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The integrity of cisplatin in aqueous and plasma ultrafiltrate media studied by ^{195}Pt and ^{15}N nuclear magnetic resonance

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Abstract ^{195}Pt and ^{15}N nuclear magnetic resonance (NMR) was used to study the chemical equilibria of cisplatin in water and plasma ultrafiltrate (PUF). Cisplatin was found to be stable for at least 2, but no longer than 5 months in a reconstituted clinical formulation, as determined by ^{195}Pt NMR. In aqueous solution, the *cis*-$PtCl_2(NH_3)_2$ ^{195}Pt and ^{15}N NMR signal intensities decreased with time and the formation of $[PtCl(H_2O)(NH_3)_2]^+$ at pH values of 3.0, 6.5, 7.5 and 9.5 was observed within 24 h of sample preparation. In addition, $[Pt(H_2O)_2(NH_3)_2]^{++}$ was observed at pH 3.0, and $[PtCl(OH)(NH_3)_2]$ and $[Pt(OH)_2(NH_3)_2]$ were observed at pHs 7.5 and 9.5. During incubation of PUF with cisplatin for 35 h, ^{15}N NMR signals for at least eight cisplatin derivatives appeared at different times, whereas only four were observed by ^{195}Pt NMR. With our NMR protocols, the detection limit for quantifiable cisplatin derivatives is estimated at 500 μM using ^{195}Pt NMR and $\leq$ 200 μM using ^{15}N NMR. In addition to providing useful information about the chemical stability of cisplatin and derivatives formed in aqueous solution, these magnetic resonance techniques, particularly ^{15}N NMR, can provide useful information about the metabolism of cisplatin in biological regimes.

Key words Nuclear magnetic resonance (NMR) · Cisplatin · Plasma ultrafiltrate

Introduction

Cis-Platinum(II)diammine dichloride, commonly referred to as cisplatin, is an important cancer chemotherapeutic agent which is particularly effective against testicular, ovarian and bladder cancer [16, 27]. Although it is associated with severe side effects (nephrotoxicity, neurotoxicity, ototoxicity, myelosuppression and emesis), and has limited activity against other major cancers, it is a principal chemotherapeutic in cancer clinics, where the doses administered are often limited by renal toxicity. A better understanding of the metabolism of cisplatin in humans could lead to the development of strategies to minimize its profound toxic side effects. Although the mechanisms of cisplatin-induced toxicity are not well known, it has been suggested that different metabolites of the drug may be responsible for its toxic and antitumor effects [11, 20].

Nuclear magnetic resonance (NMR), also referred to as magnetic resonance spectroscopy (MRS), is a technique traditionally used by chemists for the identification and structural elucidation of compounds, and investigations of their chemical equilibria in solution [28]. The main advantage of the technique is the ability to detect directly substances and their biochemical evolution *in situ*, without altering or destroying them. Furthermore, every substance giving rise to a signal can usually be detected simultaneously. It is also possible to study effects of substances by monitoring the NMR signals of host biosystems such as biofluids and tissue extracts (*in vitro* NMR), live cells, tissues and organs (*ex vivo* NMR), or whole living animals and humans (*in vivo* NMR) [9, 10]. Although NMR is currently applied in pharmacological studies [for reviews see 8, 9, 17, 21, 25], its potential is still not widely exploited, particularly with regards to direct monitoring of drugs or drug effects in host systems *in vivo*. The detection and identification of cisplatin and its derivatives, as well as the interactions of these square planar Pt(II) complexes with relevant biomolecules, has been studied in aqueous environments by ^{1}H, ^{13}C, ^{31}P, ^{15}N, and ^{195}Pt NMR [3, 5, 6, 7, 14, 15, 19, 23, 29, 30]. ^{195}Pt NMR has also been used to study the human metabolism of iproplatinum, an octahedral Pt(IV) complex related to

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cisplatin [24]. Significant NMR studies of cisplatin in biological media have involved human blood plasma (^{14}N NMR [22]), cells (^{1}H [2] and ^{15}N [4] NMR), hypopharynx tumors (^{31}P NMR [31]), and mouse kidney (^{1}H NMR [26]). The objective of this study is to assess the sensitivity and utility of ^{195}Pt and ^{15}N NMR for investigating the reactions of cisplatin in water and plasma ultrafiltrate (PUF), and represents a step towards gaining a better understanding of the metabolism of cisplatin *in vivo*.

