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Organ-specific biotransformation of ormaplatin in the Fischer 344 rat

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Abstract We examined the intracellular biotransformation products of ormaplatin [(d,l-*trans*)1,2-diaminocyclohexanetetrachloroplatinum(IV)] (formerly called tetraplatin) in liver, kidney, spleen, small intestine, and plasma of the adult male Fischer 344 rat. Previous studies have established that the rank order of ormaplatin toxicity in Fischer 344 rats is spleen ≈ gastrointestinal tract > kidney ≫ liver. Animals were given tritium-labelled drug i.v. at 12.5 mg/kg, and tissues were harvested 30 min later. The kidney was found to concentrate total and cytosolic platinum to a greater extent than any of the other tissues. The absolute amount of cytosolic platinum, in micrograms per gram tissue, that was irreversibly bound to protein and/or other macromolecules was also greatest in the kidney. However, when the amount bound was expressed as a percentage of the total cytosolic platinum, the kidney was significantly lower than any other tissue. Of the various low molecular mass platinum biotransformation species characterized, by far the most abundant were complexes of platinum with the sulfur-containing molecules cysteine, methionine, and glutathione (GSH). There was more of the methionine complex in the blood plasma than in any of the tissues except for the spleen. No significant differences among the tissues were detected for the dichloro, cysteine, methionine, or the GSH complexes. The tritium-labelled diaminocyclohexane (DACH) carrier ligand appeared to remain stably bound to the platinum while in the plasma, as there was less free DACH ligand detected in plasma ultrafiltrate than in any tissue ultrafiltrate. Among the tissues, the free DACH levels were in the range of 20% of the radioactivity recovered from the HPLC column and were not significantly different. Consequently, neither biodistribution nor tissue-specific biotransformation of ormaplatin provides a ready explanation for the tissue specificity of ormaplatin toxicity in Fischer 344 rats. However, in kidney there was much less of the reactive $PtCl_2$(DACH) species than has previously been reported for the corresponding $Pt(NH_3)_2Cl_2$ species in cisplatin-treated rats. Thus, these data suggest a possible explanation for differences in nephrotoxicity induced by cisplatin versus that by ormaplatin.

Key words Ormaplatin · Biotransformation · Rats

Abreviations *DACH* (d,l)*Trans*-1,2-diaminocyclohexane · *$PtCl_2$(DACH)* (d,l)*trans*-1,2-diaminocyclohexanedichloroplatinum(II) · *DDTC* diethyldithiocarbamate · *WR-2721* *S*-2-(3-aminopropylamino)-ethylphosphorothioic acid · *HPLC* high-performance liquid chromatography · *RP* reverse phase chromatography · *SCX* strong cation-exchange chromatography · AAS flameless atomic absorption spectroscopy · *LSC* liquid scintillation counting · *MWCO* molecular weight cutoff · *GSH* reduced glutathione

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Introduction

Ormaplatin [(d,l-*trans*)1,2-diaminocyclohexanetetrachloroplatinum(IV)] (formerly called tetraplatin), a platinum(IV) analog of cisplatin (*cis*-diamminedichloroplatinum(II)), has been shown to cause significantly

less toxicity to the kidney than cisplatin [36, 38, 39], and has recently been evaluated in phase I clinical trials. In addition, it has exhibited activity against a number of tumor cell lines known to be resistant to cisplatin [1, 35]. The ability of certain chemoprotective compounds being tested clinically, such as DDTC and WR-2721 (ethiofos), to inhibit platinum-induced organ toxicity with little or no inhibition of its antitumor activity, has led some investigators to postulate that the mechanisms underlying these two activities may be different [3]. It has also been postulated that the mechanism(s) of action of platinum complexes will ultimately be determined through identification and quantitation of the various platinum biotransformation products likely to form in biological tissues [13, 25].

Given this hypothesis, some separation of platinum-containing species derived from cisplatin in plasma ultrafiltrate and in urine has been achieved, although it is only unchanged cisplatin that can be identified with reasonable certainty [11, 12]. Daley-Yates and McBrien have, however, tentatively identified a complex of platinum with methionine and an unspecified hydrolysis product [11, 12]. A standard of this hydrolysis product prepared in vitro proved to be more nephrotoxic than the parent drug, cisplatin, when injected into test animals. The analyses of rat kidney cytosols by Mason et al. [26, 27] and by Mistry et al. [32] are the only investigations thus far of any tissue biotransformation products, and both studies involved the use of cisplatin. Only Mistry et al. [32] attempted to identify any of the high or low molecular mass species that were separated.

