Cisplatin Carbonato Complexes. Implications for Uptake, Antitumor Properties, and Toxicity

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Cisplatin is a potent cytotoxic agent used for the treatment of ovarian and testicular cancer and other malignancies. The cytotoxicity of cisplatin is thought to be related to its ability to modify cellular DNA. In plasma, where the chloride concentration is ~105 mM, cisplatin is believed to partially solvate to its aquated forms. Since aquated complexes can react with components present in blood and the cytosol, they may be important for the antitumor effects, resistance, and toxicity associated with cisplatin.

Earlier, we used HSQC NMR and low concentrations of 15N-labeled cisplatin, cis-Pt(15NH3)2Cl2 (1, Figure 1), to identify the platinum species in culture media (RPMI + 10% fetal bovine serum) in the presence and absence of Jurkat cells. We interpreted the NMR measurements as showing that 1 hydrolyzes to the monoaquo complex, 2, which is in equilibrium with the monohydrxo species, 3, as is known to occur in aqueous solution. We found that, with more than 5 × 10^6 cells present, 2 had disappeared by 0.5 h, the earliest time for which NMR measurements could be made. In the absence of cells, however, the NMR signal ascribed to 2 persisted to >20 h, which was unexpected because aquated species should react more quickly than 1 with components in the medium. To identify species having NMR signatures similar to that of 2 but with reduced reactivity, we studied the reaction of 1 in carbonate buffer and in culture media with and without carbonate ion. In this report, we show that, in the presence of carbonate, 2 reacts to form the carbonato complex, 4, which is the long-lived species observed in the cell culture studies. At long times in carbonate buffer, 4 reacts to form 5. Since carbonate is present in blood at high concentrations and it may react with Pt(II) species, the species present in the earlier reported cell culture studies is not 2 but, rather, the carbonato complex, 4.

The HSQC NMR spectrum of 65 μM cisplatin, in carbonate buffer, is shown in Figure 2. The peak for N trans to O of compound 4 is at 1H/15N, δ = 3.61/~80.5, while the peak for N trans to Cl of the compound is under the peak for 1. The peak for N trans to O of 4 disappears with time, and a new compound forms, having a peak at 1H/15N, δ = 3.70/~81.8. This has been assigned to the biscarbonato complex 5. The HSQC NMR data for 4 and 5 are consistent with assignments made for similar compounds. It is observed that the corresponding bidentate carbonate in cis-bistriethylphosphine carbonato platinum(II) and at ~4 ppm higher field than that of a bridging carbonate in a dinuclear complex of Zn2+. By analysis of UV absorption data, it was determined that compound 5 has a d–d absorption band at 268 nm, which is shifted to higher energy relative to the main d–d band for 1. Since the crystal field strength of CO3^2− is greater than that of Cl−, this shift to higher energy for 5 is expected.

Comounds 1 and 5 each have a single HSQC NMR peak, but 4 has two equal peaks, one overlapping the peak for 1. The true intensity of 1 is obtained by subtracting the apparent intensity of 4 from the apparent intensity of 1, and the true intensity of 4 is double the apparent intensity of 4. These true intensities are proportional to species concentrations, so, given the total Pt concentration, one can convert intensities to concentrations, giving the points of Figure 3. The curves in Figure 3 are best-fit curves for the reaction scheme shown in Figure 1. The rate constants for conversion of 1 to 2 and

**Figure 1.** Reaction of cisplatin with carbonate.

**Figure 2.** 1H−15N HSQC NMR spectrum of 65 μM 1 in 5 mM HCO3−, [Cl−] = 3.3 mM, 37° C, t = 11.6 h.
5. Since the carbonate concentrations in culture media, plasma, and the cytosol are similar, platinum carbonato complexes may be present in therapy, and they could be the species reacting with components in the cell. Despite the large body of work on cisplatin and its analogues, little attention has been paid to the potential role of carbonate ion in these reactions.14 Hopefully, this report will stimulate new inquiry on the role of carbonate in the mechanism of action of platinum anticancer drugs.

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Supporting Information Available: Absorption data and analysis, the kinetic model for the scheme shown in Figure 1, a 13C NMR spectrum of 5 in carbonate solution, and an HSQC NMR spectrum showing peaks for 4. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(6) Carbonate buffer at neutral pH contains an equilibrium mixture of CO2, HCO3−, and CO32−. The rate constants for conversion of 4 to 5 (by reaction with carbonate) and the reverse are parameters in the model. The fit is excellent, confirming that, in 5 mM carbonate buffer, 1 disappears to form 4, which then yields 5. In 0.5 mM carbonate, the conversion of 1 to 4 occurs at the same rate as that in 5 mM carbonate, but 5 is formed much less rapidly. This shows that the rate of conversion of 4 to 5 is carbonate-dependent.

Figure 3. Concentrations of 1 (blue, squares), 4 (red, triangles), and 5 (brown, circles) from NMR measurements, with fits to the model of Figure 1 (smooth curves).

Although 5 is observed in carbonate buffer, it is not observed in culture media. This may be due to the high chloride content of the medium which suppresses the formation of 5 and/or rapid reaction of the compound with the many possible nucleophiles present in the medium. Low concentrations of the compound would be more difficult to detect with HSQC NMR.

It is known that hydrating patients with hypertonic saline solutions reduces the renal toxicity of cisplatin.6,10 Since increased chloride concentration in blood would shift the equilibria in Figure 1 to the left, reducing the concentration of carbonate species, it may be that the latter are, in part, responsible for some of the toxic effects observed with cisplatin. However, work with relevant renal cells will be necessary in order to show if this is the case.

The fate of 4, which quickly disappears from the HSQC NMR spectrum when Jurkat cells are present, is unknown.5 However, it is important to note that the heavy metal ion Cd2+ forms carbonate complexes which are taken up by cells and that anion exchangers found in the plasma membrane may be involved in its transport.12 Work in progress will determine if a similar uptake mechanism is involved with carbonate complexes of cisplatin.

In this work, we use low concentrations of 113 to show that the earlier detected cisplatin metabolite that forms in culture media, and is taken up/modified by cells, is the carbonato complex. In carbonate buffer, 4 slowly reacts to form the biscardinato complex,