Materials and methods

The cisplatin clinical formulation was obtained as a dry powder (Bristol-Myers) and reconstituted according to the instructions provided by the manufacturer with $^{2}H_2O$ (10–20%)/$^{1}H_2O$. For every ml of solution, the clinical preparation contained 1 mg of cisplatin, 10 mg of mannitol, and 9 mg of sodium chloride. *cis*-Platinum(II)diammine dichloride (Sigma Chemical) solutions were prepared by adding 1 mg/ml (3.3 mM) of cisplatin in $^{2}H_2O$(10–20%)/$^{1}H_2O$. The pH values of some samples were adjusted to 3.0, 7.5, and 9.5 by adding NaOH or HCl and were not corrected for the small isotopic effect of deuterium in $^{2}H_2O$ (≤ 0.1 pH unit). Blood plasma from three human subjects was obtained from the Ottawa Civic Hospital Blood Bank, and ultrafiltered with Amicon membrane filters (25-kDa cut-off) while centrifuging at 4500 rpm for 20 min at ambient temperature. The PUF (pH = 7.4) was kept at 4°C until the addition of crystalline cisplatin (3.3 mM) or NH_4Cl (40 mM), and $^{2}H_2O$ (5–10% volume), approximately 1 h before examination by NMR. All solutions were protected from light and stored at room temperature between NMR experiments, in order to reduce the risk of cisplatin degradation and precipitation, respectively . ^{15}N-labelled cisplatin was prepared with $^{15}NH_4Cl$ (99.3 atom%; Isotec) according to the method described in Boreham et al. [6].

^{195}Pt and ^{15}N NMR spectra were acquired at 37°C on a Bruker AMX-500 spectrometer using a broad band frequency probe with 10-mm external diameter specimen tubes (total sample volume approximately 3 ml). Typical conditions for ^{195}Pt NMR at 107.28 MHz were: number of scans (NS) = 40 000, 90° pulse $\sim$ 30 μs, acquisition time (AT) = 0.1 s, relaxation delay (RD) = 0.1 s (total experiment time approximately 2.2 h). The atypically large ^{195}Pt chemical shift range could not be sampled in a single experiment due to instrument limitations, and specific spectral regions (SR) were individually scanned (-1000 to -1450, -1450 to -1950, -1950 to -2400, and -2400 to -2900 ppm). The ^{195}Pt chemical shifts were referenced to 0.5 M H_2PtCl_6 in $^{2}H_2O$. The ^{15}N NMR spectra were acquired at 50.68 MHz using a DEPT45 [12, 28] pulse sequence (J of ^{1}H-^{15}N set to 73 Hz [3, 23]), with and without ^{1}H decoupling, where typically NS = 3800, SR = + 20 to -120 ppm, 90° pulse $\sim$ 30 μs, AT = 2.5 s, and RD = 2.7 s (total experiment time approximately 5.5 h). The ^{15}N chemical shifts were referenced to 1.5 M $^{15}NH_4Cl$ in 1 M HCl (10% $^{2}H_2O$). Line-broadening factors of 200 and 2 Hz were applied to the ^{195}Pt and ^{15}N NMR spectra shown in the figures, respectively. The times reported refer to the interval between sample preparation and the end of the NMR experiment.

