A Sensitive Postcolumn Derivatization/UV Detection System for HPLC Determination of Antitumor Divalent and Quadrivalent Platinum Complexes

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A sensitive postcolumn derivatization/UV detection system has been developed for HPLC analysis of antitumor divalent and quadrivalent platinum complexes. It is based on the derivatization of platinum complexes by reaction with sodium bisulfite to corresponding product(s) which has enhanced absorptivity at 280—300 nm. Platinum complexes examined in this study were cisplatin, carboplatin and oxaliplatin (divalent platinum complexes) and oxoplatin and tetraplatin (quadrivalent ones). The proposed detection system was sensitive to all these complexes. Under the detection conditions optimized for individual complexes, the HPLC gave linear relationships between the complex concentration and the peak height. Detection limits at 290 nm with 100 μl injection were 20 nM for cisplatin, 40 nM for oxoplatin, 60 nM for carboplatin and tetraplatin and 100 nM for oxaliplatin ($S/N=3$ at 0.005 AUFS). The proposed system was successfully applied for the determination of cisplatin and oxoplatin in plasma and urine. Pharmacokinetic behavior of oxoplatin and its reduced product cisplatin following a single intravenous injection of oxoplatin in rabbits has been discussed.

Keywords antitumor platinum complex; HPLC determination; postcolumn derivatization; sodium bisulfite; UV detection

Platinum complexes are now a well-established class of antitumor agents. Cisplatin [*cis*-diamminedichloroplatinum(II)] and carboplatin [diammine(1,1-cyclobutanedicarboxylato)platinum(II)] have been successfully used in treatment of human malignancies, and several second-generation antitumor platinum complexes are now under clinical trial.[1)]

In spite of the important role of the platinum complexes in cancer chemotherapy, little is known about their pharmacokinetics and metabolism. Most of the pharmacokinetic studies have been carried out by atomic absorption spectrophotometry but this reveals only total platinum level; there is yet no adequate method which can discriminate between platinum-containing species in biological samples such as plasma or urine. The biodegradation products of cisplatin are known to have biological activities and pharmacokinetic behavior different from those of the parent.[2)] This would also be the case for the second-generation complexes. Of the platinum-containing species present in the body, intact species are by far the most important in terms of antitumor activity. In order to study in detail the pharmacokinetics of an antitumor platinum complex, it is necessary first to develop a method for determining the parent in biological samples.

HPLC methods have been developed for determining cisplatin and some of the second-generation agents in body fluids. However, these methods have not been widely used for pharmacokinetic studies because of the obstacles to detection. In those methods, various detection systems have been adopted: off-line atomic absorption spectrophotometry,[3)] on-line inductively coupled plasma atomic emission spectrophotometry,[4)] quenched phosphorescence detection,[5)] reductive electrochemical detection with mercury drop electrode,[6)] direct UV detection[7)] and UV detection coupled with postcolumn derivatization by potassium dichromate and sodium bisulfite.[8)] We focused on the postcolumn derivatization/UV detection method from the following standpoints. It is responsive to several divalent platinum complexes. Spectrophotometric monitoring is probably preferred for pharmacokinetic studies and the sensitivity is high among the detection systems mentioned above. This means does have disadvantages, however. It is not sensitive to quadrivalent platinum agents. The postcolumn derivatization consists of two reaction steps, preceding activation by potassium dichromate and derivatization by sodium bisulfite, and this requires the delivery of two separate reagent solutions. We thought that a thorough investigation of the reaction between bisulfite and platinum complexes would lead to resolution of these disadvantages and also to improvement in sensitivity.

This paper describes a sensitive postcolumn derivatization/UV detection system responsive to divalent and quadrivalent platinum complexes without dichromate activation. Platinum complexes used in this study are depicted in Fig. 1. Cisplatin, carboplatin and oxaliplatin