The relevance of the work reported here with ormaplatin arises from the fact that the organ distribution of total platinum after administration of cisplatin and ormaplatin is basically similar [35], yet ormaplatin is much less nephrotoxic than cisplatin [36, 38, 39]. This difference in nephrotoxicty is probably not due to differences in the valence state of the platinum in these complexes, as ormaplatin is, in effect, a prodrug which undergoes rapid ($t_{1/2} \approx 3$ s) reduction in plasma to the corresponding platinum(II) analog, $PtCl_2$(DACH) [16]. These findings suggest that cisplatin and ormaplatin may undergo different biotransformation reactions once inside the cell, and that this difference is in some way critical to the development of toxicity. Furthermore, a review of findings from a number of laboratories [3] suggests that the cytotoxic lesion from platinum compounds occurs within the first 2 h after dosing. Thus, we confined our investigations to this 2-h interval. Previous work in this laboratory has demonstrated the capability of an HPLC system to isolate and identify ormaplatin biotransformation products in cultured L1210 murine leukemia cells [28, 29], and in rat plasma both in vitro [8] and in vivo [6, 7]. The work reported here extended these studies to the whole animal. Our primary objective was to identify and quantitate the various intracellular biotransformation products of ormaplatin, and to investigate the interaction between in vivo distribution of ormaplatin and individual tissue levels of these biotransformation products. One important question is whether or not the tissue-specific toxicities exhibited by ormaplatin may be explained by tissue differences in its biotransformation and/or distributional behavior.

Subsequent to the initiation of these studies, ormaplatin has been found to possess significant neurotoxicity in clinical trials [10, 33, 37]. This neurotoxicity was not evident in the earlier preclinical studies which formed the basis for this investigation. Furthermore, most of the available evidence suggests that the neurotoxicity of both cisplatin [17, 30] and ormaplatin [33] are due to effects on peripheral nerve fibers, which provide too little material for these types of biotransformation studies. Thus, it was not possible to investigate the role that tissue-specific biotransformation may play in the neurotoxicity of ormaplatin.

Materials and methods

Materials

[4,5-$^3H_2$(n)]-(d,l-*trans*)1,2-diaminocyclohexanetetrachloroplatinum(IV)] ^{3}H-ormaplatin was prepared in the Radiosynthesis Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina at Chapel Hill. The synthesis and purity of this compound have been described elsewhere [41]. Reductive tritiation of the cyclohexene ring yields a radiolabel which is not chemically exchangeable [41]. Preparation of a solution of the drug for i.v. injection was as previously described [7]. YMT ultrafiltration membranes (MWCO 30,000) were purchased from Amicon (Danvers, Mass.). HPLC grade reagents were obtained from commercial sources, and solutions were filtered and degassed prior to use. A Barnstead Nanopure water purification system with an organics filter provided the water used with solvents and for dissolution. All other general laboratory reagents were commercial reagent grade or better and were used without further purification.

Animals

Fischer 344 adult male rats (150–200 g) were obtained from Charles River Breeding Laboratories (Raleigh, NC) and housed in clear plastic cages on a 12-h light/dark cycle with access to water and Purina rodent chow *ad libitum*. Room temperature was maintained at approximately 22°C. Animals were allowed at least a 1-week acclimatization period prior to use in experiments.

Methods

Animals were given ^{3}H-ormaplatin at 12.5 mg/kg, a dose known to be toxic [6, 38], via lateral tail vein injection under light ether anesthesia as previously described [5]. At 15, 30, 60, or 120 min postinjection, blood was collected via cardiac puncture under light ether anesthesia, and immediately centrifuged in a heparinized tube at approximately 1,000 *g* for 15 min at 4°C [13]. The plasma supernatant was aspirated, and immediately diluted 1:5 in ice-cold 0.15 *M* NaCl. The diluted plasma was then filtered over Amicon

YMT membranes by centrifugation at approximately 1,700 *g* for 45 min at 4°C (Sorvall RC2-B with SS-34 rotor, Norwalk, Ct.) [6, 40]. Aliquots from all three fractions were removed and either allowed to sit overnight in scintillation cocktail for LSC, or stored at −20°C for later AAS analysis.

Organs of interest were harvested and placed into ice-cold 0.15 *M* NaCl. Whole-body perfusion to remove residual blood from the organ vasculature was determined in a control experiment not to be necessary, as there were no discernible differences in either platinum levels of the various tissue fractions or in RP HPLC biotransformation product profiles (data not shown). Slices from several lobes of the liver, the cortex and outer medulla of each kidney, the entire spleen, and several sections of small intestine were isolated on ice, weighed, and then homogenized according to the method of Mistry et al. [32]. Cytosol and cytosolic ultrafiltrate were separated essentially as described [32]. Aliquots from homogenate, cytosol, and ultrafiltrate were removed for LSC and AAS analysis. The remainder of the ultrafiltrates from plasma and each of the tissues were frozen at −20°C until analysis by HPLC.

