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Comparisons of tetrachloro(d,l-trans)1,2-diaminocyclohexaneplatinum(IV) biotransformations in the plasma of Fischer 344 rats at therapeutic and toxic doses*

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Summary. Plasma biotransformations of tetrachloro(d,l-*trans*)1,2-diaminocyclohexaneplatinum(IV) (tetraplatin) were determined in vivo at both therapeutic (3 mg/kg) and toxic (12 mg/kg) doses in Fischer 344 rats. Tetraplatin was rapidly converted to dichloro(d,l-*trans*)1,2-diaminocyclohexaneplatinum(II) [$PtCl_2$(dach)]. This conversion was complete at the earliest time measured (7.5 min) at the therapeutic dose, but some unreacted tetraplatin was detectable in the circulation at the toxic dose. Three other major biotransformation products were observed in plasma: (d,l-*trans*)1,2-diaminocyclohexaneaquachloroplatinum(II) [$Pt(H_2O)(Cl)$(dach)]$^+$, the Pt-methionine complex, and another biotransformation product tentatively identified as either the Pt-cysteine or Pt-ornithine complex. Several other minor plasma biotransformation products were detected. Two of these were most likely formed intracellulary from tetraplatin. Two or more other platinum complexes appeared to lack the diaminocyclohexane carrier ligand and were most likely formed intracellulary by trans-labilization of the carrier ligand. Tetraplatin, $PtCl_2$(dach), and [$Pt(H_2O)(Cl)$(dach)]$^+$ all rapidly disappeared from the circulation. The other biotransformation products were persistent through at least 3 h and could be responsible for the delayed toxicity of tetraplatin. Although some minor differences were observed between tetraplatin biotransformations at the toxic vs therapeutic doses, most biotransformation products were simply present at much greater concentrations at the toxic dose than at the therapeutic dose. Thus, our data suggest that dose-dependent differences in tetraplatin toxicity are probably attributable to the amount, rather than the type, of biotransformation products present in the plasma.

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Introduction

Platinum anticancer agents are widely used to treat a variety of tumors, including testicular, ovarian, head and neck, and small-cell lung carcinomas [15]. The major toxicities of these compounds are nephrotoxicity, myelotoxicity, and gastrointestinal toxicity, with neurotoxicity becoming a serious problem at higher doses [6, 12]. There has been an obvious interest in the plasma biotransformations of these anticancer agents, since some of their biotransformation products are likely to be responsible for the therapeutic and toxic effects of the parent compounds. For example, the aquated biotransformation products have long been known to be more toxic than their parent compounds [9]. Similarly, Daley-Yates and McBrien [10] have provided evidence for one or more biotransformation products of *cis*-diamminedichloroplatinum(II) (cisplatin) in plasma that are more nephrotoxic than either cisplatin or its aquated derivatives. Both Daley-Yates and McBrien [10] and Long and Repta [16] have provided evidence for methionine biotransformation products of cisplatin in plasma, although Mistry et al. [20] could not confirm this with another HPLC separation procedure. Most available evidence suggests that the methionine biotransformation products are inert [10, 19], but Alden and Repta [1] have reported that methionine increases the nephrotoxicity of cisplatin and Basinger et al. [4] have observed that the cisplatin-methionine complex retains therapeutic effectiveness.

Tetrachloro (d,l-*trans*) 1,2-diaminocyclohexaneplatinum (IV) (tetraplatin) is a second-generation platinum complex of potential clinical interest because of its effectiveness against a variety of murine [2, 26] and human [5, 13] cell lines with natural or acquired resistance to cisplatin. Although it appears to induce less nephrotoxicity than cisplatin [2, 22, 23], its gastrointestinal toxicity and myelotoxicity are likely to be dose-limiting [7, 22]. The overall pharmacokinetics of tetraplatin and cisplatin appear to be similar [21], but the in vivo biotransformations of tetraplatin have not been studied in any detail.

We have recently developed a two-column HPLC separation system for the resolution and identification of platinum complexes with the (d,l-*trans*)1,2-diaminocyclohexane (dach) carrier ligand [17] and have used it to study tetraplatin biotransformations in rat plasma in vitro [8]. At physiological concentrations (5–10 μM) in plasma, tetraplatin was very rapidly reduced to its platinum(II) analog, dichloro(d,l-*trans*(1,2-diaminocyclohexaneplatinum(II) [$PtCl_2$(dach)]. The reduction of tetraplatin to $PtCl_2$(dach) was followed by a series of slower substitution reactions involving the chloro ligands of the latter. The aquachloro complex [$Pt(H_2O)$(dach)]$^+$ was formed transiently but was eventually converted to three more stable biotransformation products. The mono-methionine complex was identified on the basis of comigration with a Pt(methionine)(dach) standard on both reverse-phase and cation-exchange HPLC separation systems. The other two stable biotransformation products were tentatively identified as the cysteine or ornithine and the urea or citrato complexes. The citrato complex would be of particular interest, since it has shown better therapeutic activity than $PtCl_2$(dach) in some tumor cell screens [3, 24].

