext} is the extrapolated volume of distribution and is equal to the sum of V_1 and V_2.

† $\bar{X}$ ± SD; N = 3–7.

Table 2. Binding of Carboplatin and cisplatin to rat plasma *in vivo*

Time (hr)	% Free Pt	
	Carboplatin	Cisplatin
0.10	79.7 ± 4.2*	98.7 ± 2.5
0.17	—	96.2 ± 9.4
0.25	—	88.4 ± 10.3
0.50	—	74.8 ± 10.3
0.75	—	25.1 ± 11.2
1	78.7 ± 7.8	0
2	68.6 ± 6.4	0
3	53.2 ± 3.1	0
4	34.4 ± 5.9	0

* $\bar{X}$ ± SD; N = 3–5.

cisplatin is undetectable. For Carboplatin, free platinum in the plasma remains constant at about 80% during the first 60 min and decreases slowly thereafter due to irreversible binding. Comparison of platinum levels in the plasma ultrafiltrate (Table 2) with that in the TCA-soluble fraction (data not shown) indicates that reversible binding to plasma protein is negligible with cisplatin, but amounts to about 20% with Carboplatin.

The biliary excretion of Carboplatin (2.5 and 20 mg/kg) and cisplatin (2 mg/kg) in the rat is shown in Fig. 3. Neither Carboplatin nor cisplatin at these doses had any effect on the rate of bile flow. The biliary excretion rate of Carboplatin is directly related to the dose, since an eightfold increase in the dose resulted in a similar increase in the excretion rate. The maximal excretion rate of Carboplatin is observed at 15 min, after which the rate decreases progressively. For cisplatin, however, the excretion rate–time profile differs slightly. Following a small reduction (6%) in the excretion rate of cisplatin at 30 min, there is a distinctive but reproducible increase (25%) in its rate 60 min after drug administration. Thereafter, the rate declines. The excretion rate of cisplatin at all times is about two- to threefold greater than that of an equimolar dose of Carboplatin. A similar difference between the two compounds is also observed in the cumulative biliary excretion. The total excretion in 6 hr, however, is very low, amounting to only 1.2% of the administered cisplatin and 0.4–0.7% of Carboplatin.

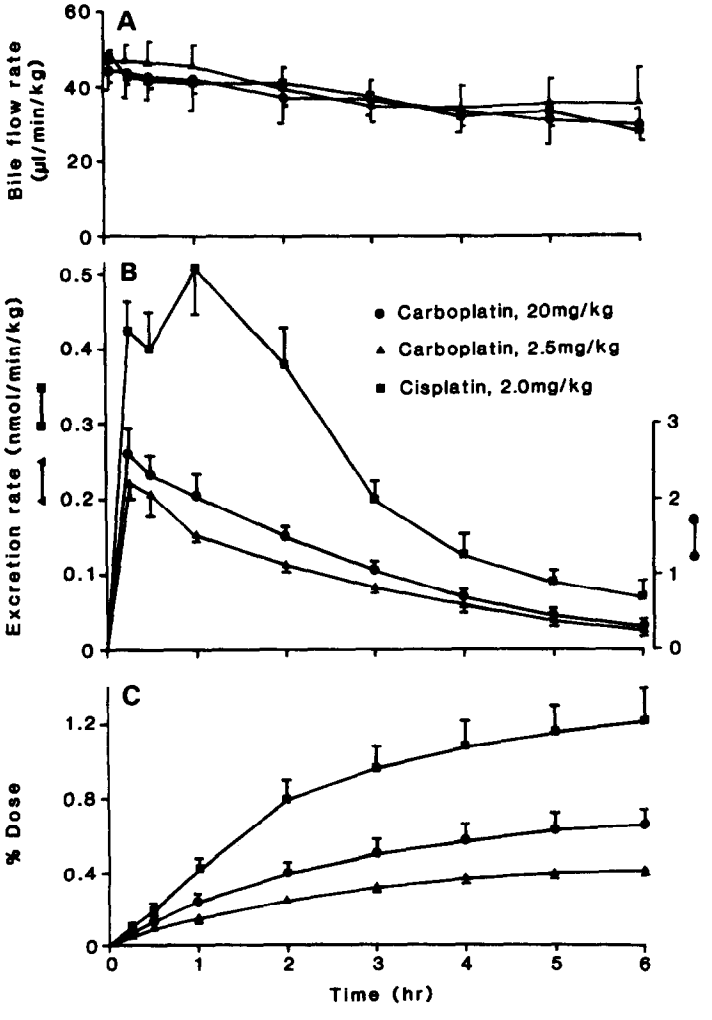


Fig. 3. Bile flow rates (A), biliary platinum excretion rate (B) and cumulative biliary platinum excretion as a % of dose (C) following intravenous Carboplatin (2.5 and 20 mg/kg) and cisplatin (2 mg/kg) administrations to female rats. Each point is a mean ± SD of three animals.

