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Inorganica Chimica Acta 389 (2012) 29–35

Contents lists available at SciVerse ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Review

Understanding how the platinum anticancer drug carboplatin works: From the bottle to the cell

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ARTICLE INFO

Article history:
Available online 24 January 2012

Dedicated to Prof. Jon Zubieta.

Keywords:
Carboplatin
Self-association
Mechanism of action
Carbonate

ABSTRACT

Carboplatin, a platinum anticancer drug used to treat many types of human cancer, contains a bidentate dicarboxylate chelate leaving ligand, a structural feature that makes it much less chemically reactive than the first-generation platinum anticancer drug cisplatin, which contains two monodentate chloride leaving ligands. In water, carboplatin exists in a monomer–dimer equilibrium with an association constant of $K\ (M^{-1}) \approx 391$, a property that accounts for the long-term stability of its ready-to-use infusion solution. When administered in the clinic, carboplatin is believed to exert its biological effects by interacting with genomic DNA and proteins. The slower substitution kinetics of carboplatin, compared to cisplatin, has prompted investigators to focus on mechanisms by which the compound can be activated *in vivo*. Carbonate, which is in equilibrium with hydrogen carbonate, carbonic acid, and dissolved carbon dioxide, is ubiquitous in biological systems, and is found in high concentrations in the blood, the interstitial fluid, and the cytosol. Activation of carboplatin by carbonate, CO_3^{2-} ($k_1 = 2.04 \pm 0.81 \times 10^{-6}$ in 24 mM carbonate buffer, pH 7.5 at 37 °C), for example, leads to the formation of platinum species that are more cytotoxic than the parent drug. This short review focuses on the reason for the unusual stability of carboplatin in its aqueous ready-to-use infusion solution, describes the reactions of the drug with biologically common nucleophiles and summarizes the activation chemistry that make the drug more reactive toward substances present in the biological system.

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0020-1693/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ica.2012.01.028

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James C. Dabrowiak *has been on the faculty of Syracuse University since 1972, where he is now a Professor of Chemistry. He was born in South Bend, IN, and received his B.S. from Purdue University, his M.S. and Ph.D. from Western Michigan University under the direction of D. W. Cooke and post doctorial study at The Ohio State University with D. H. Busch. In 1985 he was an American Cancer Society Scholar at Roswell Park Cancer Institute, Buffalo, NY and the Max Planck Institute for Biophysical Chemistry, Göttingen, Germany. He is the author of the textbook, Metals in Medicine, which address the chemistry, biochemistry and pharmacology of metal containing agents for detecting and treating disease. He has investigated the mechanism of action of a wide range of anticancer drugs, including bleomycin, daunorubicin, cisplatin, carboplatin, and oxaliplatin. Along with Prof. J. Goodisman, he developed quantitative footprinting analysis for studying drug-DNA interactions.*

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1. Introduction

Carboplatin, *cis*-diammine(cyclobutane-1,1-dicarboxylato)platinum(II), Fig. 1, is a platinum anticancer drug used for treating many types of human cancer [1–5]. While details of its molecular mechanism of action are not completely known, carboplatin is believed to exert its biological effects by interacting with cellular targets such as genomic DNA, tubulin, and other proteins [6–8]. Carboplatin was developed by Barnett Rosenberg and colleagues in the early 1970s to improve upon the clinical performance of the first-generation platinum anticancer drug cisplatin, *cis*-diamminedichloroplatinum(II), Fig. 1 [6,9]. Since carboplatin was found to be much less oto-, neuro-, and nephrotoxic than cisplatin [1,6,10], it was ultimately approved as a drug in 1989 under the brand name Paraplatin.

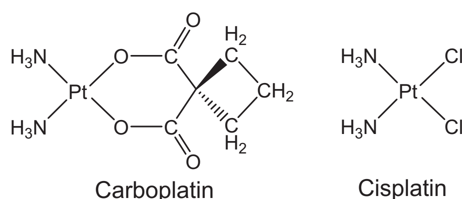


Fig. 1. Chemical structures of carboplatin and cisplatin.