Radioactivity was assessed using an LKB model 1215 scintillation counter. The difference between counts in the cytosol and in the ultrafiltrate was considered to represent macromolecular-bound platinum [6, 13], since ormaplatin (data not shown) and a number of other platinum analogs [40] have demonstrated no appreciable adsorption to YMT membranes. Counts in the ultrafiltrate, though frequently referred to as "free platinum", are more aptly termed "filterable", as it is considered likely that they represent platinum bound to low molecular mass substances from the plasma and/or cytosol [22]. AAS was performed on a Perkin Elmer Zeeman 5100 atomic absorption spectrophotometer with an HGA-600 graphite furnace. Preparation of samples for analysis was accomplished essentially according to the method of Blisard et al. [2].

The biotransformation products from the ultrafiltrates of plasma and tissues were separated by a two-column HPLC system developed by Mauldin et al. [28]. Briefly, an initial separation on a Partisil ODS-3 RP column with heptane sulfonate as the ion pairing reagent provided resolution of species as neutral paired ions on the basis of polarity. Peak fractions of interest from this separation were pooled and then injected onto a Partisil 10 SCX cation exchange column for further resolution of the positively charged species. Fractions from both separations were collected and analyzed for radioactivity by LSC. The total radioactivity of any peak of interest was expressed as a percentage of the total radioactivity recovered from the column. Individual biotransformation products were identified by comparison of their retention times on RP and SCX HPLC with those of standards prepared in the laboratory [28, 29]. While these identifications are recognized as tentative since they were not confirmed by mass spectrometry, it was considered unlikely that any major low molecular mass biotransformation product would fall outside the realm of the approximately 40 complexes already characterized under the three different conditions of our HPLC system (i.e. RP, cation exchange pH 4.0, and cation exchange pH 2.3) [28, 29].

Statistics

Determination of the statistical significance of differences among the tissues and plasma in ormaplatin biodistribution and levels of biotransformation products was by one-way analysis of variance (ANOVA), followed by Duncan's post-hoc comparison of means where appropriate. Significance was based on an alpha level of 0.05. For biodistribution data where parallel LSC and AAS data were available, a two-way ANOVA was performed to determine any possible interactive effect of tissue and method on the level of a given fraction, followed by Duncan's post-hoc comparison of means where appropriate. All statistical analyses were performed with the SAS Statistical Software package.

Results

Rahman et al. [35] have previously determined the tissue distribution of platinum in rats at various times following i.v. injections of both ormaplatin and cisplatin, and Mistry et al. [32] have reported on biotransformation of cisplatin in rat kidney. Thus, we focused this investigation on organ-specific differences in ormaplatin biotransformation. Adult male Fischer 344 rats were given ^{3}H-ormaplatin i.v. at 12.5 mg/kg (a known toxic dose) [6, 38], and at 15, 30, 60, or 120 min after administration, organ tissues were harvested and analyzed as described in Methods. Shown in Fig. 1 are RP HPLC profiles for each of these time-points from a representative tissue (kidney). Since these profiles appeared to be similar between 30 and 120 min, we felt that the major biotransformation products were comparable within this time interval. We therefore chose to concentrate our investigations on one time-point within this interval (30 min postinjection) in order to increase the statistical power of our comparisons. Thus, all subsequent data presented were obtained at this one time-point. In one control experiment, whole-body perfusion with normal saline was performed on a treated animal prior to organ harvesting. Whole-body perfusion had no effect on the biodistribution or biotransformation of ormaplatin (data not shown), so this procedure was not continued in subsequent experiments.

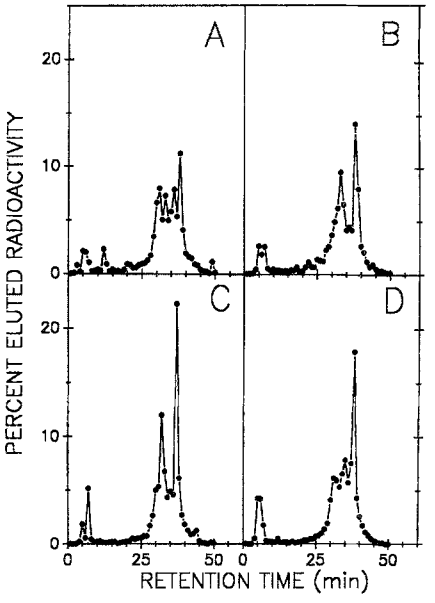

Fig. 1A–D RP HPLC profiles from tissue ultrafiltrates at various times following a toxic dose of ^{3}H-ormaplatin. ^{3}H-Ormaplatin (12.5 mg/kg) was injected i.v. into Fischer 344 rats. At 15 (**A**), 30 (**B**), 60 (**C**), and 120 (**D**) min following administration animals were exsanguinated, and tissue ultrafiltrates were prepared as described in Methods. Shown are profiles of a representative tissue (kidney) following separation of ultrafiltrates on RP HPLC as described in Methods