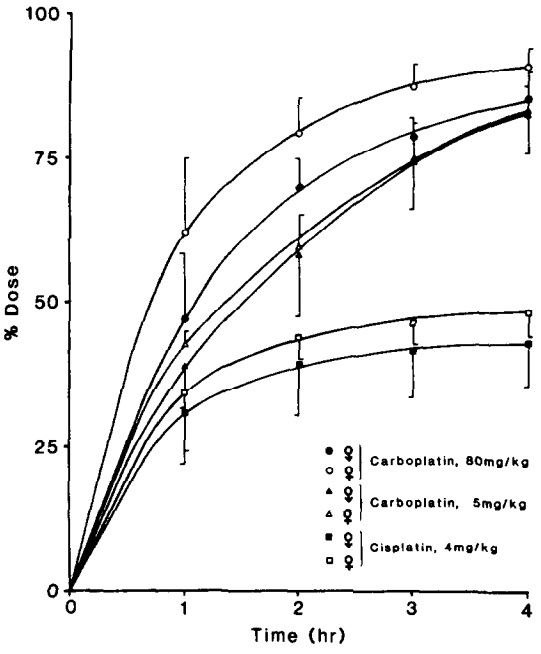


Fig. 4. Cumulative urinary platinum excretion as a % of dose following intravenous Carboplatin (5 and 80 mg/kg) and cisplatin (4 mg/kg) administrations to male and female mice. Each point is a mean ± SD of three animals.

Excretion of the two platinum complexes is predominantly via the renal route, as can be seen from Fig. 4 and Table 3. Mice receiving Carboplatin (5 or 80 mg/kg) excrete 80–90% of the dose in the urine in 4 hr (Fig. 4). Excretion of cisplatin (4 mg/kg), on the other hand, is 43–48%, about half that of Carboplatin. There appear to be neither sex nor species differences in the renal elimination of the two complexes (Table 3). In the rat urine, intact Carboplatin accounts for almost all of the excreted platinum in the 4 hr. As a comparison, the urinary excretion of co-administered inulin is also included in Table 3 and accounts for 95% of the dose in the same time. This illustrates that neither of the two platinum compounds have any short-term effects on glomerular function.

The results of the present study have been summarised in terms of clearance values, shown in Table 4. The plasma clearances of both Carboplatin (10.3 ml/min/kg) and inulin (10.1 ml/min/kg) are identical, as are their renal clearances (9.3 vs 9.6 ml/min/kg). The close similarity between the renal and plasma clearances of these two compounds indicates that their excretion is predominantly via the kidneys. The cisplatin plasma (26.1 ml/min/kg) and renal (12.3 ml/min/kg) clearances, on the other hand, are not similar. In addition, these clearances are significantly greater than those of Carboplatin and inulin. As with the renal clearance, biliary clearance of cisplatin is significantly greater than that of Carboplatin (0.27 vs 0.05 ml/min/kg). Biliary clearance of inulin is negligible (0.006 ml/min/kg). These low biliary clearances are indicative of only a minor role for the bile in the excretion of these compounds.

DISCUSSION

In this communication we have compared the early phase pharmacokinetics of Carboplatin and cisplatin for both total and free drug. This has involved measurements of total and free platinum in the plasma, and in the case of Carboplatin, estimation of the unchanged compound. It is important to differentiate plasma platinum levels into these components as only the free drug can participate in the various pharmacokinetic processes. Our finding of a rapid disappearance of free platinum after cisplatin administration, however, has been reported previously in both rats [14] and patients [15–17]. The free platinum after Carboplatin, on the other hand, is present in the plasma for more than 4 hr (Table 2).

