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Review

Speciation of platinum compounds: a review of recent applications in studies of platinum anticancer drugs

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Abstract

This is a review of investigations involving speciation studies of five important platinum-containing drugs used in cancer treatments. The information presented here is drawn from recent reports published during the period 1995–1999. The work includes detection, separations and identifications of degradation and biotransformation products. In addition, important information is reported on the number and nature of products of reactions of platinum anticancer drugs with thiol compounds. HPLC is employed effectively for separations of reaction products in speciation investigations. Information derived from speciation is very helpful in studies of pharmacokinetics as well as side effects and toxicities of the drugs as they are administered to patients.

Keywords: Reviews; Platinum anticancer drugs

1. Introduction

This review contains a selection of significant reports concerning speciation studies of five of the most important platinum anticancer drugs from the period 1995–1999. Treatments of some forms of cancer by means of platinum-containing drugs began with the discovery that cisplatin, or *cis*-diamminedichlororplatinum(II), possessed anticancer activity [1]. Since that time, many platinum compounds have been prepared and tested in cancer treatments, and some of these compounds have been approved for treatments in several countries. A detailed discussion of the current status of platinum-containing drugs is available [2]. Dosages of drugs must be monitored carefully in order to assess their effectiveness during treatments. The appearances of side effects set limits on levels of administered drugs. Changes in concentrations of platinum in body fluids, mainly blood and urine, have played an important role in establishing pharmacokinetics of the drugs. Early research work on development of suitable analytical procedures for determinations of concentrations of platinum in clinical samples involved accurate measurements of total and ultrafilterable platinum [3]. Later, speciation involving determinations of concentrations of intact drugs and identities and concentrations of biotransformation and reaction products in biological samples became necessary. Determinations of total platinum contents were carried out conveniently using flame and graphite furnace atomic absorption (GFAAS) techniques. Speciation requires separations of platinum-containing compounds and HPLC has played a leading role in this area of research. While atomic absorption spectrometry has been used both for

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detection and measurement of platinum in fractions of column effluents, on-line detection by means of UV/Vis spectrometry and inductively coupled plasma-mass spectrometry (ICP-MS) have become important in speciation studies [3]. LC–MS and comparisons of chromatographic retention times with those of known compounds have been used for identifications of reaction products.

Although many platinum compounds have been tested for their antitumor activities, the majority were related within a set of structure–activity relationships [2]. For a platinum compound to exhibit activity against tumors, the complex conformed with the general formula of either *cis*-$[PtX_2(Am_2)_2]$ or *cis*-$[PtX_2Y_2(Am)_2]$ where X is a leaving group and Am is an inert amine with at least one N-H moiety. The leaving group (X) should be an anion of intermediate binding strength. For example, the leaving group of cisplatin is Cl^-. In the presence of fluid media containing low concentrations of chloride, both a monohydrated complex, *cis*-$[PtCl(NH_3)_2(H_2O)]^+$ and a dihydrated complex *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ are formed. The monohydrated complex has proven to be the more cytotoxic species of the hydrates. The hydrated species bind to the guanosine moieties of DNA by means of intra- and interstrand cross-linkages. The majority (>80%) of the DNA adducts induced by cisplatin are intrastrand cross-links. Platinated DNA adducts have been quantitated separately by enzyme-linked immunosorbent assays (ELISHA) after appropriate sample treatments, and more recently, by HPLC procedures. In addition, thiol compounds such as L-methionine (L-met), L-cystein (L-cys) and glutathione in biological fluids react with significant proportions of cisplatin administered to patients. L-met is important because of its large concentrations and reactivity. There is evidence that the nephrotoxicity of cisplatin is increased in the presence of its products of reaction with L-met [4]. Thus, the detection and identifications of reaction products of platinum-containing drugs with thiol compounds is important in studies of toxicities. Differences in overall toxicities of some other platinum-containing drugs have been attributed to different concentrations of L-met reaction products. The results of a number of recent speciation studies are described under the individual drugs in subsequent sections of this report. The drugs listed in Table 1 have been approved for administration in various countries, or are being studied in on-going trials.