Carboplatin contains a bidentate dicarboxylate chelate leaving ligand, a structural feature that makes the compound much less chemically reactive than cisplatin [10]. The pseudo-first-order rate constant, k_1, for the reaction of carboplatin with water, a reaction that involves the displacement of one "arm" of the cyclobutane-1,1-dicarboxylate (CBDCA) chelate ring from the Pt^{2+} ion by a water molecule, spans an unusually wide range, with values of k_1 ranging from approximately $5 \times 10^{-7}\ s^{-1}$ to less than $10^{-9}\ s^{-1}$ at 37 °C [11–14]. Although there is also discrepancy in the reported rate constant for the displacement of one of the chloro ligands of cisplatin by water [12,15], the fact that k_1 for the hydrolysis of carboplatin is about two orders of magnitude smaller than the corresponding hydrolysis rate for cisplatin has prompted investigators to focus on mechanisms by which carboplatin may be activated in chemotherapy. This short review focuses on the reason for the unusual stability of carboplatin in its aqueous ready-to-use infusion solution, describes the reactions of the drug with biologically common nucleophiles and summarizes the activation chemistry that make the drug more reactive toward substances present in the biological system.

2. Self-association of carboplatin in its ready-to-use infusion solution

The ready-to-use infusion solution of carboplatin contains 10 mg mL^{-1} (approximately 27 mM) platinum complex dissolved in water alone, water containing mannitol or dextrose, or water

containing a small amount of the free CBDCA [16,17]. When the drug is administered to the patient in the clinic, the ready-to-use infusion solution is diluted in 5% aqueous dextrose or glucose to the appropriate therapeutic concentration, and the resulting solution is infused into the blood of the patient. A striking feature of the ready-to-use infusion solution is that the formulation is quite stable, with a shelf-life of 2 years [16]. While some degradation occurs during this period, it is relatively modest and independent of excess CBDCA ligand that is present in some formulations [17]. Analysis shows that the degradation products of carboplatin are ring-opened structures and hydroxo/aqua species not having the CBDCA ligand. Although the hydrolysis rate constant for carboplatin is easy to measure, the values in the literature span an unusually large range suggesting that the conditions under which the rate measurements were made, especially the total concentration of drug in solution, may influence the rate with which carboplatin reacts with water.

Earlier, we used 110 μM ^{15}N-labeled carboplatin and [^{1}H, ^{15}N] HSQC NMR to obtain a hydrolysis rate constant for carboplatin of $k_1 \approx 5 \times 10^{-7}\ s^{-1}$ (37 °C) [11], which agreed very well with the rate constant obtained by Brandšteterová et al. at the same temperature using a 300 μM solution of carboplatin [12]. The latter study also measured the rate constant at 25 °C, finding agreement with the value of k_1 measured by Tobe and coworkers who used approximately 500 μM drug to study hydrolysis [13]. An unusually small rate constant of $k_1 < 10^{-9}\ s^{-1}$, which is the only reported value that is consistent with the two year shelf life of the ready-to-use infusion solution of carboplatin, was measured by Sadler and coworkers using ^{1}H NMR and 20 mM drug [14]. While there was no mention in the early hydrolysis studies involving carboplatin that the value of k_1 may be dependent on the total drug concentration in solution, it now appears that carboplatin forms an association dimer in water which, since the dimer resists hydrolysis, is the reason for the high stability of the concentrated ready-to-use infusion solutions of the carboplatin [18].

A dimer of carboplatin was reported by Burns et al., who used liquid chromatography–mass spectrometry to analyze a solution containing approximately 300 μM drug [19]. While the existence of a dimer was only mentioned in passing in the report, the work showed that the association complex is stable in a mobile HPLC phase containing hydrogen-bond disrupting solute molecules. In another report, Guo and coworkers studied relatively high concentrations of carboplatin (3 mM) using mass spectrometry and reported the existence of a dimer, trimer, and tetramer of the drug [20]. The dimer of carboplatin was also observed in mass spectroscopic studies by Vivekanandan et al. [21] and later by us [18,22]. Although mass spectrometry is an ideal way to detect association complexes, solution samples that are injected into the spectrometer are condensed. This can lead to an increase in solute concentration thereby inducing molecular associations that may not be present under normal solution conditions.