Results

Clinical formulation of cisplatin

Figure 1 shows four ^{195}Pt NMR spectra of a clinical formulation of cisplatin obtained at the times indicated,

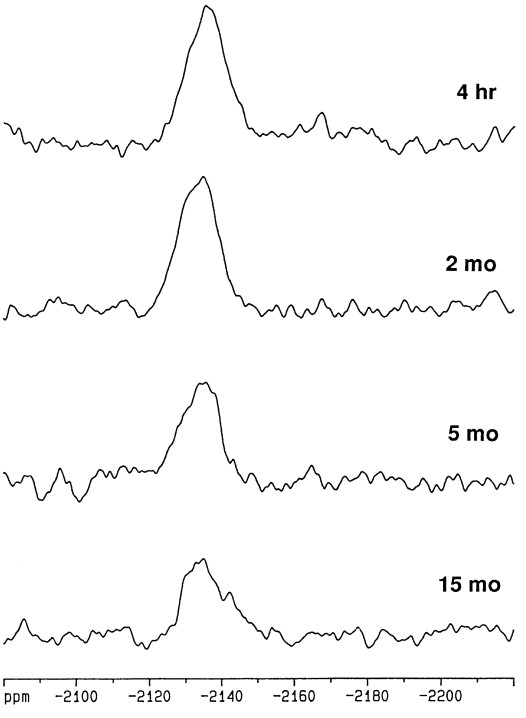

Fig. 1 ^{195}Pt NMR spectra (107 MHz, number of scans = 40 000, 37°C) of a clinical formulation of cisplatin at various times after preparation. The peak at -2135 ppm is attributed to native cisplatin

where the peak at -2135 ppm is attributed to native cisplatin [6, 15]. A reduction in signal intensity, and therefore cisplatin concentration, is observed 5 months after sample preparation. The decrease in signal intensity reflects the decomposition of the parent compound yielding other platinum derivatives and/or a slight precipitate of metallic platinum. A further reduction of the concentration of cisplatin in the clinical formulation is observed after 15 months of sample preparation. This experiment indicates that cisplatin is stable when stored in the dark at room temperature for at least 2, but no more than 5 months after reconstitution of the cisplatin clinical formulation. It was noted that the expiry date of the dry powder fell within this period.

Cisplatin in aqueous solution

Figure 2a shows three ^{195}Pt NMR spectra of crystalline cisplatin dissolved in water (1 mg/ml, pH = 6.5) obtained 4 h, 11 days, and 16 days after sample preparation. The same sample also yielded the spectra shown in Fig. 2b, obtained from another ^{195}Pt spectral region. The signal at -2135 ppm (Fig. 2a) is attributed to native cisplatin, $PtCl_2(NH_3)_2$, whereas that at -1825 ppm (Fig. 2b) is attributed to the derivative $[PtCl(H_2O)(NH_3)_2]^+$ [6, 15]. The signal intensity at -2135 ppm decreased significantly 16 days after sample preparation, whereas a parallel increase was not