In this report we describe the in vivo biotransformations of tetraplatin in Fischer 344 rat plasma. Some platinum biotransformation products are known to be more toxic than their parent compounds [4, 10]. In theory, the levels of these toxic biotransformation products could depend on the initial concentration of the drug relative to the levels of naturally occurring protective compounds in the blood. Thus, we compared tetraplatin biotransformations at therapeutic (3 mg/kg) and toxic (12 mg/kg) doses to determine whether there were any significant differences in biotransformation that might partly be responsible for the toxicity of tetraplatin at higher doses.

Materials and methods

Drugs. Tritium-labeled tetraplatin and $PtCl_2$(dach) and [^{195m}Pt]-tetraplatin were prepared as previously described [27, 28][1]. Stock solutions of tetraplatin were prepared by sonicating a suspension of the drug in 0.15 M NaCl for 1–2 h at room temperature in a dark environment. Following sonication, the undissolved material was removed by filtration through a 0.2 μm Acrodisc filter (Gelman Sciences, Ann Arbor, Mich.). The exact concentration of the stock solution (usually 2 mg/ml) was determined by counting an aliquot of the filtrate. The purity of tetraplatin was assessed by reverse-phase HPLC [11, 17] and was >95% in these experiments. The stock solutions were prepared just before each experiment and were used immediately.

Animals. Fischer 344 male rats (300–400 g) were obtained from Charles River Breeding Laboratories (Raleigh, N.C.). They were kept on a 12-h light/dark cycle in stainless steel cages at a temperature of 22° C and were allowed Purina rodent chow and water ad libitum. At least a 1-week acclimation period was allowed prior to each experiment.

Methods. Rats were anesthetized with ketamine/Rompun (25 and 5 mg/kg, respectively) and cannulated via the right jugular vein by insertion of a polyurethane tube (outside diameter, 0.037 in.) To prevent clotting, 0.1 ml of 3.3 USP units heparin/ml was injected into the cannula to remove any residual blood. A 1-day recovery period was allowed before injection of the platinum drug. Via the jugular vein catheter, eight rats were injected with 3 mg/kg [^{3}H]-tetraplatin (2.8 mCi/kg); five, with 12 mg/kg [^{3}H]-tetraplatin (11.2 mCi/kg); and one, with 10 mg/kg [^{195m}Pt]-tetraplatin (0.6 mCi/kg). This was followed by 0.1 ml 0.15 M NaCl containing 1.9 USP units heparin/ml to flush the catheter. The radioactive drugs were injected into the rats over a 1-min period. Blood samples were drawn through the catheter into a heparinized syringe at times ranging from 3.5 min to 3 h following tetraplatin administration. The volume of blood taken ranged from 100 μl at 3.5 min to 1,500 μl at 3 h. The 3.5 min sample was generally not analyzed because of the possibility that the sample might contain residual tetraplatin from the catheter. The blood ws immediately centrifuged at 3,500 *g* for 3.5 min at 4° C.

Each plasma sample was diluted 1 : 5 into cold 0.15 M NaCl and an aliquot was counted in either an LKB model 1215 liquid scintillation counter or a Nuclear-Chicago model 1185 gamma counter for [^{3}H]- and [^{195m}Pt]-tetraplatin, respectively. The remaining samples were filtered through Amicon YMT membrane filters with a molecular-weight cutoff of 30,000 Da (Amicon Corp., Danvers, Mass.) by centrifugation at 2,000 *g* for 30 min at 4° C. The filtered samples were counted as previously described and then frozen at –80° C until HPLC analysis. The difference between the counts before and after filtration was taken as the protein-bound platinum, and the material remaining after filtration was referred to as free platinum. The $t^1/_2\alpha$ values were estimated from the initial rate of clearance in semilog plots of platinum concentration versus time.

The plasma biotransformation products were resolved by reverse-phase and cation-exchange HPLC as previously described [11, 17]. Following HPLC separation, aliquots from each fraction were counted. Data from the scintillation counter were collected and converted to ASCI files with the Ultraterm program (Pharmacia-LKB, Piscataway, N.Y.). Peaks were identified and quantitated using the Chromatochart program (Interactive Microware, University Park, Pa.). The reactivity of the HPLC fractions was determined by the DNA-binding assay described previously [18].