Aliquots from tissue homogenate, cytosol (100,000 *g* fraction), and ultrafiltrate were analyzed both for radioactivity by LSC and for platinum content by AAS. As described in Methods, that portion of the cytosolic platinum and/or radioactivity that was retained on the membrane during the ultrafiltration step was considered to be bound to cytosolic macromolecules, primarily protein [6, 13]. These data are presented in Fig. 2 as absolute amounts of platinum and ^{3}H-DACH in the various tissue fractions, where tritium levels were converted to a value for platinum using the specific activity of the ^{3}H-ormaplatin. For total, cytosolic, filterable and bound platinum, the apparent rank order for the various tissues was kidney > liver > spleen > small intestine. Rahman et al. [35] showed a similar rank order of total platinum distribution (kidney > liver > spleen > small intestine) following both cisplatin and ormaplatin administration, and reported comparable amounts of tissue platinum (e.g. 29.1 μg/g in the kidney 1 h after a 3 mg/kg i.v. dose to Sprague-Dawley rats). Statistical analysis confirmed the difference between the amount in the kidneys and the amounts in all other tissues tested, for all fractions and for both methods of analysis. No other differences were detected statistically. In particular, while previous studies have demonstrated labilization of the DACH carrier ligand at later times in mice treated with ^{3}H-ormaplatin [20], statistical analysis detected no differences at 30 min in the measurement of platinum levels by the two methods, LSC and AAS.

The amount of platinum bound to macromolecules (Fig. 2D) is shown for each of the tissues as a percentage of the total amount of platinum in the cytosol in Fig. 3; the corresponding value for the percentage of total platinum in the plasma bound to plasma proteins is shown for comparison. The 70–75% binding to plasma proteins at 30 min compares favorably with the results of Carfagna et al. [6, 7] for ormaplatin in this same system. The percentages of cytosolic platinum and DACH ligand that were bound to macromolecules in the liver, spleen, and intestine were not statistically different from the corresponding percentages of plasma platinum and DACH bound to plasma proteins. The percentage bound in the kidney, in contrast, was considerably less ($P < 0.05$), of the order of 30%.

Identification and quantitation of ormaplatin biotransformation products was in accordance with previously published procedures [6, 28, 29] in which comparison was made of HPLC retention times of tissue samples with those of appropriate standards. Figure 4 shows a sequence of typical HPLC profiles of ^{3}H-ormaplatin biotransformation products from a representative tissue (kidney) illustrating how the various biotransformation products were resolved and identified. Fig. 4A is a profile derived from a RP HPLC separation, the first separation of the two-column system described in Methods. The peaks are labelled according to previously established nomenclature [6]. Peak *a* was material unretained by the column and has not been fully characterized. Peak *b* contained the reduced $PtCl_2$(DACH); any peak/shoulder eluting immediately prior to peak *b* corresponded to the unchanged parent drug, ormaplatin. Based upon the elution profiles of approximately 40 DACH-Pt standards previously characterized in this same system [28], peak *e* was predominantly the cysteine complex, peak *f* could have contained the GSH, serine, threonine,

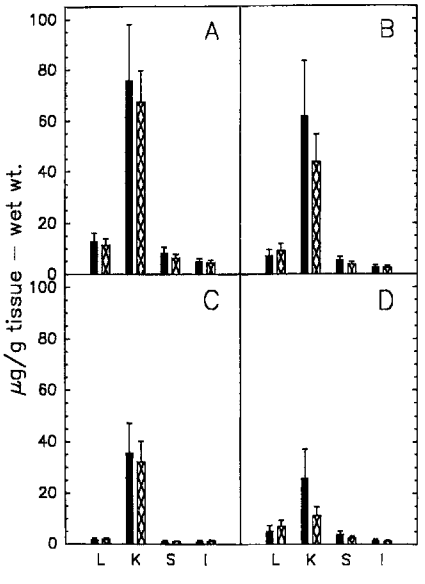

Fig. 2A–D Organ distribution and disposition of ^{3}H-ormaplatin at 30 min following a toxic dose. Tissue fractions were quantitated for ^{3}H-DACH by LSC (*hatched bar*) and for platinum by AAS (*solid bar*), and converted to μg/g tissue-wet weight (mean ± SE, $n = 3$–6) (*L* liver, *K* kidney, *S* spleen, *I* small intestine). **A** Total, **B** cytosolic, **C** filterable, **D** bound. Statistical analysis included two-way ANOVA, followed by Duncan's post-hoc comparison of means ($P < 0.05$). The difference between kidney and each of the other tissues was found to be significant for all fractions and by both methods of analysis; no other differences were found to be significant