In addition to the plasma, the role of the bile has also been examined in the overall clearance of Carboplatin and cisplatin. Biliary excretion of the two platinum complexes, however, is of minor importance in the rat. The low excretion of cisplatin in the bile found here is similar to that previously reported in the same species [18]. It is nevertheless apparent that biliary excretion of cisplatin is greater than that of Carboplatin. The unusual feature noted during cisplatin excretion was the further increase in its excretion rate after 30 min. Since cisplatin is rapidly converted to the active aquated species [19], it is possible that the increase in rate is due to these species or their reaction products. In this regard, Leroy *et al.* [19] have suggested that, in the dog, the appreciable biliary excretion of cisplatin at later times is probably due to the presence of cisplatin metabolites.

Table 3. Urinary excretion of Carboplatin, cisplatin and inulin in mice and rats

				% Dose in urine in 4 hr	
Compound	Species	Sex	Dose	Total Pt	Parent Compound
Carboplatin	Mice	M	80 mg/kg	85.1 ± 2.5*	N.D.
		F	80 mg/kg	90.6 ± 3.1	N.D.
	Rat	F	20 mg/kg	88.6 ± 3.9	85.1 ± 3.7
Cisplatin	Mice	M	4 mg/kg	43.0 ± 7.5	N.D.
		F	4 mg/kg	48.3 ± 4.1	N.D.
	Rat	F	2 mg/kg	47.3 ± 3.6	N.D.
Inulin	Rat	F	8 μCi/kg	—	95.3 ± 3.5

* $\bar{X}$ ± SD; N = 3–7.
N.D. = not determined.

Table 4. Plasma, renal and biliary clearances of Carboplatin, cisplatin and inulin in rats

Compound	Clearance of free drug (ml/min/kg)		
	Plasma	Renal	Biliary
Carboplatin	10.3 ± 0.4*	9.3 ± 0.4	0.053 ± 0.009†
Cisplatin	26.1 ± 1.4‡	12.3 ± 1.5‡	0.27 ± 0.05‡
Inulin	10.1 ± 0.5	9.6 ± 0.4	0.0062 ± 0.0006

* $\bar{X}$ ± SD; N = 3–7.
† $P < 0.05$ vs cisplatin or inulin by Student's *t*-test.
‡ $P < 0.05$ vs Carboplatin or inulin by Student's *t*-test.

Studies on the renal handling of Carboplatin and cisplatin in mice and rats clearly demonstrate the importance of the urine in the excretion of the two compounds. Furthermore, Carboplatin is excreted in rat urine mainly as the unchanged compound. Urinary excretion of cisplatin in mice during the first 4 hr (43–48% of dose) is very similar to that seen over 3 days in metabolism cages (unpublished data), indicating that the excretion of this compound is rapid and essentially complete within this short time period. This excretion, nevertheless, is much lower than the reported 80–90% in mice receiving [^{195m}Pt]labelled cisplatin [20–22]. It is possible that the lower urinary excretion found in the present study may be due to the different strain of mice used. Another possible explanation for the discrepancy between our study and those of others is the difference in the route of administration, although this may be thought unlikely since i.v. or i.p. administration to dogs has no effect on the urinary excretion of cisplatin [23]. Renal elimination of this compound in the rat, on the other hand, is in close agreement with those reported elsewhere [18, 22, 24, 25] and is similar to that seen in mice in the present study. Excretion of cisplatin in the urine, however, is about half that seen with Carboplatin in either of the two species studied, even when equimolar doses are utilised (Table 3). This lower urinary excretion of cisplatin could be due to a combination of tubular reabsorptive processes, reported recently for this compound [26], and irreversible binding to plasma (this study) and probably to other tissues.

Inulin was included in the study as a marker for the glomerular filtration rate. The values obtained for inulin clearances are identical to those found by other workers [27, 28]. No difference is found in the inulin clearance values determined by either the single i.v. injection technique, used in this study, or the standard inulin infusion method [29, 30]. The close similarity in the renal clearances of Carboplatin and inulin provides strong evidence that the urinary excretion of Carboplatin is by glomerular filtration. This finding contrasts sharply with that of Daley-Yates and McBrien [31], who have reported that renal elimination of Carboplatin in rats is by an active process, but our conclusion, however, appears to have been confirmed by data arising from clinical studies with Carboplatin [9]. The greater renal clearance rate of cisplatin than that of inulin in the present study, on the other hand, indicates that an active renal tubular transport may well be involved in the urinary excretion of this platinum complex. This *in vivo* finding is in agreement with previous reports of active transport of cisplatin in rat kidney slices [32] and in isolated perfused rat kidneys [26]. The cisplatin/inulin ratio of 1.28 for the renal clearances is similar to that reported with isolated perfused kidneys [26] and in intact rats [33]. A higher value (3.1) has also been reported [31]. In addition to the active secretory process, active reabsorption of cisplatin has also been reported in studies with the isolated kidney [26]. Since the site of active renal secretory and reabsorptive processes for most substances are confined to the proximal tubules [34], it follows then that tubular cells capable of actively transporting cisplatin intracellularly from both the plasma and the lumen of proximal tubules would contain relatively higher concentrations of the drug. This would be in keeping with the available evidence that the site of cisplatin nephrotoxicity is confined mainly to the proximal tubules [2].