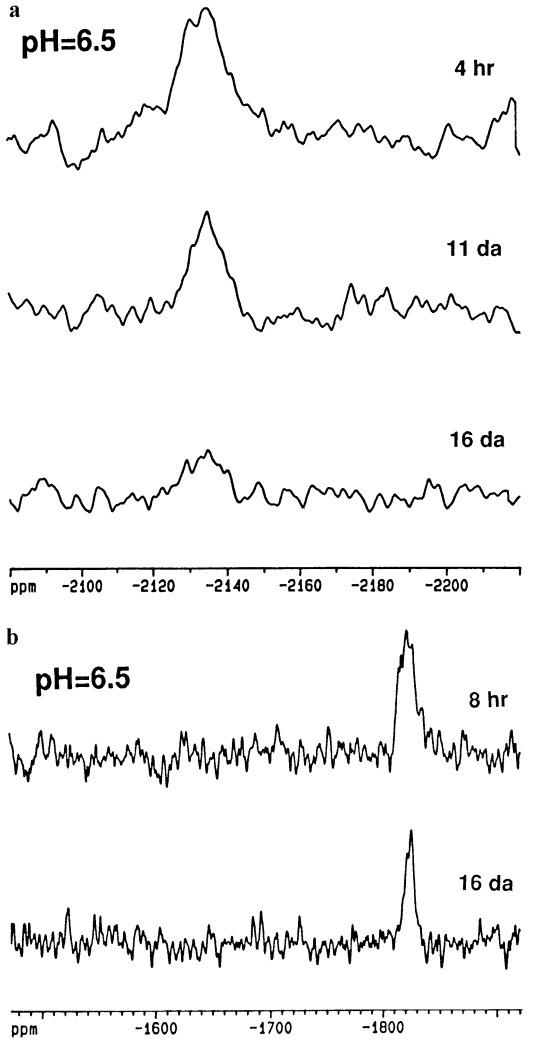

Fig. 2a, b ^{195}Pt NMR spectra (107 MHz, pH = 6.5, number of scans = 40 000, 37°C) of aqueous cisplatin at various times after preparation. **a** Native cisplatin spectral region; **b** cisplatin derivative spectral region. The peak at − 1825 ppm is attributed to $[PtCl(H_2O)(NH_3)_2]^+$

observed at − 1825 ppm. The latter result shows that the equilibrium between $PtCl_2(NH_3)_2$ and $[PtCl(H_2O)(NH_3)_2]^+$ is established rapidly, and suggests that the loss of the cisplatin signal is due, in part, to the formation of other undetected cisplatin derivatives and/or a precipitate of metallic platinum. No additional ^{195}Pt resonances were observed at pH 7.5 or 9.5, for times as long as 1 and 3 days after sample preparation, respectively. At pH = 3.0, an additional weak signal at − 1740 ppm was observed after a longer ^{195}Pt NMR experiment (11 h). This signal is most likely due to $[Pt(H_2O)_2(NH_3)_2]^{++}$, which is present as a consequence of acid hydrolysis or aquation of the chloride ligands in acid media [5, 18]. Our assignment of this resonance does not, however, agree with previously reported values of approximately − 1590 ppm for $[Pt(H_2O)_2(NH_3)_2]^{++}$ [6, 15].

Aqueous ^{15}N-labelled cisplatin solutions gave rise to ^{195}Pt NMR signals with the same chemical shifts (within a few parts per million) as their unlabelled counterparts described above; however, the signals were split into multiplets due to spin-spin coupling between the ^{195}Pt and adjacent ^{15}N nuclei (spectra not shown).All the aqueous solutions gave rise to ^{15}N NMR signals (both ^{1}H-coupled and ^{1}H-decoupled) from cisplatin at − 68.8 ppm and $[PtCl(H_2O)(NH_3)_2]^+$ at − 66.8 and − 89.8 ppm within a few hours of sample preparation (spectra not shown). After 24 h, the ^{15}N peak intensity of native cisplatin aqueous solution (pH = 7.5, 37°C) was still greater than that of the two ^{15}N peaks due to $[PtCl(H_2O)(NH_3)_2]^+$, and peaks at − 63.7 and − 85.4 ppm, assigned to $[PtCl(OH)(NH_3)_2]^+$, and − 83.9 ppm, assigned to $[Pt(OH)_2(NH_3)_2]$, were observed. After 24 h at pH 9.5, the dominant ^{15}N peak was at − 83.9 ppm, and evidence for the formation of dimeric/trimeric cisplatin-hydroxo derivatives [6, 18] was observed (small signals at − 80.7, − 81.3, and − 81.9 ppm). The ^{195}Pt-^{15}N and ^{1}H-^{15}N coupling constants measured (J = 320–370 and 73 Hz, respectively) are consistent with previously published values [1, 3, 6, 15]. The cisplatin species observed by ^{195}Pt and ^{15}N NMR in water are listed in Table 1.