Results

Male Fischer 344 rats were cannulated and injected with either therapeutic (3 mg/kg) or toxic (12 mg/kg) doses of tetraplatin. These doses were chosen on the basis of previous studies on Sprague-Dawley and Fischer 344 rats [21–23]. The basic pharmacokinetics are shown in Fig. 1. Initial levels of total (Fig. 1A) and protein-bound (Fig. 1C) platinum were much higher at the toxic dose, as might be expected. Surprisingly, the initial levels of free platinum (Fig. 1B) were only slightly higher at the toxic dose, primarily because the reaction with plasma protein was more rapid (Fig. 1D). However, the initial rate of clearance was sufficiently more rapid at the therapeutic dose than at the toxic dose, that by 60 min the steady-state levels of free platinum were 5 times lower at the therapeutic dose. The approximate $t^1/_2\alpha$ values at the therapeutic and toxic doses, respectively, were 5.5 and 37 min for total platinum and 4.5 and 8 min for free platinum. Although the initial clearance of both total and free platinum was rapid at both doses, significant steady-state levels of platinum remained in the circulation for up to 3 h after injection. The nature of the low-molecular-weight biotransformation products was determined by HPLC separations.

[1] Subsequent experience has shown that the 1,2-bis-azidocarbonylcyclohex-4-ene intermediate in the described synthesis of [^{3}H]-tetraplatin is extremely explosive, and we no longer recommend that protocol

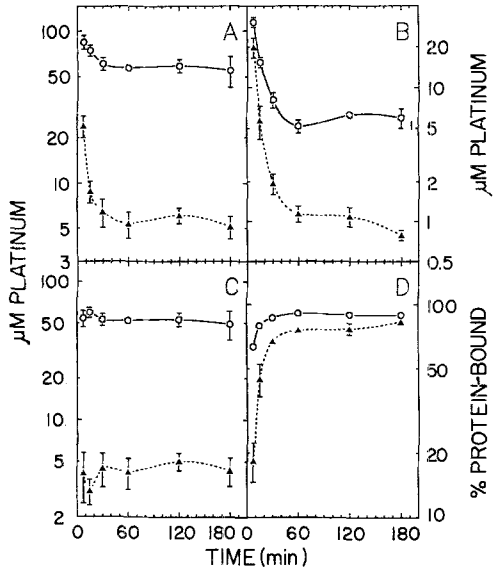

Fig. 1 A–D. Tetraplatin pharmacokinetics at the therapeutic and toxic doses. Levels of total, free, and protein-bound platinum are shown for 5 experiments at the toxic dose (○, 12 mg/kg) and for 8 experiments at the therapeutic dose (▲, 3 mg/kg). **A** Total platinum. **B** Free platinum. **C** Protein-bound platinum. **D** Percentage of protein-bound platinum. The error bars indicate the standard error of the mean (where no error bars are shown, the error range is less than the width of the symbol used)

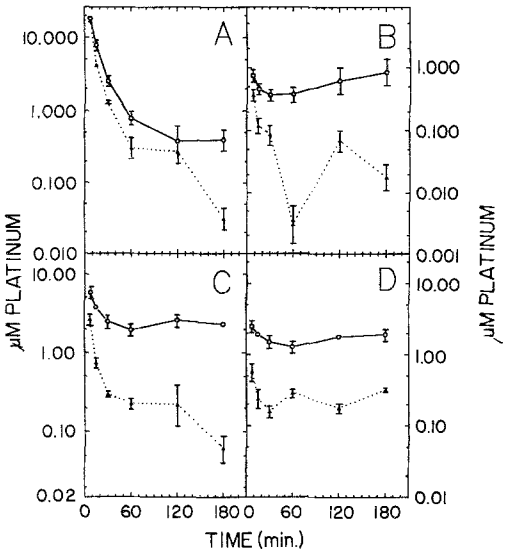

Fig. 3 A–D. Pharmacokinetics of individual biotransformation products at the therapeutic and toxic doses. The pharmacokinetics of the biotransformation products identified by reverse-phase HPLC as shown in Fig. 2 were averaged for 5 experiments at the toxic dose (○) and for 8 experiments at the therapeutic dose (▲). The error bars indicate the standard error of the mean. **A** Peak b. **B** Peak d_1. **C** Peaks e+f. **D** Peak g