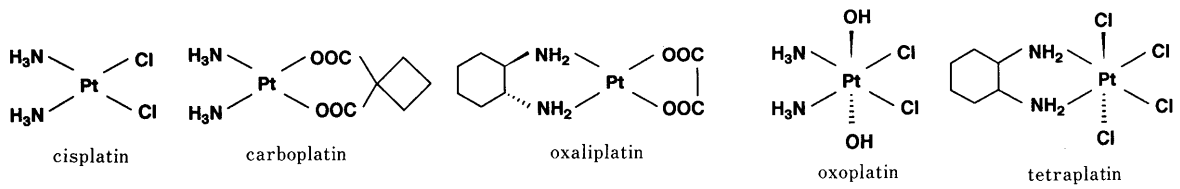

Fig. 1. Structural Formula of Platinum Complexes Used in the Present Study

[(1*R*,2*R*-diaminocyclohexane)oxalatoplatinum(II)] are divalent platinum complexes, and oxoplatin [*cis*-diammine-dichloro-*trans*-dihydroxoplatinum(IV)] and tetraplatin [(1,2-diaminocyclohexane)tetrachloroplatinum(IV)] are quadrivalent ones. Oxaliplatin, oxoplatin and tetraplatin are promising second-generation antitumor platinum complexes.[1)]

Experimental

Materials Cisplatin was purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Oxaliplatin was obtained through the courtesy of the Laboratory of Analytical Chemistry, Pharmaceutical Sciences, Nagoya City University, and used without further purification. Carboplatin, oxoplatin and tetraplatin were synthesized in manners analogous to those of Hill *et al.*,[9)] Hoeschele *et al.*[10)] and Brandon and Dabrowiak,[11)] respectively, being identified by their elemental analysis.[12)] Analysis of platinum was carried out according to a colorimetric method.[13)] Acetonitrile from Nacalai Tesque was distilled once prior to use. Distilled and deionized water was used throughout. Other chemicals were of reagent grade or better and used as received. Stock solutions (1 mM) were prepared weekly with 0.9% saline for cisplatin and tetraplatin and with water for other complexes. The stock solutions were stored under refrigeration and diluted to give the desired concentrations prior to use. Tetraplatin solutions were prepared in glassware shielded from light to prevent photoreduction.[14)] Human plasma was obtained from the Blood Transfusion Division of Kanazawa University Hospital and human urine from healthy volunteers, being kept at −20 °C until use.

Evaluation of Variables Affecting the Derivatization of the Platinum Complexes by Sodium Bisulfite The fundamental experimental procedure was as follows. Solutions of platinum complexes (60 μM) and sodium bisulfite were freshly prepared with a desired buffer solution and the pHs of those solutions were adjusted to the desired values if necessary. A platinum complex and sodium bisulfite solutions pre-incubated at a desired temperature for 10 min were mixed in equal volumes to initiate the reaction followed by incubation at the desired temperature in the dark. A portion of the reaction mixture was withdrawn at adequate intervals to measure absorbance at 290 nm against the buffer solution on a Shimadzu model 265-FW spectrophotometer (Kyoto, Japan).

HPLC The HPLC system consisted of a Shimadzu model LC-6A pump, a Rheodyne model 7125 sample injector fitted with a 100 μl loop (Cotati, CA, U.S.A.), a Shimadzu model CTO-2A column oven, a Sanuki model DM2U-1026 pump (Tokyo, Japan), a Shimadzu model SPD-6AV spectrophotometric detector, a Shimadzu model C-R3A integrator and a poly(tetrafluoroethylene) (PTFE) postcolumn reaction coil (0.5 mm i.d. × 10 m). An eluent was delivered by LC-6A pump at the flow rate of 1 ml/min and a postcolumn reagent solution by DM2U-1026 pump at 0.3 ml/min. Postcolumn reagent solution was freshly prepared each time and kept in an ice-cooled water bath. The postcolumn reaction coil was maintained at a desired temperature in a water bath. Postcolumn derivatization time was estimated to be about 1.7 min based on the retention times of platinum complexes with and without the reaction coil.

Handling of Plasma and Urine Samples A urine sample obtained was immediately subjected to HPLC without any pretreatment. An aliquot (0.5 ml) of plasma sample obtained was immediately centrifuged at 3000 × *g* for 15 min at 4 °C in an Amicon MPS-1 micropartition starter kit fitted with a YMT membrane (Danvers, MA, U.S.A.) to obtain plasma ultrafiltrate (about 0.2 ml). A portion of the plasma ultrafiltrate was immediately subjected to HPLC, and another portion to an atomic absorption spectrometer Hitachi model Z-8000 (Hitachi, Japan).

