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ORIGINAL ARTICLE

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Pharmacokinetics and biotransformations of oxaliplatin in comparison with ormaplatin following a single bolus intravenous injection in rats

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Abstract *Purpose*: Traditionally ultrafilterable Pt has been used to estimate the body exposure to platinum drugs. However, previous studies have shown that ultrafilterable Pt consists of both cytotoxic and inert biotransformation products of platinum drugs. Therefore, it has been proposed that pharmacokinetic parameters of the parent drug and its cytotoxic biotransformation products are more likely to be correlated with the drug toxicity and efficacy than those of ultrafilterable Pt. Oxaliplatin and ormaplatin are likely to form very similar biotransformation products in vivo based on previous studies. However, ormaplatin causes severe and irreversible neurotoxicity while oxaliplatin causes moderate and reversible neurotoxicity. To evaluate the hypothesis that the neurotoxicity is associated with the pharmacokinetics of active biotransformation products, we investigated the biotransformations and pharmacokinetics of oxaliplatin and ormaplatin in rats at equimolar doses. *Methods*: ^{3}H-oxaliplatin and ^{3}H-ormaplatin were administered to Wistar male rats through single bolus i.v. injections (20 μmol/kg). Blood was sampled from 3.5 min to 360 min and centrifuged at 2000 *g* to separate the plasma from red blood cells (RBCs). The RBCs were sonicated and centrifuged at 13 000 *g* to separate the cytosol from the membrane fraction. Both plasma and RBC cytosol were filtered through YMT30 membranes (M_r = 30 000 kDa), and the ultrafiltrates were analyzed using a single column HPLC technique to identify and quantitate the biotransformation products. The pharmacokinetics of oxaliplatin, ormaplatin, and their biotransformation products were characterized utilizing the curve stripping and nonlinear least-squares fitting program RSTRIP. *Results*: The decays of total, plasma, plasma ultrafilterable (PUF), RBC-bound, and plasma protein-bound Pt-dach (only Pt species with an intact dach carrier ligand were quantitated in this study) were described by biphasic curves. No significant kinetic differences between oxaliplatin and ormaplatin were observed for total, plasma, and PUF Pt-dach in the initial α decay phase. However, Pt-dach bound to plasma proteins fourfold more quickly for ormaplatin than for oxaliplatin, and the AUC for Pt-dach bound to plasma proteins was twofold higher for ormaplatin than for oxaliplatin. The concentration of RBC-bound Pt-dach was highest at the initial time-point of 3.5 min for both drugs, which suggested a very rapid RBC uptake. The binding of Pt-dach to RBCs was slightly greater initially for ormaplatin than for oxaliplatin. However, the RBC-bound Pt-dach decayed more rapidly for ormaplatin ($t_{1/2\alpha RBC}$ = 5.1 min) than for oxaliplatin ($t_{1/2\alpha RBC}$ = 15.3 min). Thus the AUC_{RBC} was slightly greater for oxaliplatin than for ormaplatin. The AUC was also slightly greater for oxaliplatin than for ormaplatin for the Pt-dach associated with the RBC membrane and RBC cytosolic proteins. However, there was no significant difference between oxaliplatin and ormaplatin for Pt-dach in the RBC cytosolic ultrafiltrate. There was also no significant difference in the AUC_{puf} between oxaliplatin and ormaplatin. Both oxaliplatin and ormaplatin produced the same types of major plasma biotransformation products including Pt(dach)Cl_2, Pt(dach)$(Cys)_2$, Pt(dach)$(GSH)_2$, Pt(dach)(GSH), Pt(dach)(Met), and free dach. The decays of oxaliplatin, ormaplatin, and their biotransformation products were described by biphasic curves. The C_{max} and AUC were 19- and 15-fold higher, respectively, for oxaliplatin than for ormaplatin. However, the C_{max}

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and AUC were 29- and 16-fold less for Pt(dach)Cl_2 derived from oxaliplatin than for that derived from ormaplatin. No significant differences were observed among the C_{max} values and AUC values for the other plasma biotransformation products. Pt-dach species formed in RBCs were also identified and quantitated. Oxaliplatin was observed in the RBC cytosol, while no ormaplatin was found. The same types of major RBC biotransformation products were observed including Pt(dach)Cl_2, Pt(dach)$(Cys)_2$, Pt(dach)(GSH), Pt(dach)$(GSH)_2$, and free dach. Among these Pt-dach species, Pt(dach)Cl_2 was present at a twofold lower concentration initially but persisted longer for oxaliplatin than for ormaplatin, while the other RBC biotransformation products behaved kinetically similarly and no significant AUC differences were observed. *Conclusion*: Our study suggests that the different toxicity and efficacy profiles between oxaliplatin and ormaplatin may be related to the different pharmacokinetic features of these two drugs, especially the different plasma concentrations of their common biotransformation product Pt(dach)Cl_2. This in turn suggests that Pt(dach)Cl_2 and its hydrolysis products may be uniquely neurotoxic.

Key words In vivo biotransformation · Pharmacokinetics · Oxaliplatin · Ormaplatin · Platinum(II)

Abbreviations *dach* (*trans*-D,L)1,2-diaminocyclohexane, *free dach* dach not attached to a Pt complex, *Pt-dach* any Pt species with an attached dach carrier ligand, *PUF* plasma ultrafiltrate, *AUC* area under the curve, *RBC* red blood cell, *PBS* phosphate-buffered saline; ***parent compounds*** *cisplatin cis*-diamminedichloroplatinum(II), *oxaliplatin*, (*trans*-L)1,2-diaminocyclohexaneoxalatoplatinum(II), *ormaplatin* (*trans*-D,L)1,2-diaminocyclohexanetetrachloroplatinum(IV); ***major biotransformation products*** *Pt(dach)*Cl_2 Pt-dach dichloro complex, *Pt(dach)*$(Cys)_2$ Pt-dach bis cysteine complex, *Pt(dach)*$(GSH)_2$ Pt-dach bis glutathione complex, *Pt(dach)(GSH)* Pt-dach mono glutathione complex, *Pt(dach)(Met)* Pt-dach mono methionine complex; ***minor biotransformation products*** *Pt(dach)*$(H_2O)(Cl)$ Pt-dach aqua chloro complex, *Pt(dach)*$(H_2O)_2$ Pt-dach diaqua complex, *Pt(dach)(citrate)* Pt-dach citrate complex, *Pt(dach)(lactate)* Pt-dach lactate complex, *Pt(dach)(Ser)* Pt-dach serine complex, *Pt(dach)(Thr)* Pt-dach threonine complex

Introduction

Oxaliplatin (*trans*-L-1,2-diaminocyclohexaneoxalatoplatinum(II); Fig. 1) has great promise as a third generation anticancer drug [25, 26, 38]. It has recently been approved in France for the treatment of colorectal cancer and is currently being evaluated in phase II clinical trials in the USA. This drug is as effective as cisplatin (*cis*-diamminedichloroplatinum(II); Fig. 1) with respect to antitumor activity against both murine and human malignant tumors [15, 21, 23]. However, it is superior to cisplatin and carboplatin in that it shows little or no nephrotoxicity and myelotoxicity. Only peripheral neuropathy occurs at high cumulative dosages [13, 21]. More importantly it shares no cross-resistance with cisplatin when tested in cisplatin-resistant tumors [35].