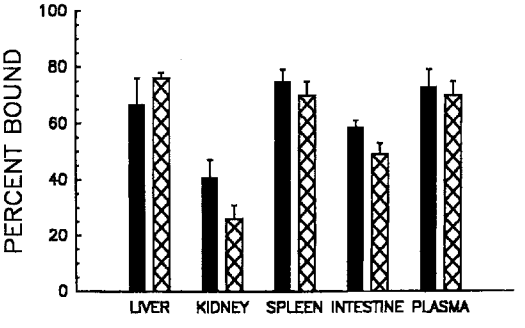

Fig. 3 Percent binding to cytosolic macromolecules. Conditions were as described for Fig. 2. Data are the amount of ^{3}H-DACH (*hatched bar*) or platinum (*solid bar*) bound to cytosolic macromolecules expressed as a percentage of the total amount in the cytosol (mean ± SE, $n = 4$–6). Statistical analysis was as described for Fig. 2. Kidney was found to be significantly different from the rest of the tissues tested by both methods of analysis

glutamine, or asparagine complexes, peak *g* was predominantly free DACH carrier ligand and the methionine complex, and peak *h* could have been free DACH or the citrato complex.

These possibilities were further resolved by means of SCX HPLC separation of selected peaks at pH 4.0 and (where necessary) pH 2.3. Peaks *e* and *f*, and peaks *g* and *h* were not always clearly resolved by RP HPLC, and therefore they were usually pooled for injection on the SCX column. Figure 4B shows the profile resulting from the injection of the *e/f* peaks of Fig. 4A onto a SCX column at pH 4.0. In this case the peak with a retention time of 7 min was most characteristic of the cysteine complex and the peak at 10 min was most characteristic of the GSH complex, although the overlap of these two even on cation exchange means that their identification should be considered tentative. The peak with a retention time of 36 min was probably from some overlap with the *g/h* peaks. Figure 4C is the profile of the *g/h* peaks from Fig. 4A subjected to the same pH 4.0 SCX separation. Figure 4D shows the profile of these same *g/h* peaks from Fig. 4A subjected to SCX at pH 2.3. Under these conditions a standard of free DACH carrier ligand elutes with a retention time of 35–36 min at both pH 4.0 and pH 2.3 [28]. The methionine standard migrates as a single peak at 35 min at pH 4.0, whereas at pH 2.3 it has a retention time of approximately 42 min [28]. As shown in Fig. 4, significant amounts of the methionine, cysteine, and GSH complexes with platinum, as well as of free DACH carrier ligand, could be identified. Smaller amounts of $PtCl_2$(DACH), unchanged ormaplatin, and other, unidentified biotransformation products were also present.

A summary of the quantitation of the major ormaplatin biotransformation products from the tissues and plasma, as a percentage of filterable platinum, is shown in Fig. 5. The presence in the plasma of the reduced $PtCl_2$(DACH) species as the major biotransformation product at 30 min confirmed previous work from this laboratory [6, 16] that ormaplatin is rapidly reduced to the platinum(II) derivative in the presence of plasma protein sulfhydryl. It is, therefore, likely that $PtCl_2$(DACH) is the predominant species to enter the cell and undergo subsequent biotransformation. However, the data shown here (Fig. 5) and previously [6, 9] suggest that, at toxic doses such as those used in this study, the plasma concentration of ormaplatin can exceed the reductive capacity of the plasma protein sulfhydryls, since some unchanged ormaplatin was still detectable in the plasma at 30 min, and to a lesser extent in the tissues. Several significant differences were observed between plasma and the tissues surveyed in this study. For example, the amount of $PtCl_2$(DACH) in the plasma was greater than in any of the tissues. There was also less free DACH ligand in plasma at 30 min than in any of the tissues tested. Finally, the