Animal Experiment Oxoplatin was dissolved at a concentration of 10 μmol/ml in a sterilized 0.9% saline and given intravenously to male white Japanese rabbits (weighing about 2.5 kg) at a dose of 10 μmol/kg. Sampling of blood and urine and data analysis were carried out according to our previous report.[15)] Platinum levels in samples were determined according to an atomic absorption spectrometric method.[16)] Oxoplatin and cisplatin concentrations in samples were determined by the HPLC methods proposed in this study. The plasma concentrations of plasma filterable platinum, oxoplatin and cisplatin were fitted to the equation $C=Ae^{-\alpha t}$, where C (nmol/ml) is the concentration at time t, and A (nmol/ml) and α (min^{-1}) are initial concentration and elimination rate constant, respectively.

Results and Discussion

Strategy of This Study In the detection system with dichromate activation previously reported,[8)] a platinum complex eluted from a column was first activated by potassium dichromate to accelerate the succeeding derivatization by sodium bisulfite. While the acceleration effect of dichromate was observed in our study, we found that the reaction between bisulfite and cisplatin proceeded rapidly without dichromate activation to give product(s) exhibiting enhanced absorptivity at 290—300 nm. Therefore, the activation step did not seemed essential. On the other hand, the previous detection system is not responsive to quadrivalent platinum complexes. As mentioned later, however, we found that oxoplatin and tetraplatin, quadrivalent platinum complexes used in this study, were rapidly reduced in the presence of bisulfite to yield corresponding divalent platinum complexes, which were found to be reactive with bisulfite. In fact, our detection system responds to both complexes, as described below. Dichromate may exert an interfering effect in cases of quadrivalent platinum complexes.

Our idea is that we could develop a novel detection system responsive to both divalent and quadrivalent platinum complexes with improved sensitivity by derivatizing platinum complexes with bisulfite but without dichromate activation. In this study, variables affecting the reaction between bisulfite and a platinum complex were optimized for individual complexes.

Variables Affecting the Reaction between Platinum Complexes and Bisulfite First of all, we examined whether the platinum complexes used in this study would react with bisulfite in the absence of potassium dichromate. The spectra of reaction mixtures of the platinum complexes and bisulfite showed increased absorbance at the 270—320 nm region, indicating that it would be possible to detect both the divalent and quadrivalent platinum complexes after derivatization by bisulfite. A spectrum of cisplatin-sodium bisulfite reaction mixture is shown in Fig. 2 as an illustration. The absorption maxima of all five complexes were observed around 290 nm.

Variables affecting the reaction between the platinum

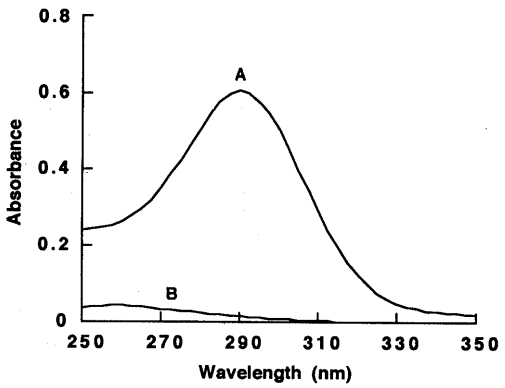

Fig. 2. UV Spectra of (A) Cisplatin–Sodium Bisulfite Reaction Mixture and (B) Sodium Bisulfite Solution

A: Reaction mixture of 30 μM cisplatin–20 mM $NaHSO_3$ was allowed to stand for 10 min at room temperature (about 20 °C). B: 20 mM $NaHSO_3$ solution. Cisplatin and $NaHSO_3$ solutions were prepared with 20 mM acetate buffer (pH 5.5) and the pH of those solutions was adjusted to 5.5 with NaOH if necessary. The spectra were measured against 20 mM acetate buffer (pH 5.5).