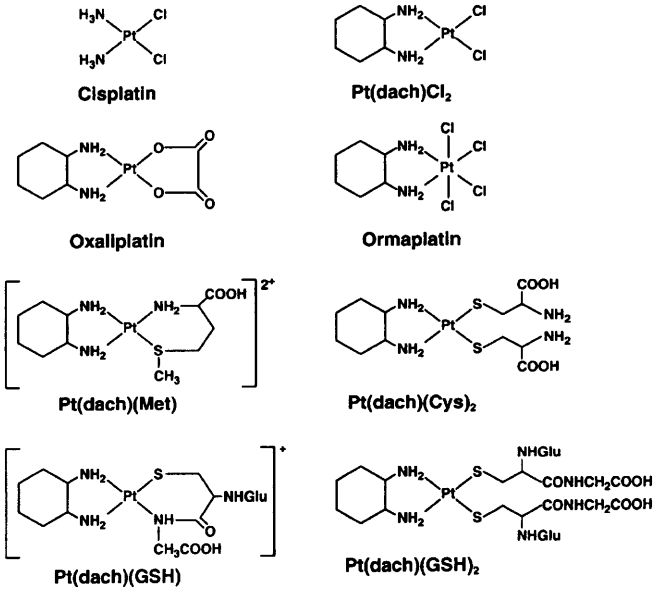

Fig. 1 Chemical structure of platinum complexes

Ormaplatin (*trans*-D,L-1,2-diaminocyclohexanetetrachloroplatinum(IV); Fig. 1) is also a third generation anticancer drug candidate that has been selected for clinical development due to its efficacy against cisplatin-resistant tumors [1, 28, 39] and reduced nephrotoxicity [1, 12, 33, 34]. Like oxaliplatin, ormaplatin has a 1,2-diaminocyclohexane (dach) carrier ligand and it is effective against a variety of cisplatin-resistant cell lines [1, 2, 25, 39]. Unlike oxaliplatin, ormaplatin is at the platinum(IV) valence state and is formulated with a mixture of *trans*-L and *trans*-D isomers of the 1,2-diaminocyclohexane ligand. As for oxaliplatin and cisplatin (with which nephrotoxicity is controlled by hydration), the dose-limiting toxicity for ormaplatin is neurotoxicity [8, 24, 25, 36]. However, the severity of ormaplatin neurotoxicity has prevented its clinical development [8, 24, 31].

It is currently not clear to what extent the parent drugs oxaliplatin and ormaplatin and/or their biotransformation products are responsible for their different efficacy and/or neurotoxicity profiles. It has been suggested that cisplatin itself may be responsible for its antitumor activity, and other Pt biotransformation products present in the plasma ultrafiltrate may be more responsible for its nephrotoxicity [9, 10, 32]. Carfagna et al. [5] have further shown for Pt-dach drugs that the plasma ultrafiltrate consists of both cytotoxic and inert biotransformation products. Therefore, they proposed that pharmacokinetic parameters of the cytotoxic biotransformation products were more

likely to be correlated with the drug toxicity and efficacy than those of plasma ultrafilterable Pt. This hypothesis is supported by a correlation of the neurotoxicity of ormaplatin with the C_{max} and AUC for $Pt(dach)Cl_2$, one of its major cytotoxic biotransformation products [30].

Previous studies of oxaliplatin in mice and rabbits have focused on the pharmacokinetics of plasma total and ultrafilterable Pt [3, 4, 17]. The pharmacokinetics of the biotransformation products of oxaliplatin remain poorly characterized. We have developed a novel single-column HPLC technique that has allowed us to identify and quantitate oxaliplatin, ormaplatin, and their major biotransformation products [19]. In order to understand how the parent drugs and/or their biotransformation products are correlated with the observed differences in neurotoxicity and efficacy between oxaliplatin and ormaplatin, we applied this newly developed HPLC method to identify and quantitate the biotransformation products for both oxaliplatin and ormaplatin formed in rat blood in vivo. Furthermore, we compared the pharmacokinetic features between ormaplatin and oxaliplatin as well as between their biotransformation products at equimolar doses. Our current study suggests that the differences in neurotoxicity of oxaliplatin and ormaplatin could be related to the different plasma concentrations of their common biotransformation product, $Pt(dach)Cl_2$.

Materials and methods

Chemicals

HPLC grade methanol, acetonitrile, 1-heptanesulfonate, and KH_2PO_4 were purchased from Fisher Scientific (Pittsburgh, Pa.). HPLC grade water was obtained with a Milli-Q water purification system (Millipore Corporation, Bedford, MA). All HPLC solutions were filtered through a 0.22-μm hydrophilic Durapore filter (GWP, Millipore Corporation, Bedford, Mass.) before use. Amino acids and glutathione were purchased from the Sigma Chemical Co. (St. Louis, Mo.). Sodium heparin (5000 units/ml) was purchased from the pharmacy store room at University of North Carolina Hospital.

Drugs

^{3}H-oxaliplatin (2.46 mCi/mmol) and ^{3}H-ormaplatin (2.65 mCi/mmol) were prepared by Dr. Steven Wyrick (Radiosynthesis Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina) as described previously [40]. Oxaliplatin stock solution (4 mg/ml, or 10 m*M*) was prepared in 5% dextrose solution. Ormaplatin stock solution (1.6 mg/ml, or 3.5 m*M*) was prepared in 150 m*M* NaCl.

Animals

Wistar male rats (6–8 weeks of age, approximately 225 g in weight) were purchased from Charles River Breeding Laboratories (Raleigh, N.C.). Prior to the experiment, 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.

Equipment

Dissection tools were purchased from Fisher Scientific. Three-way valves and cannula were purchased from Sigma.