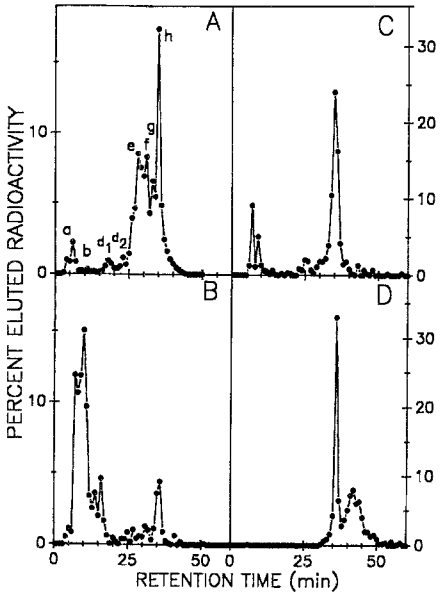

Fig. 4A–D Resolution of individual biotransformation products. Ultrafiltrates of blood plasma and tissue cytosol were prepared as described in Methods. Low molecular mass biotransformation products of ormaplatin in each of the ultrafiltrates were resolved by means of a two-column HPLC system developed in this laboratory and described previously [28]. **A** RP separation of a representative tissue ultrafiltrate (kidney). Peaks are indicated by *a*, *b*, d_1, d_2, *e*, *f*, *g*, and *h*, according to previously established nomenclature [6]. **B** SCX separation of *e/f* peaks from **A** at pH 4.0. **C** SCX separation of *g/h* peaks from **A** at pH 4.0. **D** SCX separation of *g/h* peaks from **A** at pH 2.3. Note *y*-axis scale is not uniform among graphs

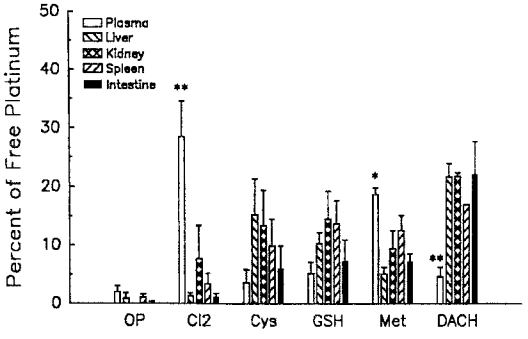

Fig. 5 Tissue-specific ormaplatin biotransformation products as a percentage of filterable platinum. Individual biotransformation products were identified based upon comparison of their elution characteristics on RP and SCX HPLC with those of standards prepared in the laboratory [28, 29]. Peaks were quantitated as a percentage of the total radioactivity (^3H) recovered from the column, and then normalized as a function of the filterable platinum present in each cytosol (mean ± SE, $n = 3$ or 4). Results of statistical analysis by one-way ANOVA: [*Cl2*]: ($F(4,15) = 9.07$, $P < 0.0006$); [*Met*]: ($F(4,10) = 6.77$, $P < 0.0066$); [*DACH*]: ($F(4,10) = 7.55$, $P < 0.0045$); no further significant effect at the $P < 0.05$ level was detected. Results of Duncan's post-hoc comparison of means ($P < 0.05$): ** significantly different from all other tissues tested; * significantly different from all other tissues but spleen. *OP* ormaplatin, *Cl2* $PtCl_2$(DACH), *Cys* Pt(cysteine)(DACH), *GSH* Pt(glutathione)(DACH), *Met* Pt(methionine)(DACH), *DACH* dissociated DACH carrier ligand

amount of methionine complex detected was greater in the plasma than in all the tissues except spleen. However, there were no statistically detectable differences among the tissues in the levels of any of the biotransformation products quantitated.

To determine the fate of that filterable platinum that had lost the DACH carrier ligand, the RP HPLC fractions from kidney ultrafiltrates were analyzed for platinum by AAS in parallel with tritium by LSC (Fig. 6). Data are plotted as percentages of the radioactivity or total platinum recovered from the HPLC column in the Fig. 6A, B. Fig. 6C shows the absolute micromolar difference between the LSC and the AAS determinations for each fraction. Recovery averaged 85% for platinum and 83% for recovered radioactivity over all analyses. In general, there appeared to be good agreement between the resulting profiles from the two analyses. The prominent exception on this particular sample was the excess of tritium in the large peak with retention time 35 min on LSC analysis and a smaller excess of platinum in the peak migrating with the void volume (retention time 4–6 min) on AAS analysis. SCX HPLC at pH 4.0 and at pH 2.3 confirmed that most of the ^{3}H-labelled peak with a retention time of 35 min in the kidney was the free DACH carrier ligand (Fig. 4C, D). The data in Fig. 6 suggested that at least a portion of the platinum lacking the DACH carrier ligand might be in the form of a low molecular mass biotransformation product(s) eluting with the void volume of the column. Subsequent experiments have suggested that this excess filterable platinum consists primarily of the $Pt(GSH)_2$ biotransformation product (manuscript in preparation).