Cisplatin in PUF

Figure 3 shows four ^{1}H-decoupled ^{15}N NMR spectra of PUF from one subject obtained consecutively up to 26 h after the addition of 1 mg/ml of ^{15}N-labelled cisplatin. All the peaks observed were located between − 65 and − 90 ppm and are listed in Table 1. The signal at − 68.6 ppm (A), assigned to the parent cisplatin, decreases as a function of time, concurrent with the appearance of several derivatives labelled B–M. Peaks B (− 67.7 ppm) and C (− 81.2 ppm) are observed within 2 h of sample preparation (spectrum not shown), reach a maximum intensity sometime between 0 and 8 h, and then decay as other signals appear. Upon degradation of cisplatin, unless both chloride ions have been replaced by identical ligands, each derivative formed will give rise to two resonances of equal intensity. The concurrent appearance of two prominent peaks at − 79.2 and − 83.0 ppm (labelled D) thus suggests that these signals originate from two NH_3 ligands in one cisplatin derivative with two other differing ligands. The peaks labelled E-H (at − 69.0, − 76.0, − 80.6 and − 86.0 ppm) become distinct simultaneously (14 h) and have similar intensities; therefore, any two of the signals could originate from the same derivative. The same condition applies to the peaks labelled I and J (− 79.8 and − 85.1 ppm) and K-M (− 69.5, − 78.1, and − 85.1 ppm). Thus, after incubating PUF with cisplatin for 26 h, at least nine cisplatin species are observed. The corresponding ^{15}N NMR spectrum of neat PUF did not display any signals (spectrum not

Table 1. ^{195}Pt and ^{15}N NMR chemical shifts of cisplatin species

Medium	^{195}Pt (ppm)	^{15}N (ppm)	Assignment[a]
Water (pH = 9.5, 7.5, 6.5)	− 2135	− 68.8[a]	$[PtCl_2(NH_3)_2]$
	− 1825	− 66.8[b], − 89.8[c]	$[PtCl(H_2O)(NH_3)_2]^+$
Water (pH = 7.5, 9.5)		− 63.7[b], − 85.4[c]	$[PtCl(OH)(NH_3)_2]$
		− 83.9	$[Pt(OH)_2(NH_3)_2]$
Water (pH = 3.0)	− 2135	− 68.8	$[PtCl_2(NH_3)_2]$
	− 1825	− 66.8[b], − 89.8[c]	$[PtCl(H_2O)(NH_3)_2]^+$
	− 1740		$[Pt(H_2O)_2(NH_3)_2]^{++}$
Plasma ultrafiltrate	− 2135	− 68.6 (A)[d]	$[PtCl_2(NH_3)_2]$
	− 1810, 1715		U[e]
	− 1645, − 1580		U
		− 67.7 (B), − 81.2 (C)	U
		− 79.2 (D), − 83.0 (D)	U
		− 69.0 (E), − 76.0 (F)	U
		− 80.6 (G), − 86.0 (H)	U
		− 79.8 (I), − 85.1 (J)	U
		− 69.5 (K), − 78.1 (L)	U
		− 85.1 (M)	U

[a] assignments based on those in references 1, 4, and 14
[b] ^{15}N *trans* to Cl
[c] ^{15}N *cis* to Cl
[d] labels as in Fig. 3
[e] Unassigned