Methods

Rats were anesthetized with ether and cannulated via the right jugular vein by insertion of a cannula of outside diameter 0.037 in. To prevent clotting, 500 μl heparinized saline (20 units/ml) was flushed into the cannula to remove any residual blood. A 1-day recovery period was allowed prior to the injection of the platinum drugs. ^{3}H-oxaliplatin (8 mg/kg) was quickly infused into rats through the cannula followed by flushing with 400 μl heparinized saline (20 units/ml). Blood samples were taken via the jugular vein cannula at the following times: 3.5, 7, 10, 20, 30, 45, 60, 90, 120, 240, and 360 min, and immediately spun at 4 °C for 5 min at 2000 *g* to separate red blood cells (RBCs) and plasma. Aliquots of plasma were diluted to 1 ml with cold 10 m*M* $NaClO_4$ solution and filtered through a YMT30 membrane (Mr 30 000 kDa cut-off; Amicon Corporation, Danvers, Mass.) by centrifugation at 2000 *g* for 15 min at 4 °C. The filtrates were frozen in dry ice and stored at −20 °C until HPLC analysis. The RBC pellets were washed three times using phosphate-buffered saline (PBS), resuspended in 1 ml 10 m*M* $NaClO_4$ solution, then sonicated on ice for 2×20 s and spun at 13 000 *g* at 4 °C for 10 min. The supernatants (RBC cytosol fractions) were filtered through the YMT30 membranes, and the resulting RBC cytosol ultrafiltrates were also frozen in dry ice and stored at −20 °C until HPLC analysis. The pellets (RBC membrane fractions) were resuspended in 1 ml 10 m*M* $NaClO_4$. To quantitate Pt-dach[1] in plasma, plasma ultrafiltrate, RBC cytosol, RBC cytosol ultrafiltrate, and RBC membrane suspension, 2×20-μl aliquots were mixed with 5 ml Scintisafe Econoz scintillation fluid (Fisher Scientific, Pittsburg, PA) and counted in an LKB 1215 liquid scintillation counter (LKB, Bromma, Sweden).

The plasma ultrafiltrates (PUFs) and RBC cytosol ultrafiltrates were analyzed by reverse-phase HPLC as described previously [19]. Briefly, 100–500-μl aliquots of the ultrafiltrate were injected onto the reverse-phase HPLC column. Fractions of 1 ml were collected throughout the whole elution. Aliquots of 0.2–0.5 ml were removed for each fraction and mixed with 5 ml Scinti Verse and counted in an LKB 1215 liquid scintillation counter to quantitate the amount of ^{3}H-labeled Pt-dach in each fraction. The chromatograms were obtained by plotting the radioactive counts of each fraction (cpm) versus time. The standard compounds used to identify the HPLC peaks have been described previously [19].

^{3}H-ormaplatin (9.1 mg/kg) was administered to rats at an equimolar dose. The in vivo biotransformations of ormaplatin were studied using the same procedure as for oxaliplatin. The in vivo biotransformations of ormaplatin have been studied previously in this laboratory [5]. The identities and relative plasma concentrations of the major biotransformation products of ormaplatin were the same in the previous study as in the current study. Since we were primarily interested in major differences in pharmacokinetic parameters (more than twofold) between oxaliplatin and ormaplatin, only one experiment was conducted for the characterization of ormaplatin biotransformations in the current study.

Pharmacokinetic parameters were calculated using the curve stripping and nonlinear least-squares fitting program RSTRIP [14]. The equation $C = A_1e^{-k_1t} + A_2e^{-k_2t}$ was fitted to the decay for total, plasma, PUF, plasma protein-bound, and RBC-bound Pt-dach of oxaliplatin and ormaplatin, as was the decay of oxaliplatin, ormaplatin, and their biotransformation products, where A_1, A_2, k_1, and k_2, are concentrations and rate constants. The truncated area under the concentration-time curve (AUC) was calculated

[1]Because the ^{3}H-labeled 1,2-diaminocyclohexane (dach) carrier ligand was used for the quantitation of oxaliplatin and ormaplatin biotransformation products, only Pt species with an intact dach carrier ligand were quantitated in this study. Thus, the term Pt-dach rather than Pt is used to describe the Pt species detected.

using the trapezoidal rule. The partitioning of Pt-dach was calculated from the corresponding AUC over the total AUC.

Results

Pharmacokinetics of oxaliplatin and ormaplatin in rat blood

^{3}H-oxaliplatin (8 mg/kg or 20 μmol/kg) was given to rats via i.v. administration. The Pt-dach levels were monitored from 3.5 min to 360 min. The decays of Pt-dach in whole blood, plasma, plasma ultrafiltrate, RBCs, and plasma protein were described by biphasic curves (Fig. 2A). The binding of Pt-dach to RBCs was extremely fast. The concentration of the RBC-bound Pt-dach was highest at the initial time-point of 3.5 min. Thus, only the decay phase of the RBC-associated Pt-dach was observed ($t_{1/2\alpha\ RBC} = 15.3$ min). The binding of Pt-dach to plasma proteins was slower than to RBCs, so both the formation phase ($t_{1/2\ formation} = 11.7$ min) and the decay phase ($t_{1/2\alpha} = 18.7$ min) were observed (Table 1). Based on the comparison of AUCs with AUC_{total}, 51% of Pt-dach was bound to RBCs, 34.5% to

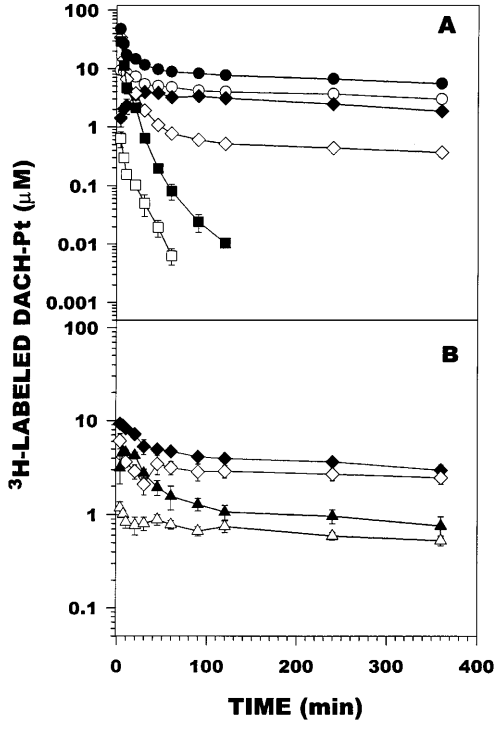

Fig. 2A, B Time course for distribution of Pt-dach from oxaliplatin in rat blood in vivo. The amounts of ^{3}H-labeled Pt-dach in the blood were determined as described in Materials and methods. Each data point represents the mean ± standard error of the mean for three experiments. Where the error bar is not visible, it is smaller than the symbol. **A** Blood: ● total Pt-dach in the blood, ○ Pt-dach bound to RBCs, ◆ Pt-dach bound to plasma proteins, ◇ Pt-dach in plasma ultrafiltrate, ■ oxaliplatin, □ $Pt(dach)Cl_2$. **B.** RBCs: ◆ Pt-dach bound to RBCs, ◇ Pt-dach in RBC cytosol, ▲ Pt-dach bound RBC membrane, △ Pt-dach in RBC cytosol ultrafiltrate