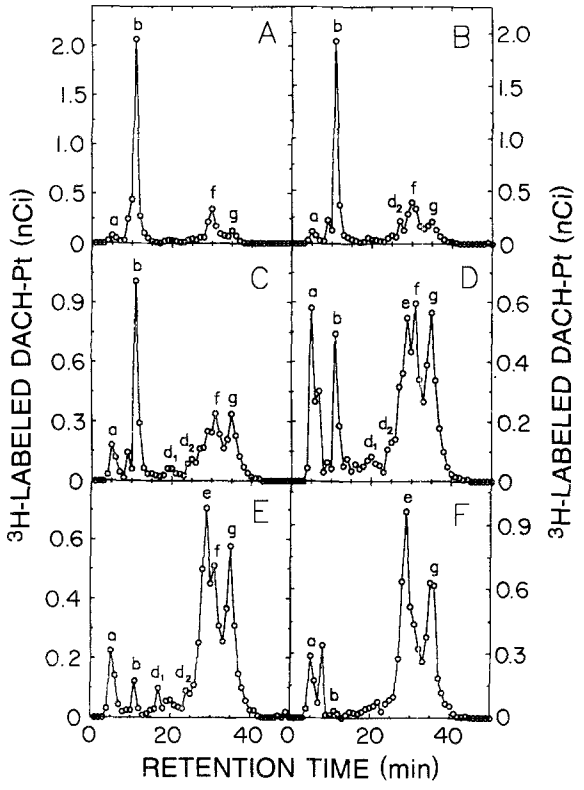

Fig. 2 A–F. Reverse-phase profiles of [^{3}H]-tetraplatin biotransformation products at the toxic dose. [^{3}H]-Tetraplatin (12 mg/kg) was injected i. v. into a Fischer 344 rat. At various times after injection, filtered plasma samples were analyzed by reverse-phase HPLC as previously described [17]. **A** 7.5 min. **B** 15 min. **C** 30 min. **D** 1 h. **E** 2 h. **F** 3 h. Note that the amount of filtered plasma sample analyzed for the 3 h sample was 1.5-fold that analyzed for the 2-h sample. Peaks are indicated by *a*, *b*, d_1, d_2, *e*, *f*, and *g* (see Fig. 3)

The reverse-phase HPLC profiles of the low-molecular-weight biotransformation products[2] were essentially the same at the toxic dose (Fig. 2) as at the therapeutic dose (data not shown), except that peak d_2 was seen only at the toxic dose. These profiles were also very similar to those previously observed after rat plasma has been incubated with tetraplatin in vitro [8], with the exception of peaks d_1 and d_2, which were seen only in vivo. The relative area in each peak was quantitated and converted to micromolar concentrations based on the column recovery and the micromolar concentration of free platinum at that time (Fig. 1 B). The data obtained from eight experiments at the therapeutic dose and from five experiments at the toxic dose are summarized in Fig. 3. As the HPLC system did not adequately resolve peaks e and f, they were quantitated together. However, it was evident from the HPLC profiles (Fig. 2) that peak f was a transient biotransformation product that had essentially disappeared by 1 h. Because the total counts were much lower at the therapeutic dose, the quantitation of individual biotransformation products was not as accurate at it was for the toxic dose. However, the data generally show that the disappearance of individual biotransformation products was similar at the toxic and

[2] The nomenclature used to describe the peaks in this separation was based on patterns originally observed in tissue-culture studies for Pt(mal)(dach) and $PtCl_2$(dach) [18, 19]. Peak a represents material eluting in the void volume and has not been characterized. Peak b corresponds to $PtCl_2$(dach). The peak/shoulder immediately preceding peak b corresponds to tetraplatin. These two species were further resolved on a Zorbax HPLC column [2]. Peak c corresponds to Pt(mal)(dach) and was not present in these experiments. Peaks d_1 and d_2 represent unknown biotransformation products that appear to form intracellularly. The characterization of the other peaks is described in this section of the present report

Table 1. Reactivity toward DNA of selected tetraplatin biotransformation products

Time after injection	Peak fraction from reverse-phase HPLC		
	d_1	e+f	g
Therapeutic dose:			
7.5 min	ND	50	ND
15 min	ND	44.9	ND
Toxic dose:			
7.5 min	ND	49.1	ND
15 min	ND	51.6	ND
30 min	10.5	33.2	23.7
1 h	2.7	11.2	13
3 h	<0.1	5.6	6.3

Peak fractions were obtained following reverse-phase HPLC at the therapeutic or toxic dose of tetraplatin (Fig. 2). Reactivity toward DNA was determined as previously described [18], except that the assay was carried out at 37° C for 30 min. The values are reported as a percentage of input counts bound to DNA. Under these assay conditions, the reactivity of standard $[Pt(H_2O)(Cl)(dach)]^2$ was 50.6% ± 1.2% (n = 4). ND, Not determined