Recently, we used proton NMR to study the self-association of carboplatin in solution by measuring the integral of the NH resonance at δ 4.17 ppm of carboplatin, associated with the two symmetry-equivalent NH_3 molecules of the drug, as a function of the total drug concentration in solution [18]. When the concentration of carboplatin is high, the NH integral is large and near the value expected for the compound (6H) but when the concentration of drug is low, the signal at 4.17 ppm integrates for fewer than six protons. In order to explain the change in the integral with drug concentration, it was postulated that carboplatin exists as an association complex at high concentration, the nature of which blocks the exchange of the NH protons with the protons on solvent. However, at low concentration, the drug exists mainly as a monomer that has exposed NH protons. These protons rapidly exchange with the protons of solvent making the NH resonance of the monomer unobservable. By fitting the magnitude of the integral as a function of drug concentration to various association models, it was found that carboplatin exists in a monomer–dimer equilibrium in water with an association constant of $K\ (M^{-1}) \approx 391$. Since the structure of the dimer prevents exchange of the NH protons with water, it is also likely to block the attack of water at the Pt^{2+} ion in hydrolysis, which is the reason that concentrated solutions of carboplatin are stable. Using the determined value of K, the ready-to-use aqueous solution carboplatin (27 mM) contains approximately 81% of the more stable dimer which is the reason for the long shelf life of the drug formulation.

A clue that carboplatin can undergo self-association in solution is evident in the single crystal X-ray structure of the drug that shows that individual molecules are connected to each other through an extensive network of intermolecular hydrogen bonds [23]. In particular, hydrogen bonds exist between the ammonia molecules of one complex and the oxygen atoms of the dicarboxylate CBDCA ligand of neighboring complexes. Since elements of this hydrogen bond network could persist in concentrated aqueous solution, it is easy to see that a drug dimer is possible and, while dilution would drive the equilibrium toward the more reactive monomeric form, a small amount of carboplatin dimer could be present in cell culture studies done with carboplatin, and it could be circulating in blood (~100 μM carboplatin) of patients receiving carboplatin in chemotherapy [24].

3. Transport of carboplatin

Xie et al. used size exclusion chromatography in combination with inductively coupled plasma mass spectrometry (SEC-ICP-MS) to measure the kinetics of carboplatin binding to human serum albumin (HSA) and γ-globulin (IgG) in the blood of cancer patients [25]. While the sites of platinum binding were not determined, the study found that the second-order rate constants for binding to HSA and IgG are k = 0.74 and 1.01 $M^{-1}\ min^{-1}$, respectively, at 37 °C, both of which are smaller than the rate constant for the reaction of cisplatin with HSA. In a more recent study, Sooriyaarachchi et al. employed size exclusion chromatography-inductively coupled plasma atomic emission (SEC-ICP-AES) to study the binding of carboplatin to proteins in plasma of healthy male and female volunteers [26]. The study showed that carboplatin binds to HSA and possibly also to α_2-macroglobin with a rate that is slower than that of cisplatin, with only 40% of carboplatin being bound to proteins after 24 h.

After passing through the blood, carboplatin appears to enter the cell through passive diffusion [27,28], although it may enter via active transport or through ion channels as well [28,29]. Work done by Osella and coworkers suggests that carboplatin enters the cell via a passive diffusion mechanism [27]. These authors demonstrated that after 24 h of exposure to carboplatin, the accumulation ratio (the ratio between intracellular [drug] and extracellular [drug]) of carboplatin in MCF-7 cells is not dependent on the initial concentration of drug present in culture medium.

The main copper transporter in human cells is the *trans* membrane protein, hCtr1. While this protein is important in the transport of copper into the cell, Howell and coworkers suggested that hCtr1 may also be involved in the transport of platinum drugs into the cell [30]. An important feature of hCtr1 is its exposed extracellular portion that contains methionine- and histidine-rich domains. This region is critical for binding copper and, as has been suggested, also the platinum drugs for their ultimate transport into the cell. In an effort to elucidate the transport mechanism involving hCrt1, Guo and coworkers [31] studied the interaction of carboplatin, cisplatin and other platinum compounds with a Met- and His-rich 20 mer peptide (hCtr1-N20)

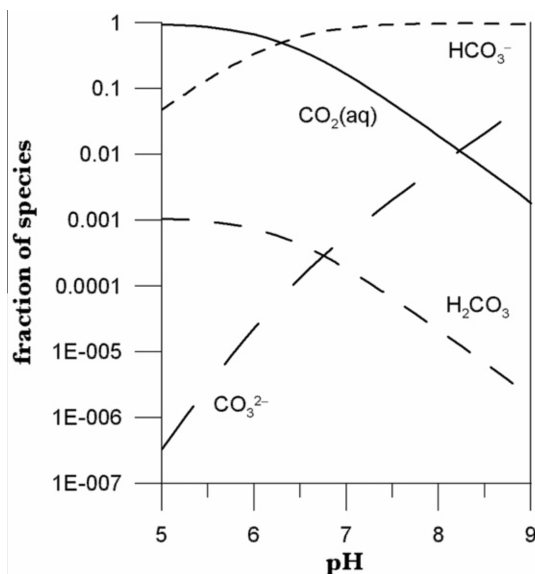


Fig. 2. Speciation in carbonate buffer as a function of pH at 37 °C.