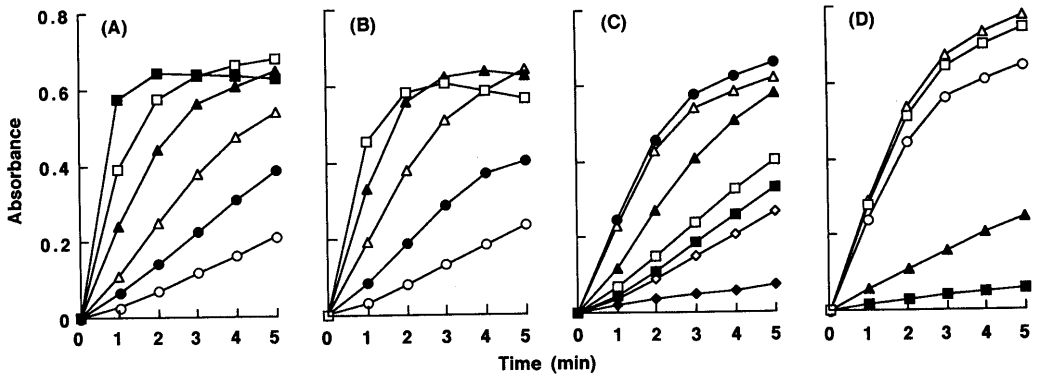

Fig. 3. Effects of (A) Sodium Bisulfite Concentration, (B) Reaction Temperature, (C) pH and (D) Organic Modifier on Derivatization of Cisplatin by Sodium Bisulfite

The fundamental reaction conditions were as follows: a reaction mixture of 30 μM cisplatin–20 mM $NaHSO_3$ prepared with 20 mM acetate buffer (pH 5.5) was allowed to stand at room temperature (about 20°C). A: $NaHSO_3$ concentration, 2 mM (○); 5 mM (●); 10 mM (△); 20 mM (▲); 50 mM (□); 100 mM (■). B: Reaction temperature, 21 °C (○); 30 °C (●); 40 °C (△); 50 °C (▲); 60 °C (□). $NaHSO_3$ concentration, 10 mM. C: pH, 3.0 (◇); 4.0 (□); 5.0 (△); 5.5 (●); 5.7 (▲); 6.0 (■); 7.0 (◆). In the case of pH 3.0, the pH was adjusted with HCl. Buffers used were 20 mM acetate buffer for pHs 4.0, 5.0, 5.5 and 5.7, 20 mM 2-morpholinoethanesulfonic acid (MES)/NaOH buffer for pH 6.0 and 20 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES)/NaOH buffer for pH 7.0. D: Organic modifier, none (○); 0.1% methanol (▲); 0.2% methanol (■), 10% acetonitrile (△); 30% acetonitrile (□).

complexes and bisulfite were evaluated by monitoring absorbance of the reaction mixtures at 290 nm at intervals. Variables evaluated were sodium bisulfite concentration, temperature, pH and organic modifier concentration. Results obtained with cisplatin are shown in Fig. 3 as an illustration. Similar features were observed for other complexes. The absorbance increased more rapidly with an increase in bisulfite concentration or temperature. The reaction was found to be highly dependent on pH, indicating that it was necessary to strictly control the pH for postcolumn derivatization in the vicinity of 5.5. A significant difference in increasing profile of absorbance was not observed when the reaction was carried out in an acetate-, citrate- or phosphate-buffered medium (pH 5.5). Next, the influence of methanol and acetonitrile was examined because carboplatin, oxaliplatin and tetraplatin are chromatographed under reversed-phase mode. Methanol markedly inhibited the derivatization at a level as low as 0.1%, while acetonitrile exhibited a slight stimulatory effect. An eluent must be free from methanol. Overall, these results indicate that even without dichromate activation, the derivatization proceeds so rapidly that it is completed within a few minutes under appropriate conditions.

It has been reported that oxoplatin and tetraplatin are reduced to yield cisplatin and (1,2-diaminocyclohexane)dichloroplatinum(II) (CHP) as the main product, respectively, in the presence of ascorbic acid.[17] Sodium bisulfite is a well-known reducing agent, and therefore it seems likely that bisulfite reduces oxoplatin and tetraplatin. Chromatographic analyses of oxoplatin- and tetraplatin-sodium bisulfite reaction mixtures demonstrated that those platinum(IV) complexes were reduced by bisulfite to give cisplatin and CHP as the principal reduced product, respectively.[18] We then examined the time courses of concentrations of oxoplatin and tetraplatin and their respective reduced products in the presence of sodium bisulfite at pH 5.5, 6 and 7. The time courses at pH 6 are given in Fig. 4 as an illustration. Although significant difference was not observed in reduction rates of oxoplatin and tetraplatin among the pHs examined, the cisplatin and