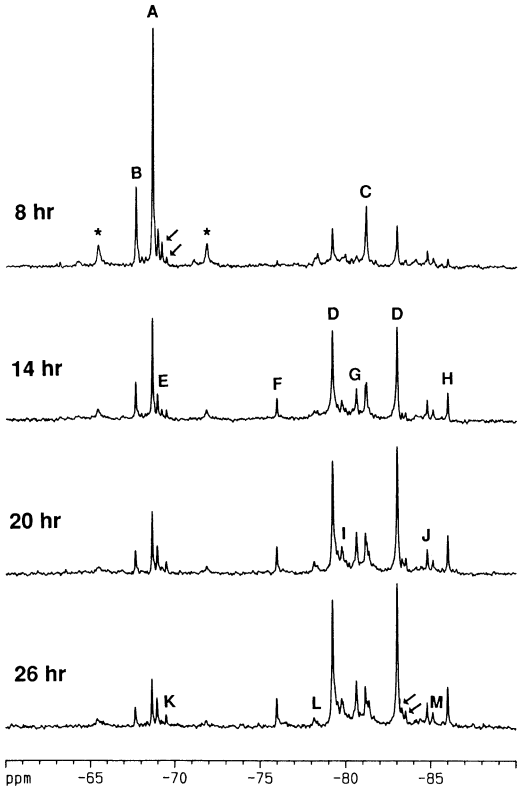

Fig. 3. ^{1}H-decoupled ^{15}N NMR distortionless enhancement by polarization transfer (DEPT) spectra of plasma ultrafiltrate incubated with ^{15}N-labelled cisplatin (51 MHz, number of scans = 3800, 37°C). Peak A is attributed to native cisplatin while peaks B–M remain unassigned. The peaks denoted with asterisks are ^{195}Pt satellites of the peak A [^{195}Pt-^{15}N coupling constant (J) = 327 Hz] and the peaks denoted with *arrows* are chemically shifted due to the small amount of ^{2}H (from $^{2}H_2O$) which is chemically exchanged with ^{1}H in some NH_3 groups

shown). Following the ^{15}N experiments, a very weak residual ^{195}Pt NMR signal is observed at − 2135 ppm, confirming the presence of a residual amount of parent cisplatin after 35 h of incubation at 37°C. A relatively strong ^{195}Pt NMR signal is observed at − 1715 ppm and there is evidence for weak signals at − 1580, − 1645, and − 1810 ppm (spectra not shown). Thus, in the region − 1000 to − 2900 ppm, four cisplatin derivatives are detected in PUF by ^{195}Pt NMR. The rapid initial formation of cisplatin derivatives B and C, and all the ^{15}N signals listed in the plasma ultrafiltrate section of Table 1, was also observed in PUF from two other subjects incubated with cisplatin. $^{15}NH_4Cl$(40 mM) was added to one sample of PUF and the ^{15}N NMR spectra were obtained under the same conditions as those in Fig. 3. Except for free NH_4^+ observed initially near 0 ppm, no ^{15}N peaks were observed within 26 h of sample preparation.

Discussion

^{195}Pt NMR is sensitive to ligand coordination of platinum, the central atom in cisplatin, and each ^{195}Pt NMR signal observed can be attributed to a different cisplatin derivative. The platinum Pt(II) or Pt(IV) valency of a derivative may be determined from its chemical shift value [15]. ^{15}N isotopic labelling of cisplatin allows the use of ^{15}N NMR, which normally is very insensitive because of a very low natural abundance of this isotope. Furthermore, application of the DEPT (distortionless enhancement by polarization transfer) pulse sequence results in an enhancement of the ^{15}N signal due to polarization transfer from the neighboring

NMR-sensitive protons [12, 28]. Since NMR signal areas are directly related to the concentrations of the corresponding species, it is possible to gain insight into their stabilities and kinetic behavior by obtaining spectra at different time intervals. We have used ^{195}Pt and ^{15}N NMR to study the chemical equilibria of cisplatin (3.3 mM) in three different environments: clinical formulation, water, and PUF. The results are discussed with respect to: (1) the cisplatin derivatives observed: (2) cisplatin stability; and (3) the overall sensitivity and utility of ^{195}Pt and ^{15}N NMR for the study of cisplatin under physiologically relevant conditions.

Cisplatin derivatives

The clinical formulation of cisplatin gave rise to a ^{195}Pt NMR signal at -2135 ppm, assigned to native cisplatin. By monitoring this signal as a function of time, as shown in Fig. 1, we find that cisplatin is stable for at least 2, but no longer than 5 months. This is consistent with data from an HPLC assay, where the concentration of cisplatin did not change within a 56-day period (Bristol-Myers, personal communication to R. Goel). A more precise ^{195}Pt NMR determination of cisplatin stability in the clinical formulation could be achieved by increasing the length of each NMR experiment, in order to increase the signal to noise ratio, and decreasing the time interval between each experiment. It seems plausible that this ^{195}Pt NMR result reflects the actual shelf-life of the reconstituted drug since NaCl and mannitol, also present in the clinical formulation, are likely more stable than the metal-containing cisplatin.