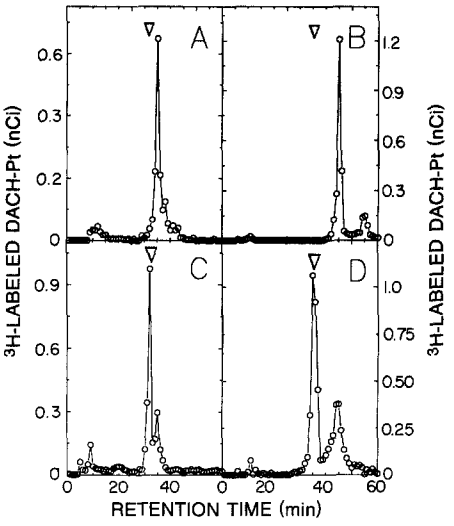

Fig. 4 A – D. Resolution of free dach and the Pt-methionine complex by cation-exchange HPLC. Peak g from the reverse-phase separations of the 3-h aliquots at the therapeutic and toxic doses (Fig. 2 F) were analyzed by cation-exchange HPLC at pH 4 and 2.3 as previously described [22]. **A** Peak g at the therapeutic dose and pH 4. **B** Peak g at the therapeutic dose and pH 2.3. **C** Peak g at the toxic dose and pH 4. **D** Peak g at the toxic dose and pH 2.3. ▽, Elution position of free dach on this column. These columns were standardized with tritium-labeled free dach on a daily basis

therapeutic doses, although the steady-state levels were significantly lower at the therapeutic dose. Peak b [primarily $PtCl_2$(dach)] disappeared rapidly from the circulation at both doses ($t^1/2\alpha \cong 4$ min for the therapeutic and 6 min for the toxic dose). Peaks e+f showed a rapid initial decrease (primarily due to a decrease in peak f), followed by relatively constant steady-state levels of peak e through 3 h. Peaks d_1 and g were also maintained at relatively constant steady-state levels through the first 3 h.

At the toxic dose, a small peak that immediately preceded peak b at early times could represent a small amount of unreacted tetraplatin in the circulation. Thus, tetraplatin and $PtCl_2$(dach) were resolved from separate aliquots of plasma filtrate by the Zorbax HPLC separation described previously [11]. These experiments confirmed the presence of a small amount of tetraplatin in early plasma samples at the toxic dose but not at the therapeutic dose (data not shown). Peak f has previously been shown to correspond to the aquachloro species $[Pt(H_2O)(Cl)(dach)]^+$ as well as several platinum-amino acid complexes [11, 17–19]. We have shown that $[Pt(H_2O)(Cl)(dach)]^+$ can be distinguished from the other possible components of peak f on the basis of its reactivity in a DNA-binding assay [11, 19]. Therefore, reactivity toward DNA was determined for several peak fractions from reverse-phase HPLC (Table 1). On the basis of this assay, peaks e+f appeared to be primarily $[Pt(H_2O)(Cl)(dach)]^+$ through the first 30 min at both toxic and therapeutic doses. There were insufficient counts to carry out this assay at later times for the therapeutic dose, but at the toxic dose it was apparent that the amount of $[Pt(H_2O)(Cl)(dach)]^+$ present in peaks e+f steadily declined with time. Some reactivity was observed in peaks d_1 and g at 30 min. It is not clear whether this was due to other biotransformation products with inherent reactivity toward DNA or due to overlap with peak f.

Finally, based on previous experiments, peak g might be expected to contain either free dach [17, 19], the Pt-methionine [17, 19], or the Pt-citrato [8] complexes. These possibilities can be resolved by cation-exchange HPLC, especially if this procedure is carried out at both pH 4 (which resolves the Pt-methionine and citrato complexes) [8] and pH 2.3 (which resolves free dach and the Pt-methionine complex) [17–19]. At the therapeutic dose, it was clear that most of peak g represented the Pt-methionine complex (Fig. 4 A, B), whereas at the toxic dose most of peak g corresponded to free dach, with smaller amounts of the Pt-methionine complex being present (Fig. 4 C, D). No citrato complex was detectable at either dose.

The analyses described above enabled resolution of the reverse-phase HPLC peaks into individual biotransformaton products. Because of low counts, these data were incomplete at the therapeutic dose. However, the results at the toxic dose are summarized in Fig. 5. It was evident that both $PtCl_2$(dach) and $[Pt(H_2O)(Cl)(dach)]^2$ were rapidly cleared from the circulation. The small amounts of tetraplatin present at the toxic dose were also rapidly cleared. In contrast, the other plasma biotransformation products appeared to be maintained at approximately constant steady-state levels, with the possible exception of d_2, which was slowly cleared from the circulation.