Table 1
Status of some platinum anticancer drugs

Drugs	Main limiting toxicity	Clinical status
Cisplatin	Nephrotoxicity, myelosuppression	Worldwide approval
Carboplatin	Myelosuppressiom	Worldwide approval
Oxaliplatin	Neuropathy	Approved in France; phase II clinical trials
Lobaplatin	Thrombocytopenia	Approved in China; phase II clinical trials.
Nediplatin	Myelosuppression	Approved in Japan
JM216	Myelosuppression	Phase III clinical trials

2. Platinum-containing drugs

2.1. Cisplatin

Cisplatin is the most important of the platinum-containing anticancer drugs. It is used in treatments of a variety of cancers, but there are limitations on dosages because of severe side effects as noted in Table 1. Cisplatin is hydrolyzed to form species which react with DNA. The most active species is the monohydrated complex, $[PtCl(NH_3)_2(H_2O)]^+$, as noted earlier. Cisplatin in plasma can react with proteins also, particularly with their sulfur groups. Analytical procedures have been developed in order to study the formation and the identities of biotransformation products. There are concerns that some of the earlier reports of studies involving speciation have been affected by conditions and reagents used in analytical procedures. Some recent research has been reported on separations and determinations of biotransformation and reaction products of cisplatin with sulfur-containing chemicals where particular attention has been given to possible interferences.

Cisplatin and its monohydrated complex in plasma were separated by HPLC using ion-exchange col-

umns [5]. A coupled column system consisting of a strong cation-exchange column, (Nucleosil SA), and a strong anion-exchange column, (Nucleosil SB), was effective in separations in aqueous solutions. Analyses of biological samples required the use of individual columns to separate the species because of interfering natural substances. Cisplatin was isolated on the anion-exchange column while the monohydrated complex was separated on the cation-exchange resin column. Additional details of the work are found in Table 2, Ref. [5]. Calibration curves were linear over concentration ranges of 0.14–78 μ*M* for cisplatin and 1.3–130 μ*M* for the monohydrated complex. The analytical procedure was applied in a study of the pharmacokinetics of cisplatin and its monohydrated complex in humans [6]. Possible reactions between cisplatin and its hydrated complexes and constituents used in mobile phases of HPLC procedures were investigated [7]. Formic acid did not react with the analytes. However, phosphoric acid in the mobile phase reacted with hydrolysis products of cisplatin. Two phosphate compounds were formed with the monohydrated complex. Conditions used in the work are shown in Table 2, Ref. [7]. Another report also noted reactions of components of HPLC mobile phases [8]. Acetonitrile reacted with the hydrated complexes, while methanol proved to be a satisfactory reagent. This work showed also that reactions occurred with carboxylic acids used for adjustments of pH. Triflic acid (trifluoromethanesulfonic acid) was recommended as being nonreactive. Neither hexanesulfonic acid nor sodium dodecyl sulfate (SDS) reacted with cisplatin hydrated complexes. The chromatographic conditions recommended for separation of cisplatin and its hydrated complexes are shown in Table 2, Ref. [8]. About 20 min was required for each chromatographic run.

Kinetic studies of reactvities between L-met with cisplatin and its monohydrated complex in aqueous and saline solutions were carried out [4]. After an incubation period of 24 h, five reaction products containing platinum were detected by means of ICP-MS. LC–MS was used to identify the products which included $Pt[(NH_3)_2(met)Cl]$, $Pt[(NH_3)(met)Cl]$, $[Pt(NH_3)_2(met)]$ and $Pt(met)_2$. Reaction products of cisplatin with glutathione in phosphate buffer solution were investigated [9]. Two glutathione adducts which did not contain phosphate were separated by HPLC. The conditions included a Lichrosorb RP18 (250×4.6 mm) column and a mobile phase of 15 m*M* phosphoric acid, pH 2.2. A UV detector was used to monitor the separations, and products were identified by LC–MS. However, these glutathione adducts were not found in plasma ultrafiltrates of patients and rats when samples were analyzed after administrations of cisplatin and glutathione.

Table 2
Cisplatin transformation and reaction products: HPLC conditions[a]

Sample	Compounds	Column	Mobile phase	Detection	Ref.
Plasma	Cisplatin, monohydrated complex	Nucleosil SA, 50×3.2 mm Nucleosil SB 150×4.6 mm	0.125 *M* succinic acid–CH_3OH (2:3); pH 5.2	Postcolumn deriv. DDTC, 344 nm	[5]
Aqueous saline solution	Cisplatin, monohydrated complex, reaction products	Spherisorb ODS 150×4.63 mm	15 m*M* formic acid or 15 m*M* phosphoric acid	ICP-MS or 210 nm	[7]
Aqueous solution	Cisplatin, hydrated complexes	Bonda pak C_{18} 300×3.9 mm	3% (v/v) CH_3OH–H_2O, 0.05 mM SDS; pH 2.5 (triflic acid)	305 nm	[8]
Urine	Cisplatin, transformation products	Microsorb-MV C_{18} 250×4.6 mm	0.1% TFA, 7 m*M* HS, 5% CH_3OH; pH 2.6	ICP-MS	[10]

[a] DDTC=diethyldithiocarbamate; HS-1=heptanesulfonate; TFA=trifluoroacetic acid.