Table 1 Pharmacokinetic parameters of oxaliplatin (*OX*) and ormaplatin (*OP*). The values of the pharmacokinetic parameters for oxaliplatin are the average (± SEM) of the values from three individual experiments. The values of the pharmacokinetic parameters for ormaplatin were obtained from a single experiment

Pt species	$C_{max}(\mu M)$		AUC (min μM)		$t_{1/2\ formation}$ (min^{-1})		$t_{1/2\alpha}$ (min^{-1})		$t_{1/2\beta}$ (min^{-1})	
	OX	OP	OX	OP	OX	OP	OX	OP	OX	OP
Total	109 ± 9	67	2836 ± 68	3417	–	–	2.7 ± 0.2	2.9	258 ± 18	240
Plasma	105 ± 8	46	1364 ± 66	2313	–	–	2.2 ± 0.1	2.8	201 ± 15	364
Plasma ultrafiltrate	96 ± 7	45	385 ± 17	357	–	–	2.5 ± 0.1	2.9	137 ± 11	260
Plasma proteins	3.7 ± 0.2	7.4	979 ± 85	1956	11.7 ± 0.5	2.9	18.7 ± 0.6	49.5	495 ± 15	693
RBC	10.4 ± 0.1	17.4	1454 ± 24	1103	–	–	15.3 ± 0.4	5.1	805 ± 21	152
RBC cytosol	10.8 ± 0.4	19.8	999 ± 69	705	–	–	2.6 ± 0.2	2.4	156 ± 55	166
RBC membrane	4.8 ± 0.3	4.5	471 ± 28	397	–	–	31 ± 4	26	537 ± 21	1388
RBC cytosolic proteins	10.0 ± 0.9	18.9	761 ± 79	441	–	–	2.2 ± 0.2	2.2	1077 ± 65	96
RBC cytosol ultrafiltrate	1.9 ± 0.2	2.1	238 ± 28	264	–	–	2.3 ± 0.2	2.2	524 ± 19	426
Oxaliplatin	86 ± 5	–	154 ± 4	–	–	–	2.1 ± 0.1	–	14.2 ± 0.5	–
Ormaplatin	–	4.6	–	10.2	–	–	–	2.4	–	11.8
$Pt(dach)Cl_2$	1.7 ± 0.5	49.1	5.1 ± 0.1	82.2	–	–	2.1 ± 0.3	1.9	14.8 ± 1.5	11.0
$Pt(dach)(GSH)_2$	0.13 ± 0.08	0.18	18.2 ± 0.9	28.1	8.8 ± 0.5	2.3	16.0 ± 1.0	8.9	475 ± 33	320
$Pt(dach)(Cys)_2$	0.16 ± 0.06	0.15	49.7 ± 2.7	24.9	2.0 ± 0.4	1.9	20.5 ± 1.5	18.3	577 ± 41	284
Pt(dach)(Met)	0.12 ± 0.04	0.07	12.2 ± 0.4	10.4	8.4 ± 0.3	1.8	13.5 ± 1.3	29.5	212 ± 13	433
Pt(dach)(GSH)	0.04 ± 0.01	0.02	4.6 ± 0.5	4.3	12.4 ± 0.6	9.7	15.8 ± 1.5	23.1	377 ± 23	122
Dach	0.09 ± 0.03	0.04	17.4 ± 1.1	9.5	6.1 ± 0.7	14.3	54.3 ± 7.0	73.1	389 ± 31	597
Peak e	0.18 ± 0.05	0.14	19.1 ± 2.0	10.6	4.3 ± 0.5	4.2	40.8 ± 4.1	35.5	693 ± 56	496
Peak f	0.06 ± 0.02	0.11	7.0 ± 0.3	11.1	12.6 ± 1.5	2.1	13.3 ± 1.5	22.4	447 ± 58	477
Peak j	0.06 ± 0.01	0.02	5.4 ± 0.2	2.1	7.6 ± 1.1	2.4	15.7 ± 0.8	24.5	248 ± 32	450

plasma proteins, and only 13.6% was ultrafilterable (Fig. 2A). Of the Pt-dach bound to RBCs, 32.4% was bound to the RBC membrane, 52.3% to RBC cytosolic proteins, and 16.3% was in the RBC cytosol ultrafiltrate (Fig. 2B).

^{3}H-ormaplatin was administered to rats at an equimolar dose compared to oxaliplatin (9.1 mg/kg or 20 μmol/kg). The decays of Pt-dach in whole blood, plasma, plasma ultrafiltrate, RBCs, and plasma protein were also best described by biphasic curves (Fig. 3A). There were no significant differences in AUCs (less than twofold) between oxaliplatin and ormaplatin for Pt-dach in whole blood, plasma, and plasma ultrafiltrate (Fig. 4 and Table 1). As with oxaliplatin, only a decay phase was observed for the RBC-associated Pt-dach, probably due to a similar rapid RBC uptake of Pt-dach. The C_{max} was twofold higher for the RBC-bound Pt-dach for ormaplatin than for oxaliplatin, probably suggesting a more rapid initial uptake by RBCs for ormaplatin than for oxaliplatin (Table 1). However, the decay of the RBC-bound Pt-dach was threefold faster for ormaplatin ($t_{1/2\alpha\ RBC}$ = 5.1 min) than for oxaliplatin ($t_{1/2\alpha\ RBC}$ = 15.3 min); therefore, the AUC_{RBC} was slightly greater for oxaliplatin than for ormaplatin. Of the Pt-dach associated with the RBCs, the AUCs for Pt-dach bound to the RBC membranes and cytosolic proteins (Table 1) were also slightly greater for oxaliplatin than for ormaplatin. However, the AUCs for Pt-dach in the RBC cytosolic ultrafiltrates were virtually identical for oxaliplatin and ormaplatin. The rate of binding of Pt-dach to plasma proteins was also greater for ormaplatin ($t_{1/2\ formation}$ = 2.9 min) than for oxaliplatin ($t_{1/2\ formation}$ = 11.7 min). In addition, the rate of decay for Pt-dach bound to plasma proteins was slower for ormaplatin ($t_{1/2\alpha}$ = 49.5 min) than for oxaliplatin ($t_{1/2\alpha}$ = 18.7 min). This lead to a greater AUC of the Pt-dach bound to plasma proteins for ormaplatin than for oxaliplatin. The Pt-dach in the PUF for ormaplatin decayed approximately as quickly as for oxaliplatin and the AUC_{PUF} was about the same (Table 1).