The earlier data (Fig. 4) had shown significant amounts of free dach in the circulation at the toxic dose of tetraplatin. This free dach most likely arose through trans-labilization of the carrier ligand [14, 18, 25] and suggested the existence of some platinum biotransformation products that would not have been detected in the earlier experiments due to loss of the labeled carrier ligand. Thus, the experiments were repeated using [^{195m}Pt]-tetraplatin synthesized as previously described [28]. As the synthesis did not provide sufficient material for the 12-mg/kg dose,

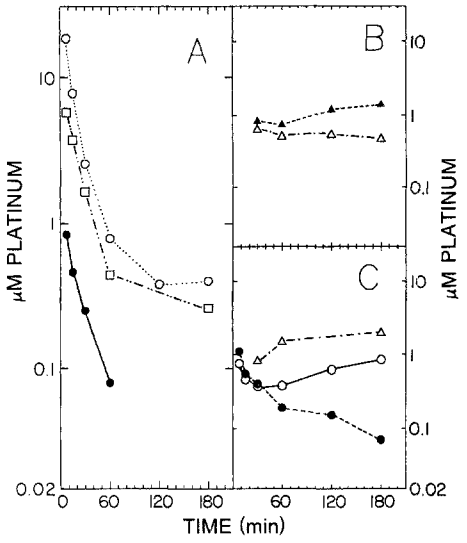

Fig. 5 A–C. Concentrations (μM) of platinum biotransformation products at the toxic dose of tetraplatin. Tetraplatin and $PtCl_2$(trans-dach) were quantitated by reverse-phase HPLC on a Zorbax column [11]. The other biotransformation products were quantitated by reverse-phase HPLC on a Whatman Partisil 5 ODS-3 column (Fig. 2). The contribution of $[Pt(H_2O)(Cl)(dach)]^2$ to peaks e+f was determined by the DNA-binding assay (Table 1); the material remaining was assumed to represent peak e. The contributions of free dach and the methionine complex to peak g (Fig. 2) were resolved by cation-exchange HPLC at pH 4.0 and 2.3 (Fig. 4). The data points represent the mean of 5 experiments; the standard error of the mean was generally ≤% or, at the lower concentrations, ≤.1 μM. **A** ○·····○, $PtCl_2$(dach); □–··–□, $[Pt(H_2O)(Cl)(dach)]^+$; ●——●, tetraplatin. **B** △–·–△, Pt-methionine complex; ▲– – –▲, free dach. **C** ○——○, peak d_1; ●– – –●, peak d_2; △–·–△, peak e

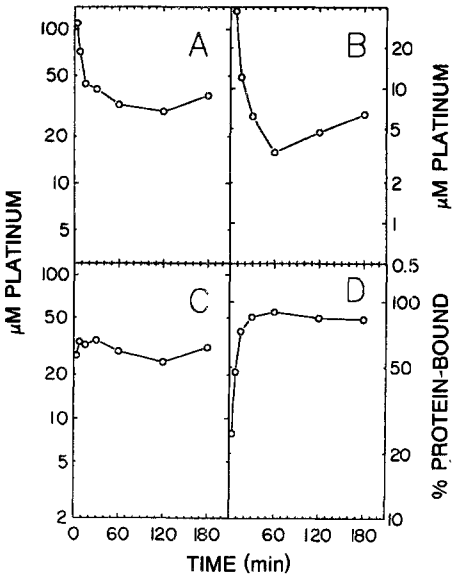

Fig. 6 A–D. [^{195m}Pt]-Tetraplatin pharmacokinetics. [^{195m}Pt]-Tetraplatin (10 mg/kg) was injected i.v. into a rat at time 0. Plasma samples were obtained at 3.5, 7.5, 15 and 30 min and at 1, 2, and 3 h and were analyzed as described in Materials and methods. Platinum concentrations were determined based on the specific activity of the [^{195m}Pt]-tetraplatin (27.1 mCi/mmol), allowing for decay ($t^1/_2$ = 4.02 days). **A** Total platinum biotransformation products. **B** Free platinum biotransformation products. **C** Protein-bound platinum biotransformation products. **D** Percentage of platinum biotransformation products that were protein-bound