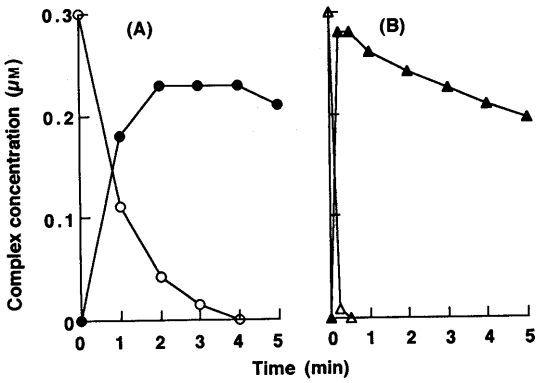

Fig. 4. Time Courses of Concentrations of Parent Complex and Its Reduction Product in (A) Oxoplatin– and (B) Tetraplatin–Sodium Bisulfite Reaction Mixtures

A: Oxoplatin (○), cisplatin (●). B: Tetraplatin (△), CIP (▲). Reaction mixture of 30 μM oxoplatin– or tetraplatin–10 mM $NaHSO_3$ prepared with 20 mM MES/NaOH buffer (pH 6.0) was allowed to stand at room temperature (about 20 °C). A portion of the reaction mixture was withdrawn at intervals and subjected to HPLC analysis. Oxoplatin, cisplatin and tetraplatin were determined under their individual HPLC conditions listed in Table I. CIP was determined using the same HPLC conditions as for cisplatin.

CHP levels determined by HPLC were the lowest at pH 5.5 followed by pH 6 and 7. This indicates that the yielded cisplatin and CHP disappeared most rapidly at pH 5.5 *via* the reaction with bisulfite, being consistent with the results shown in Fig. 3C. The results obtained in this experiment suggest that bisulfite reduces oxoplatin and tetraplatin first and subsequently reacts with the yielded platinum(II) complexes.

Chromatographic and Detection Conditions Chromatographic and detection conditions were optimized with the five platinum complexes based on the information obtained in the above investigations. In particular, determination of cisplatin and oxoplatin in plasma and urine were successfully achieved. The conditions obtained are listed in Table I.

In the analysis of cisplatin, an anion exchange chromatographic system was adopted because of the following unique chromatographic properties.[7a] Although cisplatin is a non-charged compound, it is retained on an

TABLE I. Chromatographic and Detection Conditions Optimized for Each of the Platinum Complexes Studied

	Cisplatin	Oxoplatin	Carboplatin, oxaliplatin, tetraplatin
Column	MCI gel CDR10 (4.6 mm i.d. × 8 cm)	MCI gel CPK08 (4.6 mm i.d. × 8 cm)	Inertsil ODS-2 (4.6 mm i.d. × 25 cm)
Column temperature (°C)	40	40	40
Eluent (1 ml/min)	0.1 M Na_2SO_4–30% CH_3CN–10 mM acetate buffer (pH 5.5)	50 mM K_2SO_4 (pH 3.5 with acetic acid)	5% CH_3CN–10 mM acetate buffer (pH 5.5)
Postcolumn reagent solution (0.3 ml/min)	40 mM $NaHSO_3$–10 mM acetate buffer (pH 5.5)	40 mM $NaHSO_3$–10 mM acetate buffer (pH 5.5)	40 mM $NaHSO_3$–10 mM acetate buffer (pH 5.5)
Postcolumn reaction temperature (°C)	60	60	60
Detection wavelength (nm)	290	290	290

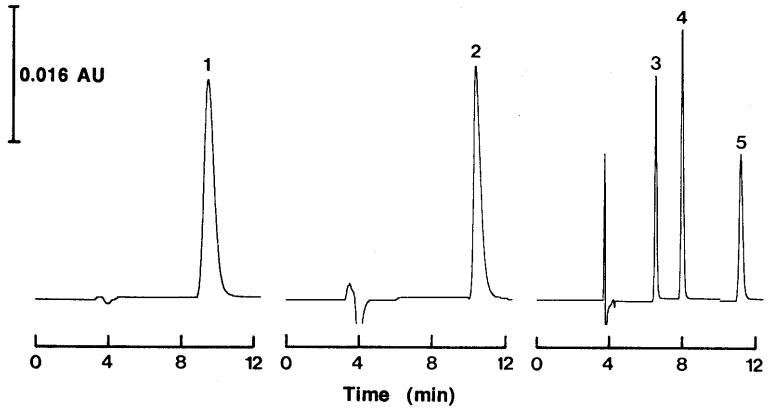

Fig. 5. Typical Chromatograms of Authentic Samples of Cisplatin, Oxoplatin, Carboplatin, Oxaliplatin and Tetraplatin
Peaks: 1, cisplatin (10 μM); 2, oxoplatin (20 μM); 3, carboplatin (50 μM); 4, tetraplatin (50 μM); 5, oxaliplatin (50 μM).