An investigation of the species formed in reactions of cisplatin with creatinine was reported [10]. Concentrations of creatinine, a product of creatine metabolism, in urine and serum are indicators of renal function. Kidney damage is indicated by elevated levels of creatinine in blood. Speciation of platinum compounds in urine samples of patients treated with cisplatin were studied using HPLC with detection by means of coupled ICP-MS. Details of the conditions for the separations are shown in Table 2, Ref [10]. The major component of the samples was cisplatin. The monohydrated complex of cisplatin and a 1:1 Pt–creatinine complex, *cis*-$[Pt(NH_3)_2Cl(creat)]^+$ were identified. The latter was the second most abundant platinum-containing species. Other chromatographic peaks were those of cisplatin-urea and cisplatin-uric acid reaction products. Identifications were made by comparisons of chromatographic retention times of reaction products with those of known compounds. Another part of the investigation involved analyses of samples from patients treated with carboplatin, or *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II). Analyses of urine samples were carried out on the same columns that were used for cisplatin, but the mobile phase included 5 m*M* 1-heptanesulfonate (in place of 7 m*M*) and 10% methanol (in place of 5%). The main platinum-containing species was carboplatin. None of the minor species corresponded with those detected in samples from cisplatin treatments. Three weeks after treatments had ceased, one of the samples contained both cisplatin and its monhydrated complex.

The separation and quantitation of major platinum–DNA adducts formed by cisplatin using HPLC and ^{32}P-postlabeling has been reported [11]. The major intrastrand cross-links are *cis*-$Pt(NH_3)_2$d(GpG), or Pt-GG, constituting 40–70% of the products, and *cis*-$Pt(NH_3)_2$d(ApG), or Pt-AG. ApG denotes 2′-deoxyandenylyl(3′→5′)-2′-deoxyguanosine, and GpG denotes 2′-deoxyguanylyl(3′→5′)-2′-deoxyguanosine. Sample preparation for HPLC was complex and it will not be described in detail here. However the steps in the procedure include sample digestion, purification, removal of platinum and labeling with ^{32}P. Sample components were then separated on a C_{18} column (Intersil ODS-80A, 4.6×150 mm) by isocratic elution at 40°C with 0.1 *M* KH_2PO_4/4.5% methanol at pH 5.5. An on-line flow radioisotope detector was used. The lower limits of quantitation were 87 and 53 amol μg^{-1} DNA for Pt-GG and Pt-AG respectively.

2.2. Carboplatin

Carboplatin is an analogue of cisplatin. Administration of the drug is easier because it is less toxic than cisplatin at standard dosages. One of the differences in toxicitiy is attributed to the substitution of a leaving group, namely 1,1-cyclobutanedicarboxylato, with more binding strength in carboplatin than the chloro substituents in cisplatin. This results in different pharmacokinetics. However, the reaction mechanism of carboplatin with DNA is similar to that of cisplatin. Carboplatin exhibited cross-resistance with cisplatin. Cross-resistance indicates that carboplatin is active against the same types of tumors as cisplatin.

The mechanism of reaction, at 37°C, of L-met with carboplatin was compared with that of cisplatin [12]. The reaction products, or adducts, were separated by HPLC using conditions noted in Table 3, Ref. [12]. LC–MS was employed for identifications of the adducts. The reactions were studied in both aqueous and phosphate buffer solutions. In the presence of phosphate, the initial carboplatin reaction product was $[(NH_3)_2$(met)(1,1-cyclobutanedicarboxylic acid)Pt. The final reaction products were the same as those formed by reaction of L-met with cisplatin, namely $[(NH_3)_2$(met)]Pt and two isomers $[(met)_2]$Pt. The isomers had different chromatographic retention times. In the presence of sodium chloride solution (150 m*M*), both carboplatin and cisplatin reacted with L-met to yield five methionine–platinum adducts, the same number and compositions for each drug. This observation supported results from studies carried out by others that carboplatin is transformed to cisplatin in this medium. The work showed also that the modes of reactions of the drugs are dependent upon the compositions of the reaction media.