Apart from being handled selectively by the active renal secretory process, a second distinctive feature of cisplatin relative to Carboplatin is the discrepancy between plasma clearance and the combined renal and biliary clearances. The latter two pathways of cisplatin clearance together represent just under half the total (plasma) clearance, and this is indicative of the existence of at least another mechanism for cisplatin clearance. It is highly likely that the intracellular irreversible binding of cisplatin to macromolecules, similar to that demonstrated here and elsewhere [14, 19] for plasma proteins, could represent such a mechanism of clearance.

One other factor to be considered in regard to nephrotoxicity is the relative stabilities of the two platinum complexes [35] and the effect this has on irreversible binding and potential toxicity. It has been reported that the rate-limiting step in cisplatin binding is its conversion to the active aquated species [19]. Because of the chelate effect of the bidentate cyclobutane dicarboxylate ligand, the rate of formation of the active species from Carboplatin will be slower than that from cisplatin. Thus, the irreversible chemical–biological interactions taking place in the plasma, kidney and other tissues may occur at a much greater rate with cisplatin than Carboplatin. It is possible that the greater rate of proximal tubular platination, exacerbated by the existence of active tubular transport mechanisms which increase the intracellular platinum concentrations, may explain why cisplatin is nephrotoxic. It is apparent that transport and binding studies in isolated renal tubules would clearly aid in furthering our limited knowledge of cisplatin-induced nephrotoxicity.

In conclusion, differences in the *in vivo* renal handling of cisplatin and Carboplatin have been identified in the present study. Excretion of cisplatin appears to involve active renal tubular mechanisms resulting in its net secretion into the urine. The absence of any such active processes for Carboplatin and its greater chemical stability may be important in explaining its lack of nephrotoxicity.

Acknowledgements—The authors wish to thank Miss Ann Robinson for typing the manuscript. This work was supported by grants from The Medical Research Council and The Cancer Research Campaign, U.K.