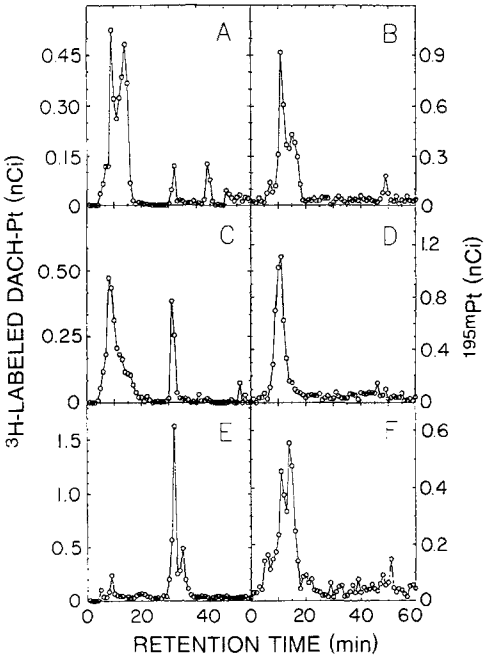

Fig. 7 A–F. Comparison of cation-exchange profiles of reverse-phase peaks e, f, and g from rats treated with [^{3}H]- and [^{195m}Pt]-tetraplatin. Cation-exchange HPLC [17] at pH 4.0 was used to characterize peaks e, f, and g from reverse-phase HPLC. **A** [^{3}H]-Tetraplatin, 7.5 min, peak f. **B** [^{195m}Pt]-Tetraplatin, 7.5 min, peak f. **C** [^{3}H]-Tetraplatin, 3 h, peak e. **D** [^{195m}Pt]-Tetraplatin, 2 h, peak e. **E** [^{3}H]-Tetraplatin, 2 h, peak g. **F** [^{195m}Pt]-Tetraplatin, 2 h, peak g

10 mg/kg was used. The pharmacokinetics (Fig. 6) and reverse-phase HPLC elution profiles (data not shown) were very similar to those observed with the tritium-labeled drug. The cation-exchange profiles were also very similar for peaks f (Fig. 7 A, B) and e (Fig. 7 C, D); however, they were very different for peak g (Fig. 7 E, F). The peak corresponding to free dach in plasma from animals treated with tritium-labeled drug was replaced with some earlier eluting platinum biotransformation products in plasma from animals treated with [^{195m}Pt]-tetraplatin.

Discussion

This is the first detailed comparison of the plasma biotransformations of a platinum complex at both a therapeutic and a toxic dose. Several differences were observed. Some unreacted tetraplatin was present at early times at the toxic but not at the therapeutic dose. Free dach and biotransformation product d_2 were also found in the circulation only at the toxic dose. Otherwise, the biotransformations were quite similar except that plasma levels of all of the platinum biotransformation products detected were generally higher at the toxic than at the therapeutic dose (Fig. 3).

The lack of unreacted tetraplatin in the plasma at the therapeutic dose is consistent with previous in vitro data [8]. When rat plasma was incubated with tetraplatin in vitro at concentrations (5–20 μM) corresponding to the therapeutic dose, the reduction of tetraplatin to $PtCl_2$(dach)

was extremely rapid ($t_{1/2}$ = 3 s), with plasma sulfhydryl being the principal reducing agent. However, total plasma sulfhydryl amounted to only about 200 μM. At the toxic dose, the initial levels of tetraplatin in the circulation could easily match or exceed the plasma sulfhydryl concentration. The free dach most likely arose from the trans-labilization of carrier ligand by high intracellular sulfhydryl levels [14, 25]. We did not see any significant loss of carrier ligand when tetraplatin was incubated with rat plasma in vitro [8] or with tissue-culture medium [11]. However, we have observed intracellular labilization of the dach carrier ligand in L1210 cells incubated with $PtCl_2$(dach) [18]. The unidentified biotransformation products d_1 and d_2 are also most likely formed intracellularly. Previous experiments in our laboratory have shown that extracellular tetraplatin can be taken up and converted to product d_2 intracellularly. Biotransformation product d_1, in turn, can be formed intracellularly either from d_2 or from $PtCl_2$(dach) (Chaney et al., submitted for publication). The previous data also suggested that most of products d_1 and d_2 diffused out of the cell. The present findings are consistent with those data. No d_1 or d_2 was seen in plasma incubated with tetraplatin in vitro [8]. However, small amounts of d_1 were seen in plasma from animals treated with therapeutic doses of tetraplatin (data not shown), and larger amounts of both d_1 and d_2 were found in plasma of animals treated with toxic doses (Fig. 2).

The significance of these differences is not clear. Free dach is not likely to be toxic in itself, but its presence in plasma coincides with the formation of intracellular and plasma biotransformation products that may not occur at the therapeutic dose. Similarly, unreacted tetraplatin is not likely to be significantly more toxic than $PtCl_2$(dach), but its presence in the plasma does enable the formation of product d_2. Although either d_1 or d_2 could be responsible for some of the toxicities observed at higher doses of tetraplatin, both our present (Table 1) and our previous data (Chaney et al., submitted for publication) suggest that these biotransformation products are largely unreactive.