corresponding to the N-terminal domain of hCtr1. Electrospray ionization (ESI) mass spectrometry (MS) measurements as a function of time showed that carboplatin and cisplatin react with the Met residues of the peptide and, after initial binding of Met thioether ligands to Pt^{2+}, the bound thioether groups cause the rapid loss of the *cis*-ammonia ligands of carboplatin and cisplatin via the well known *trans effect*. As expected, the presence of the CBDCA ligand causes the reaction of carboplatin with the peptide to be slower than the corresponding reaction with cisplatin and there was no evidence for a stable ring-opened species in the reaction of carboplatin with hCrt1-N20.

Franz and coworkers [32] also studied the interaction of carboplatin with peptide fragments having sequences corresponding to the N-terminal (extracellular) domain of hCrt1. A combination of liquid chromatography and mass spectrometry confirmed that carboplatin is slower to react than cisplatin with the Met residues of the peptide. After approximately 48 h, carboplatin completely reacted with the peptide in the process, releasing the CBDCA and ammonia ligands from the Pt^{2+} ion of the drug. Since the binding interaction of carboplatin with hCrt1 is too slow to be clinically relevant and all ligands originally bound to the Pt^{2+} ion are displaced by peptide donors, transport of carboplatin into the cell via the copper transporter hCrt1 appears unlikely.

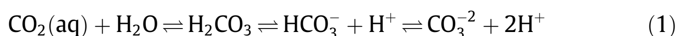
4. Activation of carboplatin

4.1. Chloride and phosphate as activating agents

In an effort to study reactions that carboplatin might undergo in the body that would provide a basis for activating the drug through displacement of part or all of the CBDCA ligand, Sadler and coworkers investigated the reaction of carboplatin with chloride and phosphate, which in blood and the cytosol have concentrations of 105 and 5, and 10 and 80 mM, respectively [14,33]. These researchers found that the pseudo first order rate constants at pH 7, 37 °C for the reaction of carboplatin with chloride and phosphate are $k_1 = 7.7 \times 10^{-7}\ s^{-1}$ and $k_1 = 4.3 \pm 0.2 \times 10^{-7}\ s^{-1}$, respectively, showing that under these conditions reaction of carboplatin with chloride is slightly faster than with phosphate (14). They also showed that the reaction of carboplatin with chloride produces *cis*-$[Pt(CBDCA\text{-}O)(Cl)(NH_3)_2]^-$, while its reaction with phosphate produces *cis*-$[Pt(CBDCA\text{-}O)(HPO_4)(NH_3)_2]^{2-}$. Sadler and coworkers measured similar rate constants for the reaction of carboplatin with 5′-GMP in the presence and absence of 140 mM chloride in phosphate buffer, and suggested that activation by chloride, i.e., displacement of the CBDCA ligand, is not important in this reaction. Beck and coworkers showed that aging carboplatin in NaCl, which displaces the CBDCA ligand, produced platinum species that were more toxic to *E. coli* PQ37 than was un-aged carboplatin, and that increasing the incubation time increased the cytotoxicity of the carboplatin solution [34].

4.2. Activation in carbonate buffer

The most important hydrogen ion controlling buffer in the body is the carbonate buffer system which has concentrations in blood and the cytosol of ~24 and ~12 mM, respectively [35,36]. This buffer consists of dissolved carbon dioxide, CO_2, carbonic acid H_2CO_3, hydrogen carbonate, HCO_3^-, and carbonate, CO_3^{2-}, all of which are in equilibrium with one another according to Eq. (1)



$$CO_2(aq) + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \rightleftharpoons CO_3^{-2} + 2H^+ \quad (1)$$

In aqueous solution, the concentration of dissolved or aquated carbon dioxide is related to the partial pressure of CO_2 in the atmosphere above the solution by Henry's law, Eq. (2), where K_H at 25 °C is 0.03416 mol kg^{-1} atm^{-1}