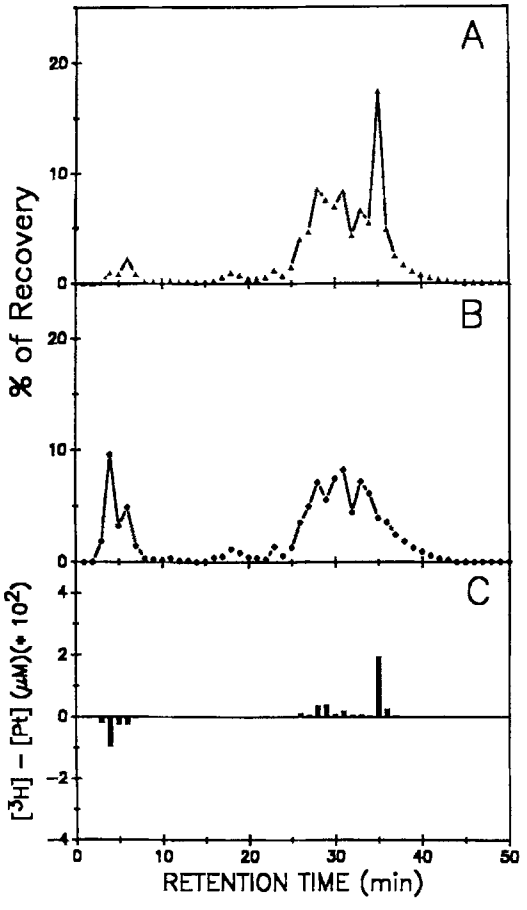

Fig. 6A–C Comparison of LSC and AAS analysis of HPLC fractions. RP fractions as described in Fig. 4A from selected animals were subjected in parallel to LSC for determination of tritium and AAS analysis for total platinum. **A**, **B** HPLC profiles for the same tissue (kidney) plotted as a function of recovered radioactivity (**A**) and total platinum (**B**). **C** The absolute micromolar concentration difference between LSC and AAS determinations for each fraction. The specific activity of the ^{3}H-ormaplatin was used to calculate the molar concentration from LSC data

Discussion

The studies reported here represent an extension of previous investigations by this laboratory into the biotransformations of DACH-Pt compounds [5–9, 28, 29] to include a direct comparison of the tissue distribution and tissue differences in the biotransformation of these agents. This goes beyond any in vivo analysis previously attempted for platinum-containing compounds. Other published studies have examined organ distribution of platinum alone [35, 36, 38], the distribution of platinum from cisplatin into various molecular mass subfractions of rat kidney cytosol [26, 27], cisplatin biotransformations in rat plasma [12] and in rat kidney [32] with more limited HPLC separation methods, or the pharmacokinetics of radioactivity versus platinum in mice given ^{3}H-labelled ormaplatin [20]. The objective of this work was to identify and quantitate the major intracellular biotransformation products of ormaplatin in selected target and non-target organ tissues in the whole animal. Findings from this work may be relevant, in practice, to two separate phenomena: the tissue specificity of ormaplatin toxicity, and the relative effects of ormaplatin versus cisplatin on particular tissues.

In this and in previous studies [35], the rank order of total platinum distribution appears to be kidney > liver > spleen > small intestine for both ormaplatin and cisplatin. Furthermore, at comparable doses, the levels of tissue platinum appear to be similar for both drugs [35, 38]. However, the tissue specificity for toxicity is very different for these two drugs. For cisplatin, the rank order of toxicity is kidney $\gg$ gastrointestinal tract $\approx$ spleen $\gg$ liver [4, 18, 24]. For ormaplatin, the rank order appears to be spleen $\approx$ gastrointestinal tract > kidney $\gg$ liver [5, 38, 39]. The investigations of Smith et al. [38] are particularly revealing because the toxicities of ormaplatin and cisplatin were compared on an equimolar basis. In those studies, comparable extents of renal toxicity required approximately

four times more ormaplatin than cisplatin. Thus, biodistribution of platinum does not appear to predict either the tissue specificity of ormaplatin toxicity or the differences in nephrotoxicity between cisplatin and ormaplatin.

Our results (Fig. 3) reveal platinum to be extensively bound by 30 min to plasma proteins with a molecular mass > 30,000. The 70% bound platinum in the plasma is comparable to the proportion found by Carfagna et al. [6] previously in this laboratory. It is also comparable to results obtained by Rahman et al. [35] with ormaplatin in Sprague-Dawley rats; in contrast, percentage binding at 30 min is significantly less in these same rats treated with cisplatin [35]. Similarly, our data for platinum binding to protein in the kidney appear to be consistent with the findings of Mason et al. [26, 27], and of Mistry et al. [32] obtained with cisplatin. However, our data (Fig. 3) also show that the percentage protein binding was significantly less in the kidney than in other tissues. This finding could be indicative of several things. One possibility is that the considerably greater amount of total platinum in the kidney has saturated existing protein- and/or nonprotein-bound sulfhydryl groups, a phenomenon previously noted for cisplatin [15, 21]. Another possibility is that a portion of the total platinum found in the kidney at this time derives from contamination of the homogenate preparation by formative urine containing platinum in the process of being excreted in the kidney tubules. Most of any urinary platinum would be expected to be filterable in our system. This possibility, likewise, has been previously postulated as an explanation for high renal platinum levels at early times following cisplatin administration [23, 32].