2.3. Oxaliplatin

Oxaliplatin, or trans-L-1,2-diaminocyclohexane-oxalatoplatinum(II), has been approved in France for treatment of colorectal cancer, and is being evaluated

Table 3
Carboplatin and anticancer drugs, transformation and reaction products: HPLC conditions

Sample	Compounds	Column	Mobile phase	Detection	Ref.
Aqueous L-methionine	Carboplatin, reaction products	ODS 150×4.6 mm	15 m*M* formic acid–CH_3OH (70:30)	210 nm	[12]
Plasma	Oxaliplatin, 6 transformation products	Partisil ODS-3, 250×4.6 mm	Gradient; A=5 m*M* HS; pH 3.4; B=90% CH_3OH–H_2O	^3H scintillation; AAS	[13]
Plasma	Lobaplatin diastereoisomers	Hypersil ODS, 250×4 mm	1.4 g KH_2PO_4 dissolved in acetonitrile–H_2O–triethylamine 26:974:0.3 (v/v/v); pH 6.4	210 nm	[15]
Plasma	JM216, 5 transformation products	PLRP-S, 250×4.6 mm	Gradient; A=acetonitrile; B=H_2O	ICP-MS	[19]
Plasma	JM216, 3 transfomation products	Prodigy C_8, 150×4.6 mm	Gradient; A=25% CH_3OH–0.0.1% orthophosphoric acid, pH 2.5; B=CH_3OH	ICP-MS	[20]
Aqueous saline, glucose	Carboplatin, hydrolysis products	Nucleosil-120-S 250×4 mm	0.5×10^{-3} *M* H_2SO_4 and 2×10^{-2} *M* Na_2 SO_4^- CH_3OH (90:10).	229 nm	[22]

in phase II clinical trials in the USA (Table 1). It exhibited no cross-resistance with cisplatin when tested in cisplatin-resistant tumors, and showed little nephrotoxicity. Platinum complexes containing 1,2-diaminocyclohexane carrier ligands are called dach complexes. A HPLC procedure has been described for studies of biotransformation products of oxaliplatin using ^3H-labeled oxaliplatin and ^{35}S-labeled nucleophiles for the detection of Pt–dach biotransformation products [13]. Atomic absorption spectrometry was used also in order to detect any unlabeled Pt compounds. Plasma samples were obtained from the blood of rats which had been treated with the drug. HPLC conditions are listed in Table 3, Ref. [13]. Major biotransformation products were identified as Pt–dach bis cystein complex, Pt–dach mono methionine complex and free dach. Minor products included Pt–dach dichloro complex, Pt–dach diglutathione complex and Pt–dach monoglutathione complex. Chromatographic retention times of unknown components were compared with those of prepared compounds used as standards for identifications of eluted products. In addition, mono methionine Pt–dach complex was identified by LC–MS. The results of this work were applied in a study of the pharmacokinetics of oxaliplatin in comparison with ormaplatin, or trans-D,L-1,2-diaminocyclohexanetetrachloroplatinum(IV). Clinical trials of ormaplatin have been abandoned because of its unpredictable neurotoxicity. Both drugs produced the same number of major biotransformation products under identical experimental conditions [14]. The results of the study suggested that the differences in toxicity between the two drugs may be related to different concentrations of the biotransformation product Pt–dach dichloro complex, because a larger concentration of this toxic substance was formed from ormaplatin. This complex and its hydrolysis products may be uniquely neurotoxic [14].

In a study of the reaction mechanism of L-met with oxaliplatin in the presence of sodium chloride, four reaction products were formed and then were separated by HPLC [12]. The conditions were the same as those for carboplatin listed in Table 3, Ref. [12], except the composition of the eluent was 50:50 (v/v) and not 70:30 (v/v).The initial reaction species, [(OH)(Cl)(dach)]Pt], reacted with L-met to form [(met)(Cl)(dach)]Pt] and [(met)(dach)]Pt. The observation that reaction products of L-met with oxaliplatin differed from those of both cisplatin and carboplatin with L-met could partially explain differences in toxicities of these drugs.

2.4. Lobaplatin

Lobaplatin, or 1,2-diamminomethylcyclobutane-platinum(II) lactate, is a water-soluble compound that is being used in China while still being evaluated elsewhere as noted in Table 1. The drug is a mixture (about 1:1) of two diasterioisomers. These are designated as LP–D1=*RRS* configuration and LP–D2=*SSS* configuration. An advantage of lobaplatin observed in a number of cancer studies is its lack of cross-resistance with cisplatin. A specific assay for both lobaplatin isomers in plasma ultrafiltrates of patients was developed [15]. The data from the analyses were used to evaluate the pharmacokinetics of the isomers, particularly in treatments of patients suffering from impaired liver and kidney functions [16]. The HPLC procedure used for separations of the isomers involved a prior extraction and concentration of lobaplatin on a C_{18} Sep Pak cartridge, and its subsequent elution in methanol. Details are found in Table 3, Ref. [15]. Standard curves were linear over ranges of 0.07–9 μM. Recoveries of isomers ranged from 97 to 106%, and between-day accuracies were 98–101%.