anion exchange column. Further, the capacity factor of cisplatin is larger on the column with higher ion exchange capacity, while it is little affected by varying salt concentration in eluent. The column was a MCI gel CDR10 because of its high ion exchange capacity (0.3 meq/g). The eluent was 0.1 M sodium sulfate–30% acetonitrile–10 mM acetate buffer (pH 5.5). Sodium sulfate was used as an eluting agent since it gave the largest capacity factor of cisplatin among the salts examined and did not interfere with the derivatization by bisulfite at 0.1 M level. When plasma and urine samples were subjected to HPLC with an eluent of 0.1 M sodium sulfate–10 mM acetate buffer (pH 5.5), a few peaks arising from plasma and urine constituents appeared around the retention time of cisplatin. The retention times of those interfering peaks were decreased by adding an organic modifier, acetonitrile, to the eluent instead of increasing sodium sulfate concentration. The postcolumn derivatization conditions were evaluated to maximize the signal-to-noise ratio of cisplatin. Both the eluent and postcolumn reagent solution were buffered at pH 5.5 with 10 mM acetate, which was the optimal pH, consistent with the results shown in Fig. 3C. The sodium bisulfite concentration in postcolumn reagent solution and reaction temperature were examined in ranges of 10 mM to 0.1 M and 40 °C to 70 °C, respectively. The maximal signal-to-noise ratio was obtained when sodium bisulfite concentration and reaction temperature were 40 mM and 60 °C, respectively.

In the oxoplatin analysis, a cation exchange chromatographic system was adopted. As oxoplatin serves as a monobasic acid in a pH range of 3 to 7, it can be chromatographed under acidic conditions on an octadecyl silica (ODS) column in the presence of an ion-pairing agent and a cation exchange column.[19] Although the theoretical plate of oxoplatin was greater on an ODS column than on a cation exchange column, analysis of oxoplatin in plasma and urine were achieved on the latter. The column was a MCI gel CPK08 (0.5 meq/g). Potassium sulfate was used as an eluting agent and the eluent was acidified with acetic acid. The capacity factor of oxoplatin increased with decreasing eluent pH and decreased with increasing eluent potassium sulfate concentration. Potassium sulfate concentration and pH of the eluent were chosen to be 50 mM and 3.5 so that oxoplatin was separated from plasma and urine constituents detectable at 290 nm, as shown in Fig. 7. Since the eluent was acidic, postcolumn reagent solution was made alkaline with sodium acetate. The maximal signal-to-noise ratio of oxoplatin was obtained on the postcolumn reagent solution containing 20 mM sodium acetate. The pH of detector effluent was about 5.5. Other conditions such as sodium bisulfite concentration and postcolumn derivatization temperature were determined similarly to the case of cisplatin.

Carboplatin, oxaliplatin and tetraplatin were chromatographed on an ODS column with water/acetonitrile eluent because this reversed-phase chromatographic system gave much larger capacity factors for the complexes than others examined in this study, including cation exchange, anion exchange and adsorption chromatographic systems. Postcolumn derivatization conditions were evaluated for

each complex to maximize the signal-to-noise ratio as in the case of cisplatin. The optimized conditions for the three complexes were identical. In this study, a postcolumn reagent solution freshly prepared was kept in an ice-cooled water bath, the sensitivity being sustained unchanged for at least 15 h.

Figure 5 shows typical chromatograms of the authentic samples of the five platinum complexes obtained under the optimized conditions. Linear relationships were obtained with the five complexes between the peak height and the complex concentration in ranges of 0.05 to 20 μM for cisplatin (9 plots), 0.1 to 20 μM for oxoplatin (8 plots) and 0.2 to 20 μM for carboplatin, oxaliplatin and tetraplatin (7 plots). Correlation coefficients were not less than 0.997. Table II lists the detection limits of the complexes (100 μl injection, $S/N=3$ at 0.005 AUFS). Coefficients of variation of intra-day (29 determinations) and inter-day assays (5 determinations) were less than 5% with the five complexes at 0.5 and 5 μM levels. Of the complexes used in this study, cisplatin and carboplatin had been analyzed by the method with dichromate activation (dichromate activation method).[8] The present method is more sensitive by a factor of about 5 for cisplatin and about 50 for carboplatin than the dichromate activation method. While direct UV detection methods have been reported for carboplatin[7c] and oxaliplatin,[7a] the present method is also more sensitive than those direct methods. As for oxoplatin and tetraplatin, this is the first study of their HPLC determination.