In addition to focusing on differences at the toxic vs therapeutic doses, we tentatively identified several of the plasma biotransformation products formed. The identifications were based on comparisons of the retention times of these compounds on at least two different HPLC systems with those of 37 platinum(II) [8, 17–19] and 13 platinum(IV) standards (Chaney et al., submitted for publication) previously prepared in this laboratory. It is clear that most of the tetraplatin was rapidly converted to $PtCl_2$(dach), as previously observed in vitro [8]. The next biotransformation product observed (peak f) was $[Pt(H_2O)(Cl)(dach)]^+$. It was identified on the basis of its elution profiles on both reverse-phase [17] (Fig. 2) and cation-exchange HPLC [17] (Fig. 7A, B) as well as its reactivity toward DNA [18] (Table 1). Of 37 Pt(dach)(II) complexes studied to date, only 9 have HPLC elution profiles consistent with peak f [17–19]. Of the latter, only $[Pt(H_2O)(Cl)(dach)]^+$ shows significant reactivity toward DNA [18]. The $[Pt(H_2O)(Cl)(dach)]^+$ complex was also observed when tetraplatin was incubated with rat plasma in vitro [8], as were the other two major biotransformation products seen in these experiments. The Pt-methionine complex was found in peak g and was identified on the basis of reverse-phase (Fig. 2) and cation-exchange elution profiles [17] (Fig. 4). The reverse-phase (Fig. 2) and cation-exchange profiles (Figs. 7C, D) of peak e have enabled the tentative identification of this biotransformation product as the Pt-cysteine or Pt-ornithine complex [8]. Of these two, the Pt-cysteine complex is more likely because if forms more rapidly under physiological conditions. One other major biotransformation product was observed in vitro and has tentatively been identified as the Pt-citrato or Pt-urea complex [8]; this product was not detectable in the present in vivo experiments. Either its formation was too slow to be observed in vivo or the complex was cleared from the circulation as rapidly as it was formed.

The present data indicate significant differences in the clearance of different biotransformation products from the circulation. Obviously, tetraplatin, $PtCl_2$(dach), and $[Pt(H_2O)(Cl)(dach)]^+$ were cleared from the circulation very rapidly (Fig. 5), although in the latter case it would be difficult to distinguish between clearance from the circulation and conversion to more stable biotransformation products. However, other biotransformation products such as the Pt-methionine and the Pt-cysteine or Pt-ornithine complexes were cleared from the circulation only slowly, if at all (Fig. 5). The same was true for free dach and the unknown biotransformation products d_1 and d_2. It is not known whether this slow clearance was due to very slow uptake by the tissue and limited filtration by the kidney or whether it represented the continuing intracellular formation and subsequent efflux of those compounds. Both mechanisms may contribute. Previous experiments have suggested that both free dach [11, 18] and products d_1 and d_2 are most likely formed intracellularly and readily diffuse out of the cell. The significance of these persistent biotransformation products is not known, but they could, in theory, be responsible for some of the delayed toxicity associated with platinum chemotherapy.

These data are fully consistent with previous studies of cisplatin biotransformations by Daley-Yates and McBrien [10]. These authors found that cisplatin was rapidly cleared from the circulation, whereas the Pt-methionine complex persisted through at least 3 h. Their data also suggested that the aquachloro complex was persistent, but their identification of this complex was based on reverse-phase retention times only. Since peaks e and f overlap, we could easily have reached a similar conclusion if our identification of the $[Pt(H_2O)(Cl)(dach)]^+$ complex had been based on reverse-phase HPLC only. Finally, it should be noted that the $t^1/_2\alpha$ estimates in the present experiments were slightly faster than those reported previously [21]. Although the labilization of the tritium-labeled carrier ligand could have led to subsequent pharmacokinetic inaccuracies, the experiment using [^{195m}Pt]-tetraplatin showed the same overall pharmacokinetics (Fig. 6).

In summary, our data show that there are some differences in the types of biotransformation products present at toxic vs therapeutic doses of tetraplatin. However, the major differences in toxicity are most likely due to the amount, rather than the type, of biotransformation products present in plasma. We identified $PtCl_2$(dach), $[Pt(H_2O)(Cl)(dach)]^+$, and the Pt-methionine complexes as

the major plasma biotransformation products of tetraplatin in vivo. We also provided tentative identification of another biotransformation product as the Pt-cysteine or Pt-ornithine complex. Tetraplatin, $PtCl_2$(dach), and $[Pt(H_2O)(Cl)(dach)]^+$ are rapidly cleared from the circulation, whereas the other biotransformation products are persistent and could be responsible for delayed toxicity. Experiments are currently under way in our laboratory to confirm the identity of these plasma biotransformation products and to determine their toxicity and therapeutic effectiveness.

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