The major intracellular biotransformation products of ormaplatin at 30 min following a toxic dose are the unchanged parent drug, ormaplatin, the dichloro species, $PtCl_2$(DACH), complexes of Pt(DACH) with cysteine, methionine, and GSH, and the dissociated DACH carrier ligand itself. They ranged in relative abundance from approximately 2–3% of filterable platinum for the unchanged ormaplatin, up to the almost 30% of the filterable platinum for $PtCl_2$(DACH) in the plasma. Levels of $PtCl_2$(DACH) and Pt(methionine)(DACH) were significantly greater in the plasma than in the tissues. This most likely reflects the fact that $PtCl_2$(DACH) is more rapidly metabolized intracellularly ($t_{1/2} = 15$ min) [29] than in plasma ($t_{1/2} = 1.5$ h) [8], and that methionine is present at greater concentrations in plasma than intracellularly [3]. Free DACH levels, in contrast, were significantly less in the plasma than intracellularly. These findings suggest either that the N–Pt bond of ormaplatin is more stable in the blood than in the intracellular milieu, or that the free DACH ligand is very rapidly cleared from the circulation. Of these two possible models, our data would appear to provide strongest support for the former, as we have shown the dissociation of the DACH ligand from the platinum to be more rapid intracellularly than in the plasma [8, 29].

What remains problematic is a determination of which reactions lead to a labilization of the DACH carrier ligand, and furthermore, what is the fate of the platinum complexes thus formed which lack carrier ligand. It is known that trans-labilization of the carrier ligand is most likely to result from reaction with sulfur-containing nucleophiles [14]. Statistical analysis of our data at 30 min detected no differences in the levels of platinum assessed by AAS in comparison with levels assessed by LSC (Figs. 2 and 3). Similarly, Kido et al. [20] reported a ^{3}H/Pt ratio approaching unity in the urine of mice treated with ^{3}H-ormaplatin at times of up to 90 min. These findings would suggest that at this early time the platinum that has lost the DACH carrier ligand which we detect by HPLC has done so primarily as a result of binding to nonprotein-bound (i.e. filterable) sulfhydryl, of which GSH is generally regarded to be the most abundant [31]. Our data (manuscript in preparation) suggest that in the kidney, at least, (Fig. 6) a significant portion of this platinum may be in the form of a $Pt(GSH)_2$ complex. An active efflux system for this complex has previously been reported by Ishikawa and Ali-Osman [19]. However, Kido et al. [20] also noted ^{3}H-DACH to be present in the urine in amounts disproportionately greater than platinum at 24 h after dosing. Their data suggest that the intracellular free DACH detected in our experiments will eventually enter the circulation and be excreted via the kidneys.

The major intracellular products detected in these studies are similar to those seen previously when L1210 cells were incubated with $PtCl_2$(DACH) [29] and are similar to the biotransformation products found in kidney cytosols of cisplatin-treated rats by Mistry et al. [32]. There were no significant differences among the tissues in the amounts of any of the biotransformation products detected when viewed as a percentage of the filterable platinum. This is somewhat surprising since levels of protein- and nonprotein-bound sulfhydryls are known to vary considerably among the tissues studied. In particular, levels of both are known to be higher in the liver of this species than in the kidney [15, 34]. On this basis alone, one might predict that levels of total platinum in the liver would be at least equal to, if not greater than, levels in the kidney. However, since nonprotein-bound sulfhydryl comprises, on average, only 20% of the total tissue sulfhydryl content in this species [15], it is possible that differences among these filterable species are being obscured by the small amounts present. Thus, there was no apparent correlation between intracellular biotransformations and the tissue specificity of ormaplatin toxicity. There was, however, an interesting contrast between the biotransformations of ormaplatin and cisplatin in the kidney cytosol. Mistry et al. [32] found unchanged cisplatin to be the major species present in kidney cytosol at 1 h. Given

the rapid reduction of ormaplatin to $PtCl_2$(DACH) [6, 8, 16], this would be equivalent to finding $PtCl_2$(DACH) to be predominant intracellularly at 30 min, and this was not true for any of the tissues examined in the present investigations. The higher intracellular levels of cisplatin may reflect the fact that a higher percentage of plasma platinum is unbound [32], and thus is free to enter the tissues. These data could also explain why cisplatin is more nephrotoxic than ormaplatin.

In summary, we have provided detailed information on the biodistribution, percentage protein binding, and tissue-specific biotransformation of ormaplatin in the Fischer 344 rat. The biotransformation studies suggest a rationale for the greater nephrotoxicity of cisplatin compared with ormaplatin, but do not adequately explain the organ specificity of ormaplatin toxicity.

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