Table II. Detection Limits Obtained for the Platinum Complexes Studied

	Detection limit (nM)
Cisplatin	20
Oxoplatin	40
Carboplatin	60
Oxaliplatin	100
Tetraplatin	60

As mentioned above, determinations of cisplatin and oxoplatin in plasma and urine were achieved by investigating chromatographic and postcolumn derivatization conditions. Figures 6 and 7 represent typical chromatograms of cisplatin and oxoplatin in plasma and urine. Peaks arising from plasma and urine constituents did not interfere with the determination of the complexes. Calibration curves for cisplatin and oxoplatin in plasma and urine made with a peak height showed good linearity in ranges of 0.05 to 20 μM for cisplatin (9 plots) and 0.1 to 20 μM for oxoplatin (8 plots) with correlation coefficients of not less than 0.998. Detection limits of these complexes in plasma and urine were the same as in Table II. Recoveries of the complexes were more than 95% and the loss during sample treatment was negligible. Coefficients of variation of intra-day (5 determinations) and inter-day assays (5 determinations) were less than 5% at 0.5 and 5 μM levels. Several HPLC methods for determining cisplatin in plasma and urine samples have been reported,[3-6,7b,8] including the dichromate activation method[8] and an amperometric method with a dropping mercury electrode and ammonium-enhanced catalytic proton reduction[6c] which is currently the most sensitive. Sensitivity of the present method for cisplatin in plasma was at least 10 times higher than that of the dichromate activation method and comparable to that of the amperometric method.[6c] Furthermore, these two methods are applicable only to plasma samples.

Pharmacokinetic Behavior of Oxoplatin in Rabbits We next studied the pharmacokinetic behavior of oxoplatin following a single intravenous injection in rabbits. It has been suggested that quadrivalent platinum complexes are reduced to corresponding divalent platinum complexes in the body. In this study, cisplatin was found in rabbit plasma and urine, indicating that oxoplatin was actually reduced to yield cisplatin in the rabbit body. Cisplatin was not produced when oxoplatin was added to rabbit

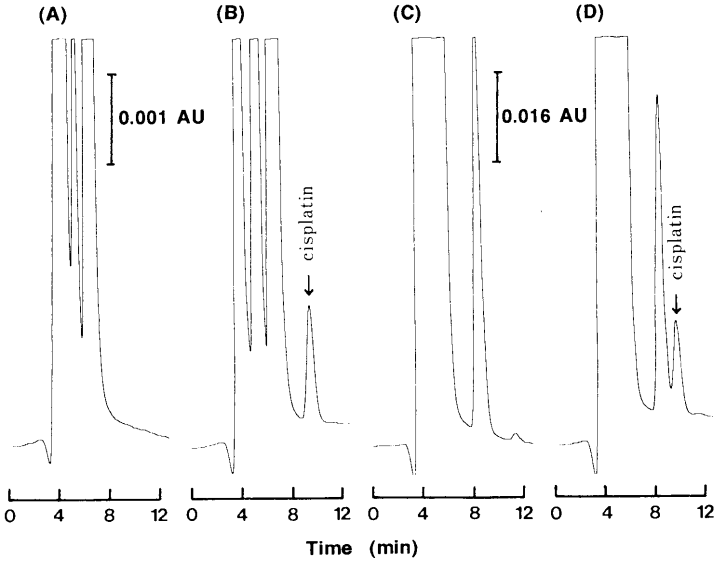

Fig. 6. Typical Chromatograms of Cisplatin in Human Plasma Ultrafiltrate and Urine Samples

A, a blank plasma ultrafiltrate sample; B, a plasma ultrafiltrate sample freshly spiked with cisplatin at 1 μM; C, a blank urine sample; D, a urine sample freshly spiked with cisplatin at 10 μM.