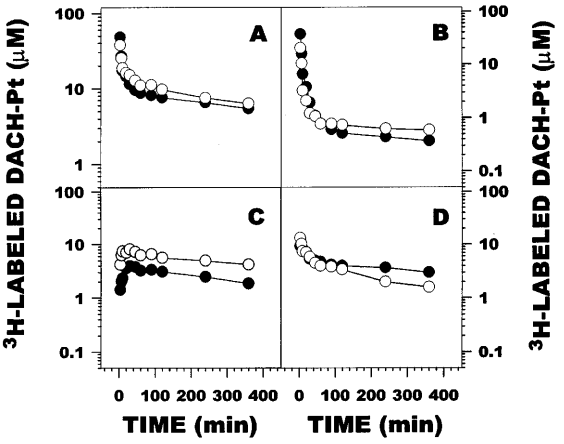

Fig. 4A–D Time course for decays of Pt-dach species of oxaliplatin and ormaplatin in rat plasma in vivo. The amounts of ^{3}H-labeled Pt-dach species in plasma were determined as described in Materials and methods. **A** ● total Pt-dach of oxaliplatin, ○ total Pt-dach of ormaplatin; **B** ● Pt-dach of oxaliplatin in plasma ultrafiltrate, ○ Pt-dach of ormaplatin in plasma ultrafiltrate; **C** ● Pt-dach of oxaliplatin bound to plasma proteins, ○ Pt-dach of ormaplatin bound to plasma proteins; **D** ● Pt-dach of oxaliplatin in RBCs, ○ Pt-dach of ormaplatin in RBCs

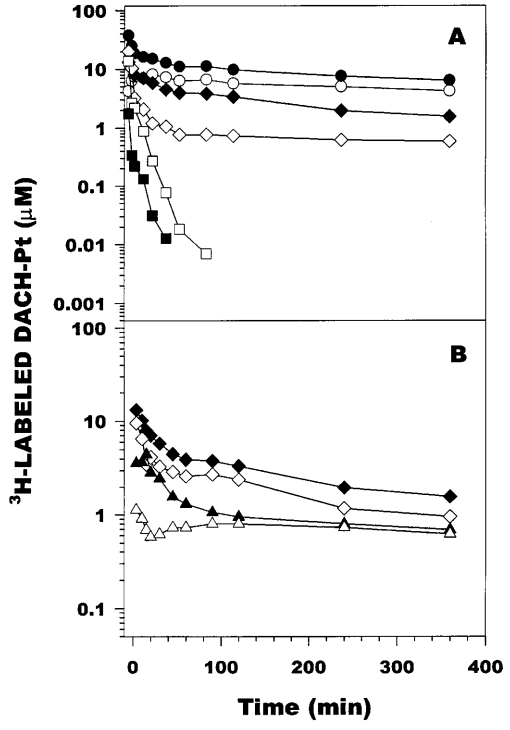

Fig. 3A, B Time course for distribution of Pt-dach from ormaplatin in rat blood in vivo. The amounts of ^{3}H-labeled Pt-dach in the blood were determined as described in Materials and methods. Each data point represents the mean ± standard error of the mean for three experiments. Where the error bar is not visible, it is smaller than the symbol. **A** Blood: ● total Pt-dach in the blood, ◆ Pt-dach bound to plasma proteins, ○ Pt-dach bound to RBCs, ◇ Pt-dach in plasma ultrafiltrate, ■ ormaplatin, □ $Pt(dach)Cl_2$; **B** RBCs: ◆ Pt-dach bound to RBCs, ◇ Pt-dach in RBC cytosol, ▲ Pt-dach bound RBC membrane, △ Pt-dach in RBC cytosol ultrafiltrate

Based on an estimation from AUCs, the Pt-dach associated with RBCs, plasma proteins, and PUF accounted for 32.2%, 57.2%, and 10.4% of total Pt-dach of ormaplatin (Fig. 3A). Thus, there was slightly less Pt-dach associated with RBCs and slightly more associated with plasma proteins for ormaplatin than for oxaliplatin (Fig. 3A, Table 1). However, there was little difference between ormaplatin and oxaliplatin with respect to the amount of Pt-dach in the PUF. Of the Pt-dach from ormaplatin bound to RBCs, 36.0% was found on the RBC membrane, 40.0% in RBC cytosolic proteins, and 24.0% in the RBC cytosolic ultrafiltrate (Fig. 3B).

Pharmacokinetics of the biotransformation products of oxaliplatin and ormaplatin in rat blood

The PUFs were analyzed by a single column HPLC technique developed previously [19]. Based on a detailed

comparison of residence times (RTs) for peaks found in the HPLC chromatograms of ^{3}H-Pt-dach compounds in PUFs and RTs of standard compounds, we identified oxaliplatin and its major biotransformation products (Fig. 1) as Pt(dach)Cl_2, Pt(dach)$(GSH)_2$, Pt(dach)$(Cys)_2$, Pt(dach)(Met), Pt(dach)(GSH), and free dach (Fig. 5A, C, E). There were three minor peaks comigrating with more than one standard compound. They were peak *e*, which comigrated with Pt(dach)(H_2O)Cl, Pt(dach)(citrate), and Pt(dach)(lactate); peak *f*, which comigrated with Pt(dach)(Thr) and Pt(dach)(Ser); and peak *j*, which comigrated with Pt(dach)$(H_2O)_2$ and some unidentified Pt-dach complexes (Fig. 5A, C, E and Table 1).

The biotransformations of ormaplatin were also characterized using the same procedure as for oxaliplatin (Fig. 5B, D, F, and Table 1). It was found that oxaliplatin and ormaplatin produced the same types of major biotransformation products. However, significant kinetic and quantitative differences were observed among oxaliplatin, ormaplatin, and Pt(dach)Cl_2, one of their biotransformation products (Fig. 6). Oxaliplatin was detected as the major Pt-dach compound in the PUF of oxaliplatin-treated rats at the initial time-point of 3.5 min (peak *b1*, Fig. 5A). It decayed below the detection limit by 120 min with a $t_{1/2\alpha}$ of 2.1 min. Pt(dach)Cl_2 was the only biotransformation product detected at 3.5 min (peak *a*, Fig. 5A), and it also quickly decayed below the detection limit by 90 min with a $t_{1/2\alpha}$ of 2.1 min. The C_{max} was 51-fold higher for oxaliplatin than for Pt(dach)Cl_2. In contrast, ormaplatin was present at very low levels in the PUF of ormaplatin-treated rats at 3.5 min (peak *b2*, Fig. 5B). Not only was Pt(dach)Cl_2 the only biotransformation product detected at 3.5 min, but it was also present as the major Pt-dach compound in the plasma of ormaplatin-treated rats at early times (peak *a*, Fig. 5B). The C_{max} was tenfold greater for Pt(dach)Cl_2 than for ormaplatin. Ormaplatin decayed below the detection limit within 45 min with a $t_{1/2\alpha}$ of 2.4 min while Pt(dach)Cl_2 remained in the blood circulation for at least 90 min with a $t_{1/2\alpha}$ of 1.9 min. The C_{max} for Pt(dach)Cl_2 was 29-fold greater and the AUC was 16-fold greater following ormaplatin infusion than following oxaliplatin infusion. Based on the $t_{1/2}$ of formation, Pt(dach)$(GSH)_2$ and Pt(dach)(Met) were