In water, we detected a few platinum-containing species by ^{195}Pt NMR and ^{15}N NMR. The parent cisplatin, $[PtCl_2(NH_3)_2]$, and the cisplatin aquated derivative $[PtCl(H_2O)(NH_3)_2]^+$ ($pK_a = 6.41$; [5]) were observed at pH values of 3.0, 6.5, 7.5, and 9.5. At pH 3.0, a signal most likely due to $[Pt(H_2O)_2(NH_3)_2]^{++}$, formed as a consequence of acid hydrolysis of the chloride ligands in acid media ($pK_a = 5.37$ and 7.21; [5]), was observed [18]. At pH values of 7.5 and 9.5, signals attributed to the hydroxo cisplatin derivatives $[PtCl(OH)(NH_3)_2]$ and $[Pt(OH)_2(NH_3)_2]$ were observed and evidence for the formation of polymeric cisplatin-hydroxo derivatives was found at pH 9.5 [6, 18]. In a previous study, several aqueous and hydroxo cisplatin derivatives were observed by both ^{195}Pt or ^{15}N NMR [6]; however, the medium was not identical to ours and the concentrations of cisplatin were much higher (0.1–1 M nitrate salts of platinum complexes); this also explains the slight differences in the reported ^{15}N and ^{195}Pt chemical shifts of the cisplatin derivatives. If other cisplatin derivatives were present in our aqueous solutions, their concentrations were below the detection limit of our ^{195}Pt and ^{15}N NMR protocols.

Several NMR signals were observed in PUF incubated with cisplatin for over 1 day, of which only the parent cisplatin peak was readily assignable based on previous ^{195}Pt and ^{15}N NMR studies. We detected at least eight cisplatin derivatives by ^{15}N NMR, whereas evidence for only four was found by ^{195}Pt NMR. There are three observations that suggest the ^{15}N NMR signals observed after incubating PUF with cisplatin originate from ^{15}N directly bonded to Pt(II): (1) the actual chemical shifts of the ^{15}N resonances fall between -65 and -87 ppm, completely characteristic of ^{15}N bound to Pt(II) in cisplatin-like species [1, 6, 15], (2) an equivalent ^{15}N NMR spectrum of neat PUF did not contain any resonances, and (3) incubation of PUF with $^{15}NH_4Cl$ (40 mM) did not give rise to any peaks which might have been attributed to species derived from free ammonia released from cisplatin. In this latter experiment, free $^{15}NH_4^+$ was detected near 0 ppm at the onset, but after 26 h of incubation, no signals were observed. Therefore, signals originating from $^{15}NH_4^+$ in PUF are either outside the 20 to -120 ppm region examined or broadened beyond detection due to binding with macromolecules. The ^{15}N resonances observed in PUF are presently unassigned, however, some clues about their origin are obtained in considering that ^{15}N shifts of amines bonded to Pt(II) appear to be determined largely by the nature of the *trans* ligand [1, 15]. For instance, the resonance at -67.7 ppm (labelled B in Fig. 3), may be due to ^{15}N *trans* to Cl^- in a cisplatin derivative [1, 15]. Peaks D, at -79.2 and -83.0 ppm, most likely originate from a cisplatin species with two NH_3 ligands and two others which are not the same and not Cl^-. Peaks E and K may be due to ^{15}N trans to Cl^- or NH_3, while the peaks between -75 and -87 ppm may be due to $^{15}NH_3$ *trans* to H_2O, OH^- or COO^- [1, 15]. Some signals are potentially due to cisplatin derivatives involved with methionine [11, 22, 23], glutathione [3, 4, 20] or cysteine [20]. At least seven unknown cisplatin derivatives were observed by HPLC in ultrafiltered plasma obtained from rats injected with cisplatin [11]. The same derivatives were also observed when cisplatin was incubated in rat plasma, subsequently ultrafiltered [11].