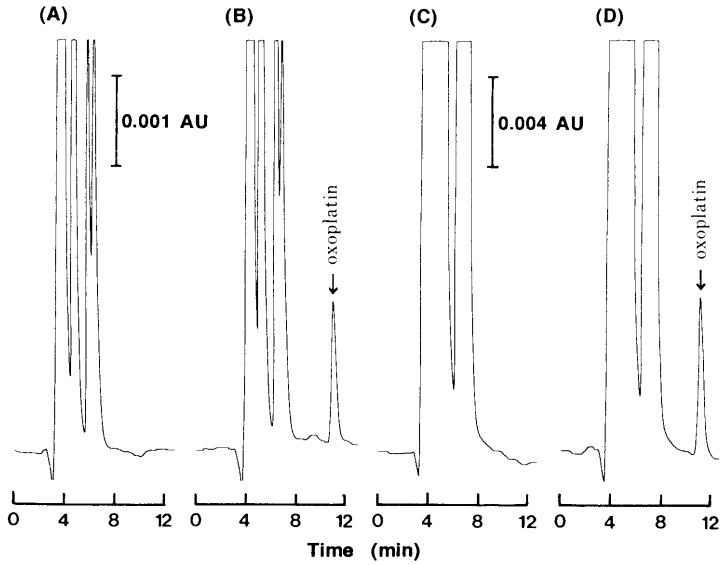

Fig. 7. Typical Chromatograms of Oxoplatin in Human Plasma Ultrafiltrate and Urine Samples

A, a blank plasma ultrafiltrate sample; B, a plasma ultrafiltrate sample freshly spiked with oxoplatin at 2 μM; C, a blank urine sample; D, a urine sample freshly spiked with oxoplatin at 10 μM.

TABLE III. Pharmacokinetic Parameters of Filterable Platinum, Oxoplatin and Cisplatin in Plasma Following a Single Intravenous Injection of Oxoplatin in Rabbits

Platinum species	A (nmol·ml^{-1})	α ($\times 10^{-2}$ min^{-1})	AUC ($\times 10$ nmol·min·ml^{-1})	X_u (%)	CL_R (ml·min^{-1}·kg^{-1})
Filterable platinum	55.3	1.08	512	87.4	1.7
Oxoplatin	55.4	1.21	458	71.9	1.6
Cisplatin	0.63	0.41	15.4	4.5	2.9

Data represent mean values obtained for two rabbits.

plasma or urine and incubated at 37 °C for a few hours. The pharmacokinetic parameters of the filterable platinum, oxoplatin and cisplatin in plasma are listed in Table III, where AUC is the area under the concentration–time curve, X_U is the percentage of platinum amount excreted in urine for up to 5 h postinjection against the dose, and CL_R is the renal clearance. A and α were described in Experimental. Plasma filterable platinum and oxoplatin were detectable for up to 5 h postinjection and decayed in a monoexponential fashion. While cisplatin in plasma was detected as early as 15 min postinjection, its concentration decreased monoexponentially with time. As for urinary excretion, more than 80% of platinum amount excreted in urine for up to 5 h postinjection was found to be oxoplatin and about 6% of it was cisplatin. The platinum amount excreted in urine for up to 5 h was about 90% of the dose. Comparing the pharmacokinetic behavior of cisplatin following a single intravenous injection in rabbits[15] revealed certain features of oxoplatin pharmacokinetics: plasma-decay of oxoplatin was much slower than that of cisplatin; the fraction of intact species in the platinum amount excreted in urine was greater for oxoplatin than for cisplatin. Quadrivalent platinum complexes with axial hydroxy ligand are known to be inert in ligand exchange.[17a,20] The pharmacokinetic features of oxoplatin indicate that it is more stable than cisplatin in the biological milieu and would be associated with the inertness of oxoplatin. Details concerning the pharmacokinetics of oxoplatin and cisplatin will be reported elsewhere in the near future.

A sensitive postcolumn derivatization/UV detection system for HPLC analysis of antitumor divalent and quadrivalent platinum complexes has been developed, and determination of cisplatin and oxoplatin in plasma and urine was successfully achieved. The present study on behavior of oxoplatin in rabbits has demonstrated that tracing the behavior of intact species is very important and informative in clarifying the pharmacokinetics of platinum complexes. The detection system developed in this study should be of use in development of HPLC methods for determining a variety of second-generation antitumor platinum complexes.

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