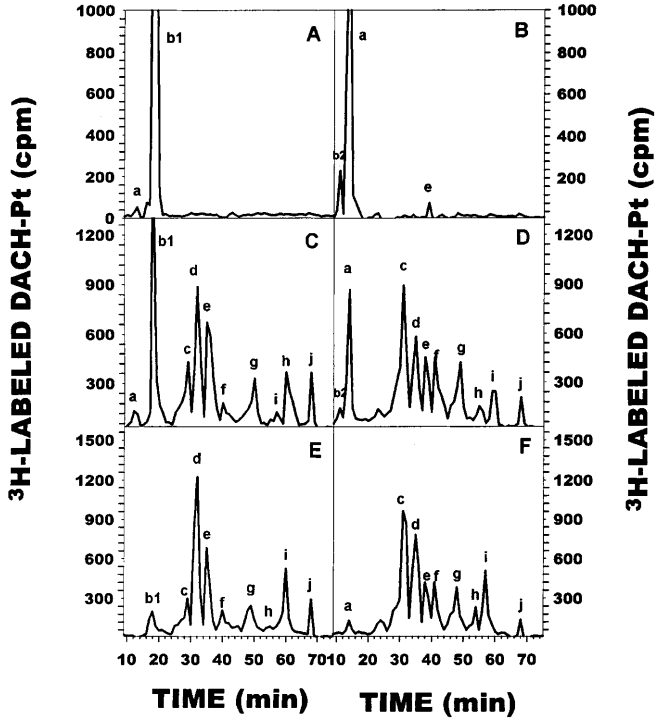

Fig. 5A–F HPLC analysis of the plasma biotransformation products of oxaliplatin (**A, C, E**) and ormaplatin (**B, D, F**) in rat blood in vivo. [^{3}H]Oxaliplatin (8 mg/kg i.v.) or ormaplatin (9.1 mg/kg i.v.) were injected into Wistar rats. At 3.5 min (**A, B**), 45 min (**C, D**), and 90 min (**E, F**), aliquots were removed and spun at 2000 *g* for 5 min to separate plasma and RBCs. Plasma aliquots were further diluted with cold 10 m*M* $NaClO_4$, filtered through Amicon YMT 30 membranes, and frozen at −20 °C. Platinum biotransformation products were resolved by the reverse-phase HPLC procedure described previously [19]. Based on previous characterization of the HPLC separation technique and comigration with standards, the identities of the major peaks are: *a* Pt(dach)Cl_2; *b1* oxaliplatin; *b2* ormaplatin; *c* Pt(dach)$(GSH)_2$; *d* Pt(dach)$(Cys)_2$; *e* Pt(dach)(H_2O)Cl, Pt(dach)(lactate), Pt(dach)(citrate); *f* Pt(dach)(Ser), Pt(dach)(Thr), *g* Pt(dach)(Met); *h* Pt(dach)(GSH); *i* dach; *j* dach + Pt(dach)$(H_2O)_2$ + unknown Pt species

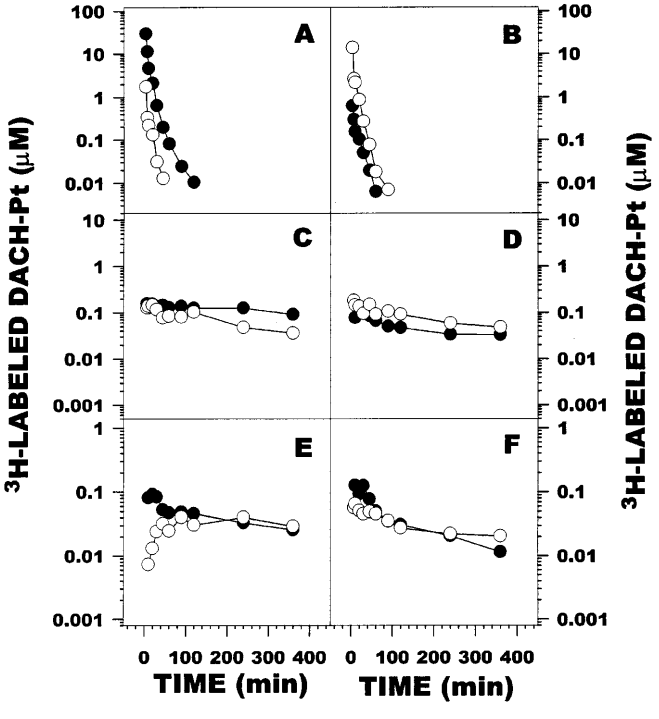

Fig. 6A–F Time course for the decay of the biotransformation products of oxaliplatin and ormaplatin in rat plasma in vivo. The biotransformation products were identified and quantitated as described in Materials and methods. **A** ● oxaliplatin, ○ ormaplatin; **B** ● Pt(dach)Cl_2 from oxaliplatin, ○ Pt(dach)Cl_2 from ormaplatin; **C** ● Pt(dach)$(Cys)_2$ from oxaliplatin, ○ Pt(dach)$(Cys)_2$ from ormaplatin; **D** ● Pt(dach)$(GSH)_2$ from oxaliplatin, ○ Pt(dach)$(GSH)_2$ from ormaplatin; **E** ● dach from oxaliplatin, ○ dach from ormaplatin; **F** ● Pt(dach)(Met) from oxaliplatin, ○ Pt(dach)(Met) from ormaplatin

formed fourfold more quickly for ormaplatin than for oxaliplatin, and the Pt-dach compound(s) in peak *f* were formed sixfold more quickly for ormaplatin than for oxaliplatin. Free dach was formed approximately twofold more slowly for ormaplatin than for oxaliplatin. No other significant differences between oxaliplatin and ormaplatin were observed with respect to the formation rate, C_{max}, or AUCs for the other biotransformation products (Fig. 6, Table 1).