2.5. JM216

JM216, or bis(acetato)amminedichloro(cyclohexylamine)platinum(IV), was developed as an anticancer drug that could be administered orally. Oral administration offers advantages of convenience for patients and lower costs of delivery of the treatment. Research programs showed that JM216 possessed antitumor activity equivalent to that of both cisplatin and carboplatin. Its toxicological properties resembled those of carboplatin, as noted in Table 1. In studies involving pharmacokinetics of JM216, low concentrations of biotransformation products of the drug in plasma ultrafiltrates (<100 ng ml^{-1}) and the number of these products required the development of sensitive analytical procedures for purposes of detection and identification. Plasma ultrafiltrate samples were analyzed by means of HPLC. Information about the procedures and detectors is contained in Table 3. Up to six biotransformation products were detected and identified. LC–MS was employed for identifications of separated constituents. The major metabolites were JM118, or amminedichlorocyclohexylamineplatinum(II), JM383, or bis(acetato)amminedihydroxo(cyclohexylamine)platinum(IV), JM518, or bis(acetato) amminechloro(cyclohexylamine)-hydroxoplatinum(IV) and JM559, the isomer of JM518 [17].

When GFAAS was used for detection of platinum-containing compounds in the column effluent and for quantitative analyses [18], detection limits were in the range of 10–50 ng ml^{-1}. The separation conditions were the same as those described in Table 3, Ref. [19]. Disadvantages of the GFAAS method of detection were slow rates of analyses because of the limitations imposed by an off-line method and insufficient sensitivity. Detection of individual species at concentrations as low as 1 ng ml^{-1} was desired. Subsequently, a more sensitive and efficient procedure was achieved by coupling HPLC to ICP-MS [19]. Limits of detection of 0.6 ng ml^{-1} were obtained. In addition, a new interface linking the chromatograph to the quadrupole mass spectrometer was developed. Effluent from the column was nebulized and introduced to a heated cyclone spray-chamber. Solvent was separated by means of both a Nafion membrane drier maintained at a temperature of 7°C and a cryogenic condenser. A total desolvation of about 89% was recorded. With this interface, solvent gradients used in the procedure resulted in minimal changes in the baseline of the ICP-MS. HPLC conditions are shown in Table 3, Ref. [19]. In another report on analyses of biotranformation products [20], a new HPLC procedure was applied that was based upon a methanol/water gradient, as noted in Table 3, Ref. [20]. No desolvation of the effluent was necessary and this allowed a direct connection to be made from the chromatograph to the mass spectrometer. Limits of quantitation were 1–2 ng ml^{-1} and accuracies ranged from 89 to 103%.

3. Sample storage and stability

Special attention is necessary for preparation and storage of samples of blood, plasma, ultrafiltrates and urine in order to preserve the concentrations of the species intact for later analyses. Most workers have centrifuged blood samples within 1 h of collection. Blood samples have been stored at a temperature of 0°C prior to ultrafiltration. Ultrafiltrates were stored

at −10°C, and some at −70°C. A small quantity of monohydrated complex of cisplatin was formed when cisplatin was mixed with plasma at 37°C and pH 7.4 [21]. The half lives of cisplatin and its monohydrated complex were 1.43 and 0.05 h respectively in blood and 0.88 and 0.05 h respectively in plasma at 37°C. Urine samples were stored at −20°C.

Reports prior to 1995 on stabilities of aqueous solutions of cisplatin and carboplatin have been summarized [3]. Some subsequent studies are noted here. Hydrolysis products of carboplatin in 0.9% sodium chloride solution, and in 5% glucose infusion solution were separated using HPLC [22]. The conditions are listed in Table 3, Ref. [22]. The products were cisplatin and diamminechloro(1-carboxylato-1-carboxycyclobutane)platinum(II). After 168 h storage at 23°C, 10% degradation of the original carboplatin had occurred in the salt solution, and only 5% in the glucose medium. In another investigation [23], degradation products of carboplatin in water and in 5% glucose solution stored at 25°C were identified as 1,1-cyclobutanecarboxylate anion, its protonated forms and the dihydrated complex of cisplatin. The results of the two investigations disagree.

4. Conclusions

The problems of speciation in studies of five important platinum anticancer drugs have been addressed in a number of research reports over the period of 1995–1999. The principle method of separation of degradation, biotransformation and other reaction products has been HPLC. Knowledge of the species derived from anticancer drugs has assisted in studies of their activities during treatments of patients. The recognition of relationships of the species formed and toxic effects observed in drug therapies has been important.

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