Cisplatin stability

The slower decomposition of cisplatin in the clinical formulation than in pure water, as monitored by ^{195}Pt NMR, can be explained by the presence of Cl^- (from NaCl) in the clinical formulation, which inhibits hydrolysis of the parent compound. In pure water at pH values 3.0, 6.5, and 7.5, both the ^{195}Pt and ^{15}N NMR results indicate that the aquated cisplatin derivative $[PtCl(H_2O)(NH_3)_2]^+$ is formed within a few hours of sample preparation, and significant native cisplatin signals are still observed 1 and several days after sample preparation, respectively. At pH 9.5, cisplatin

was significantly degraded after 26 h of incubation at 37°C and, in PUF, disappeared almost completely by 35 h, as evidenced by ^{15}N and ^{195}Pt NMR. In PUF, the first cisplatin derivatives observed, labelled B and C in Fig. 3, reached a maximum level sometime between 0 and 8 h of incubation. These derivatives subsequently decomposed, as noted by a decrease in their peak intensities, while the heights of several other peaks, labelled D–M in Fig. 3, increased. This suggests that some or all of the derivatives D–M originate directly from B or C, and are thus involved in three-species (or more) equilibria with native cisplatin.

Sensitivity/utility of ^{195}Pt and ^{15}N NMR under physiologically relevant conditions

The signal to noise ratio of the NMR signal depends primarily on the magnetic field strength (typically 4.7–11.7 Tesla for *in vitro* NMR), the concentration of the observed nuclei, the length of experiment time, and intrinsic nuclear properties. Our results were obtained at a relatively high field (11.7 Tesla), an overall concentration of platinum equal to 3.3 mM (the concentration of cisplatin in the clinical formulation), and typical NMR acquisition times of 2.2 and 5.5 h for ^{195}Pt and ^{15}N NMR, respectively. By a qualitative examination of Fig. 1, where the initial spectrum of the clinical formulation reflects 3.3 mM cisplatin, the ^{195}Pt NMR limit of detection can be estimated at 500 μM. Since ^{15}N-labelled cisplatin degraded in all the solutions studied, none of our ^{15}N resonances represent a known concentration of species. Nevertheless, based on ^{15}N NMR spectra acquired at times less than 8 h, it can be determined that peak A at 8 h in Fig. 3 reflects less than half of the initial cisplatin concentration of 3.3 mM and, therefore, the ^{15}N NMR limit of detection of quantifiable cisplatin derivatives may be estimated at 200 μM or less. Cisplatin species with a significantly lower concentration than the estimated limits above might be detected, but species giving rise to those peaks may not be quantifiable. Unless extremely long NMR times are envisaged, it is unlikely that ^{195}Pt or ^{15}N NMR would be useful in quantitatively monitoring the relatively low physiological concentrations of cisplatin with current NMR technology; with clinical doses of 100 mg/m2, the peak concentration of cisplatin in humans reaches 30–35 μM [13]. Nevertheless, ^{195}Pt and ^{15}N NMR can provide important information on the stability and pharmacokinetics of cisplatin, as well as on the identity of the derivatives formed in biological media. We conclude that ^{15}N NMR is more sensitive than ^{195}Pt NMR for the study of cisplatin in biological media, partly due to the enhancement of the ^{15}N NMR signals achieved by using the DEPT pulse sequence. Furthermore, since the whole ^{15}N spectral region relevant to cisplatin derivatives can be observed at once, ^{15}N NMR is less time-consuming than ^{195}Pt NMR.

This study of cisplatin equilibria in plasma ultrafiltrate provides a basis for further understanding the metabolism of cisplatin *in vivo*. Future studies will consist of identifying the cisplatin derivatives formed in biological media, and a detailed monitoring of their pharmacokinetics. The relationships between the cisplatin derivatives and the toxic side effects of cisplatin, will also be explored.

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