The biotransformation products of oxaliplatin and ormaplatin in RBC cytosol were also identified and quantitated in our study. When oxaliplatin was administered, oxaliplatin and its biotransformation products Pt(dach)Cl_2, Pt(dach)$(Cys)_2$, Pt(dach)$(GSH)_2$, Pt(dach)(GSH), and free dach were observed in RBCs. These Pt-dach compounds were the same as those observed in RBCs when oxaliplatin is incubated with rat blood in vitro [20]. When ormaplatin was administered, the same types of biotransformation products were observed in RBCs except that ormaplatin itself was not found. In RBC cytosol, Pt(dach)Cl_2 was present at twofold higher concentrations at the initial time-point of 3.5 min and decayed more quickly for ormaplatin than for oxaliplatin (Fig. 7). However, there were no significant differences in concentrations and AUC from 3.5 to 360 min for Pt(dach)$(Cys)_2$, Pt(dach)(GSH), Pt(dach)$(GSH)_2$, and free dach between oxaliplatin and ormaplatin. The concentrations of these compounds reached a plateau around 10 min and remained relatively constant until 360 min (Fig. 7).

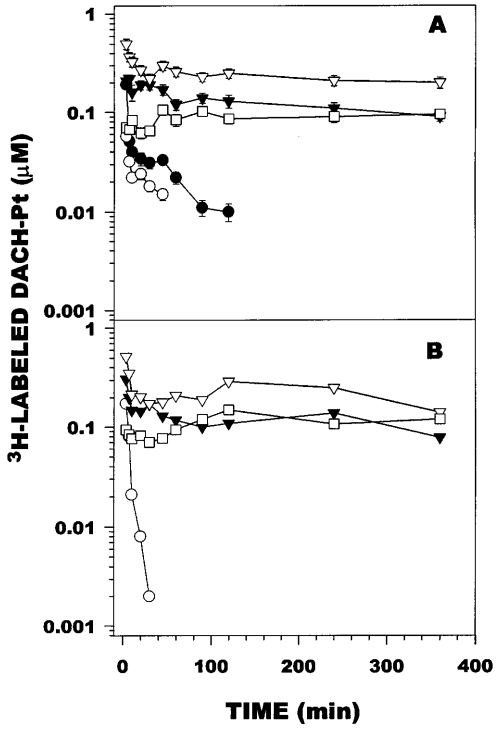

Fig. 7A, B Time course for the decay of the biotransformation products of oxaliplatin and ormaplatin in rat RBCs in vivo. The biotransformation products were identified and quantitated as described in Materials and methods. **A** biotransformation products of oxaliplatin observed in RBCs; **B** biotransformation products of ormaplatin observed in RBCs (● oxaliplatin, ○ Pt(dach)Cl_2, ▼ Pt(dach)$(Cys)_2$, ▽ Pt(dach)(GSH)/$(GSH)_2$, □ dach)

Discussion

Total and PUF Pt have been measured in previous pharmacokinetic studies of oxaliplatin in mice and rabbits [4, 17] and ormaplatin in mice and rats [5, 16, 29]. Similar biphasic decays have been observed for both drugs. The $t_{1/2\alpha}$ values were slightly shorter and the C_{max} values were slightly less in our study than those reported in the previous studies. However, we started sampling at 3.5 min and had more time-points in the α decay phase than the previous studies. Those studies did not start sampling until 7 to 10 min, which could affect the estimation of both $t_{1/2\alpha}$ and C_{max}. If the $t_{1/2\alpha}$ and C_{max} were estimated using our data with a first time-point of 7 min, the values obtained would be very comparable to those reported in the previous studies. In addition, we used ^{3}H for detection of Pt species and, therefore, only quantitated Pt species containing the dach carrier ligand, whereas previous studies used atomic absorption and quantitated all Pt species. However, we have shown that only about 5–10% of the dach carrier ligand is labilized during the first 4 h when ^{3}H-oxaliplatin is incubated with rat plasma in vitro [20]. Furthermore, Kido et al. [16] have determined the pharmacokinetics of ^{3}H-ormaplatin in mice by both ^{3}H and atomic absorption measurements. Their data show that the ^{3}H:Pt ratio does not change for PUF-Pt for at least the first 2 h, and that the $t_{1/2\alpha}$ and C_{max} for PUF-Pt are essentially identical whether measured by ^{3}H or atomic absorption. Therefore, the differences in pharmacokinetic parameters for PUF-Pt ($t_{1/2\alpha}$ and C_{max}) between the current study and previous studies are likely due to differences in the blood sampling schemes. In addition, these data suggest that the pharmacokinetic parameters determined for individual plasma biotransformation products in this study are likely to be correct.

While previous studies only characterized the pharmacokinetics of total and ultrafilterable Pt for oxaliplatin, in the current study we also evaluated the pharmacokinetics of the biotransformation products of oxaliplatin. Biphasic decays were observed for oxaliplatin and all of its biotransformation products. The same types of plasma biotransformation products were identified in the current in vivo study as have been seen in a previous in vitro study of oxaliplatin biotransformations in rat blood [20]. Pt(dach)(Met), Pt(dach)$(Cys)_2$, and free dach were major stable plasma biotransformation products in both studies, while Pt(dach)$(GSH)_2$ was a major stable plasma biotransformation product only in vivo. Data from our previous studies suggest that the free dach ligand is likely to be derived from *trans*-labilization caused by intracellular glutathione [19, 20]. When oxaliplatin is incubated with rat blood in vitro, the plasma concentration was higher

for Pt(dach)(Met) than for either Pt(dach)$(Cys)_2$ or free dach [20]. However, the plasma concentration of Pt(dach)(Met) was significantly lower than that of either Pt(dach)$(GSH)_2$ or Pt(dach)$(Cys)_2$ in the current in vivo study. These results suggest that Pt(dach)(Met) is either preferentially eliminated or taken up by tissue. It is also possible that cellular biotransformations might contribute to the higher plasma concentrations of Pt(dach)$(GSH)_2$ and Pt(dach)$(Cys)_2$ observed in vivo.