REFERENCES

1. A. W. Prestayko, J. C. D'Aoust, B. F. Issell and S. T. Crooke, *Cancer Treat. Rev.* **6**, 17 (1979).
2. N. E. Madias and J. T. Harrington, *Am. J. Med.* **65**, 307 (1978).
3. F. H. Lee, R. Canetta, B. F. Issell and L. Lenaz, *Cancer Treat. Rev.* **10**, 39 (1983).
4. K. R. Harrap, M. Jones, C. R. Wilkinson, H. M. Clink, S. Sparrow, B. C. V. Mitchley, S. Clarke and A. Veasey, in *Cisplatin. Current Status and New Developments* (Eds. A. W. Prestayko, S. T. Crooke and S. K. Carter), pp. 193–212. Academic Press, New York (1980).
5. A. H. Calvert, S. J. Harland, D. R. Newell, Z. H. Siddik, A. C. Jones, T. J. McElwain, S. Raju, E. Wiltshaw, I. E. Smith, J. M. Baker, M. J. Peckham and K. R. Harrap, *Cancer Chemother. Pharmac.* **9**, 140 (1982).
6. B. S. Levine, M. C. Henry, C. D. Port, W. R. Richter and M. A. Urbanek, *J. natn. Cancer Inst.* **67**, 201 (1981).
7. Z. H. Siddik, D. R. Newell, M. Jones and F. E. Boxall, *Proc. Am. Ass. Cancer Res.* **23**, 168 (1982).
8. M. M. Abou-El-Makarem, P. Milburn, R. L. Smith and R. T. Williams, *Biochem. J.* **105**, 1289 (1967).
9. S. J. Harland, D. R. Newell, Z. H. Siddik, R. Chadwick, A. H. Calvert and K. R. Harrap, *Cancer Res.* **44**, 1693 (1984).
10. A. F. LeRoy, M. L. Wehling, H. L. Sponseller, W. S. Friauf, R. E. Solomon, R. L. Dedrick, C. L. Litterst, T. E. Gram, A. M. Guarino and D. A. Becker, *Biochem. Med.* **18**, 184 (1977).
11. J. Sampson, *Non-Linear Least Squares Programme BMDX85*, p. 177. University of California Press, Berkeley, CA (1969).
12. J. G. Wagner (Ed.), *Fundamentals of Clinical Pharmacokinetics*, pp. 38, 88. Drug Intelligence Publications, Hamilton, IL (1975).
13. D. J. Greenblatt and J. Koch-Weser, *New Eng. J. Med.* **293**, 702 (1975).
14. C. L. Litterst, *Toxic. appl. Pharmac.* **61**, 99 (1981).
15. J. B. Vermoken, W. J. F. Van Der Vijgh, I. Klein, H. E. Gall and H. M. Pinedo, *Eur. J. Cancer Clin. Oncol.* **18**, 1069 (1982).
16. W. R. Crom, W. E. Evans, C. B. Pratt, N. Senzer, M. Denison, A. A. Green, F. A. Hayes and G. C. Yee, *Cancer Chemother. Pharmac.* **6**, 95 (1981).
17. K. J. Himmelstein, T. F. Patton, R. J. Belt, S. Taylor, A. J. Repta and L. A. Sternson, *Clin. Pharmac. Ther.* **29**, 658 (1981).
18. P. A. DeSimone, R. S. Yancey, J. J. Coupal, J. D. Butts and J. D. Hoeschele, *Cancer Treat. Rep.* **63**, 951 (1979).
19. A. F. LeRoy, R. J. Lutz, R. L. Dedrick, C. L. Litterst and A. M. Guarino, *Cancer Treat. Rep.* **63**, 59 (1979).
20. J. D. Hoeschele and L. Van Camp, in *Advances in Antimicrobial and Antineoplastic Chemotherapy*, Vol. 2, pp. 241–242. University Park Press, Baltimore, MD (1972).
21. R. C. Lange, R. P. Spencer and H. C. Harder, *J. Nucl. Med.* **13**, 328 (1972).
22. C. L. Litterst, A. F. LeRoy and A. M. Guarino, *Cancer Treat. Rep.* **63**, 1485 (1979).
23. R. G. Pretorius, E. S. Petrilli, C. Kean, L. C. Ford, J. D. Hoeschele and L. D. Lagasse, *Cancer Treat. Rep.* **65**, 1055 (1981).
24. M. F. Pera, Jr., B. C. Zook and H. C. Harder, *Cancer Res.* **39**, 1269 (1979).
25. C. L. Litterst, I. J. Torres and A. M. Guarino, *J. Clin. Hematol. Oncol.* **7**, 169 (1976).
26. P. T. Daley-Yates and D. C. H. McBrien, *Biochem. Pharmac.* **31**, 2243 (1982).
27. A. M. Harvey and R. L. Malvin, *Am. J. Physiol.* **209**, 849 (1965).
28. E. A. Lock, *Toxic. appl. Pharmac.* **48**, 327 (1979).
29. M. D. Blaufox and A. Cohen, *Am. J. Physiol.* **218**, 542 (1970).
30. J. E. Hall, A. C. Guyton and B. M. Farr, *Am. J. Physiol.* **232**, F72 (1977).
31. P. T. Daley-Yates and D. C. H. McBrien, *Biochem. Pharmac.* **34**, 1423 (1985).
32. C. Jacobs, K. McGarry, L. Rich and M. W. Weiner, *Proc. Am. Ass. Cancer Res.* **23**, 126 (1982).
33. N. M. Osman and C. L. Litterst, *Cancer Lett.* **19**, 107 (1985).
34. E. E. Selkurt, in *Physiology* (Ed. E. E. Selkurt) pp. 489–533. Little, Brown, Boston, MA (1971).
35. M. J. Cleare, P. C. Hydes, D. R. Hepburn and B. W. Malerbi, in *Cisplatin. Current Status and New Developments* (Eds. A. W. Prestayko, S. T. Crooke and S. K. Carter), pp. 149–170. Academic Press, New York (1980).