At equimolar doses the C_{max} was 17-fold less and the AUC was 15-fold less for ormaplatin than for oxaliplatin. These observations are consistent with the previous reports that ormaplatin is quickly reduced to Pt(dach)Cl_2 in rat plasma [5, 6]. The biotransformation of oxaliplatin is a much slower process than the rapid reduction of ormaplatin, which could partially account for the large differences in the C_{max} and AUC between ormaplatin and oxaliplatin themselves. However, this provides only a partial explanation for the pharmacokinetic differences between ormaplatin and oxaliplatin. Since the conversion of ormaplatin to Pt(dach)Cl_2 is rapid ($t_{1/2} = 3$ s) and quantitative, ormaplatin and Pt(dach)Cl_2 can be considered as a single kinetic entity. If this is assumed to be the case, the sum of the C_{max} for ormaplatin plus Pt(dach)Cl_2 is still twofold less than that of oxaliplatin. Similarly the sum of the AUCs for ormaplatin plus Pt(dach)Cl_2 is 1.7-fold less than the AUC for oxaliplatin. It is therefore likely that other biological processes, such as cellular uptake, protein binding, and excretion are also involved in the quicker decay for ormaplatin plus Pt(dach)Cl_2 than for oxaliplatin in rat blood. The cellular uptake of ormaplatin and Pt(dach)Cl_2 has been studied in the L1210 mouse leukemia cell line [7]. Uptake has been further compared among ormaplatin, oxaliplatin, and Pt(dach)Cl_2 in a human colon HT-29 cell line and in rat and human RBCs [7, 27, 41]. The uptake rank has been established as ormaplatin > Pt(dach)Cl_2 > oxaliplatin in all three types of cells. In addition, the binding of Pt-dach to plasma proteins is fourfold faster following ormaplatin infusion than following oxaliplatin infusion. Therefore, preferential cellular uptake for both ormaplatin and Pt(dach)Cl_2 and more rapid binding to plasma proteins for ormaplatin are likely to be major causes for the lower plasma concentrations (both C_{max} and AUC) of ormaplatin plus Pt(dach)Cl_2 than of oxaliplatin. Previous studies have shown that urinary excretion is relatively slow for both oxaliplatin and ormaplatin [17, 29]. In this study, no significant AUC difference was observed for the ultrafilterable Pt-dach between oxaliplatin and ormaplatin, probably reflecting a similar level of excretion for these two drugs.

Previous studies have shown that oxaliplatin is sequestered by RBCs both in vitro and in vivo [4, 27]. It has also been observed that RBC uptake of oxaliplatin is greater than that of cisplatin and carboplatin [4, 27, 37]. However, the RBC uptake of ormaplatin has not been measured in previous pharmacokinetic studies of that drug. It was of interest to determine whether the RBC uptake is a unique feature of oxaliplatin or a common property of Pt-dach drugs. Therefore, we compared the in vivo RBC uptake for oxaliplatin and ormaplatin in this study. Both oxaliplatin and ormaplatin were rapidly taken up by RBCs. Based on the C_{max}, ormaplatin had a greater initial RBC uptake than oxaliplatin, which is consistent with our in vitro RBC uptake study [20]. However, based on both $t_{1/2\alpha}$ and AUC, the Pt-dach associated with RBCs decayed more quickly for ormaplatin than for oxaliplatin. This might suggest that the Pt-dach taken up by RBCs is more readily effluxed back to the plasma for ormaplatin. This is consistent with our previous study showing that a large percentage of the ormaplatin taken up by cells is converted to inactive biotransformation products that are not retained by cells [7].

The sequestration of oxaliplatin by RBCs also leads to the possibility that RBCs might act as a reservoir of cytotoxic Pt complexes. Therefore, we studied the biodistribution and biotransformations of oxaliplatin and ormaplatin within RBCs. For both oxaliplatin and ormaplatin, the majority of the Pt-dach associated with RBCs was retained by the RBC membrane and RBC cytosolic proteins, while the Pt-dach in the RBC cytosol ultrafiltrate only accounted for a small proportion of Pt-dach associated with RBCs. The major biotransformation products formed in the RBC cytosol in vivo were identified for the first time in our study; most of them were the sulfur-containing Pt-dach complexes. An in vitro neurotoxicity study [18] and cytotoxicity data (manuscript in preparation) have shown that the sulfur-containing Pt-dach complexes are neither neurotoxic nor cytotoxic. Therefore, RBCs are unlikely to be a drug reservoir for cytotoxic Pt complexes even though significant amounts of Pt-dach complexes are taken up by RBCs.

A major goal of this study was to determine whether there are any significant differences in the pharmacokinetics and biotransformations of oxaliplatin and ormaplatin which could explain their different neurotoxicity profiles. We observed that both oxaliplatin and ormaplatin produced the same types of major plasma biotransformation products. Thus, the different neurotoxicity profiles between oxaliplatin and ormaplatin cannot be explained on the basis of the type of biotransformation products formed. However, the AUC was 16-fold higher and the C_{max} was 29-fold higher for the Pt(dach)Cl_2 formed from ormaplatin than for the Pt(dach)Cl_2 from oxaliplatin. Since Pt(dach)Cl_2 can be further hydrolyzed into aquated species Pt(dach)$(H_2O)Cl$ and Pt(dach)$(H_2O)_2$, which are very cytotoxic [6, 10, 11], it is possible that the different plasma concentrations of Pt(dach)Cl_2 might cause the different neurotoxicity profiles of ormaplatin and oxaliplatin. Even though Pt(dach)Cl_2 is not likely to be hydrolyzed into Pt(dach)$(H_2O)Cl$ and Pt(dach)$(H_2O)_2$ in the plasma due to the high concentration of chloride (108 m*M*) [22], it will be readily converted into aquated Pt-dach once it is taken into cells where the chloride concentration is much lower (3 m*M*). Thus, the higher plasma concentration of

$Pt(dach)Cl_2$ following ormaplatin infusion is likely to lead to the higher cellular concentrations of $Pt(dach)Cl_2$ and its hydrolysis products, which in turn could lead to more severe toxicity. This hypothesis is consistent with the findings of a previous study performed in conjunction with a clinical trial of ormaplatin, which suggest a correlation between the plasma concentration of $Pt(dach)Cl_2$ and the severity of neurotoxicity [30].

In a companion study, we have developed an E-19 embryonic dorsal root ganglia (DRG) explant culture to study the neurotoxicity of Pt-dach compounds in vitro [18]. This study has shown that $Pt(dach)Cl_2$ is more neurotoxic than either oxaliplatin or ormaplatin while Pt(dach)(Met) and Pt(dach)(GSH) are not neurotoxic, suggesting a direct causal relationship between neurotoxicity and the plasma concentration of $Pt(dach)Cl_2$. We have also studied the antitumor activity for oxaliplatin, ormaplatin, and their biotransformation products (manuscript in preparation). Our data suggest that oxaliplatin itself is primarily responsible for its antitumor activity, while the biotransformation product $Pt(dach)Cl_2$ is more likely responsible for the antitumor activity and neurotoxicity of ormaplatin. It is believed that these data could be useful for the design of both clinical treatment protocols and the next generation of platinum antitumor drugs.

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