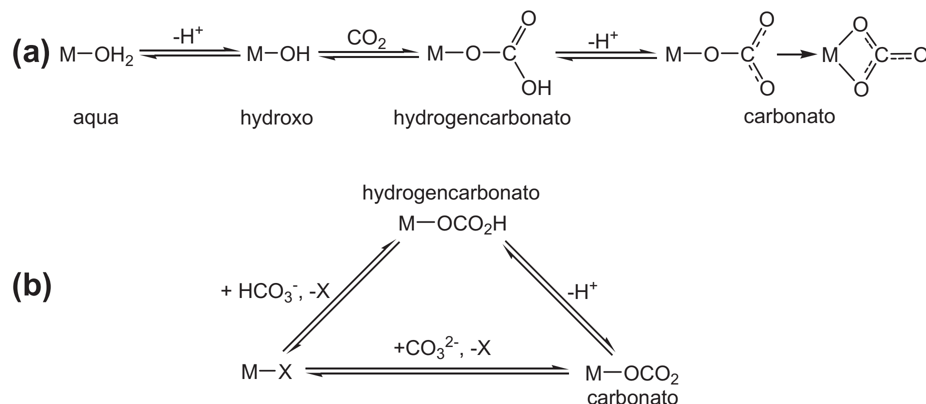


Fig. 3. Reactions of carbonate buffer components with metal complexes. (a) Reaction of carbon dioxide with a metal hydroxo complex to produce a hydrogen carbonato complex. The latter is in proton equilibrium with a carbonato complex that can be mono- or bi-dentate toward the metal ion. (b) Reaction of hydrogen carbonate/carbonate with a metal complex through a ligand displacement reaction.

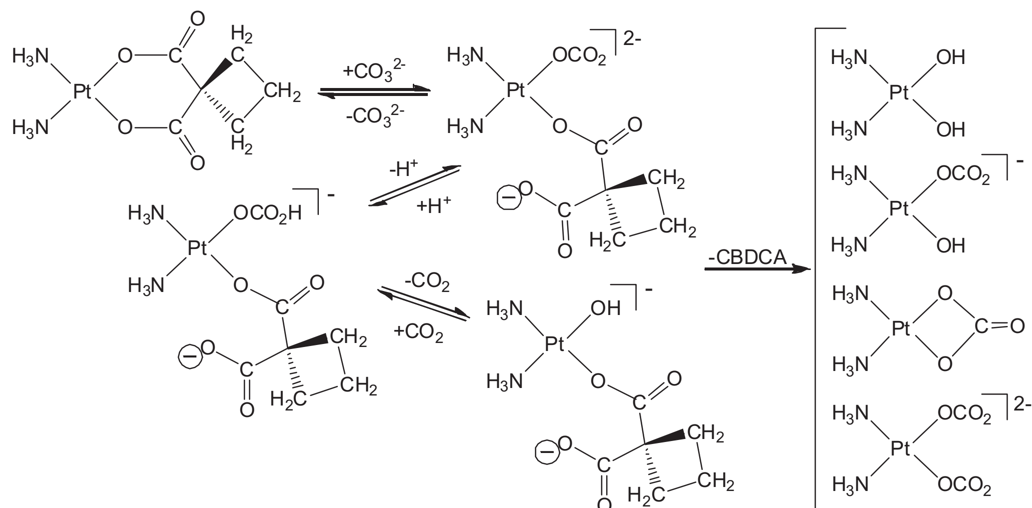


Fig. 4. Reaction of carboplatin with carbonate.

$$\frac{[CO_2(aq)]}{P_{CO_2}} = K_H \quad (2)$$

In biological fluids, some of the dissolved CO_2 reacts with water (see Eq. (1)) to form carbonic acid, H_2CO_3, according to the equilibrium expression in Eq. (3)

$$\frac{[H_2CO_3]}{[CO_2(aq)]} = K_x \quad (3)$$

Values of $1/K_x$ have been reported [37] for NaCl solutions at temperatures between 15.0 and 32.5 °C. Linear extrapolation of the results for 27.5–32.5 °C gives $K_x = 0.0011145$ at 37 °C. Since K_x is so small, $[H_2CO_3]$ is small, and often the sum of $[CO_2]$ and $[H_2CO_3]$ is simply denoted by $[CO_2']$ or $[CO_2^*]$. The first ionization constant, $K_a = 10^{-6.35}$ at 25 °C, commonly referred to as the acid ionization constant of carbonic acid H_2CO_3, actually refers to the equilibrium in Eq. (4)

$$\frac{[H^+][HCO_3^-]}{[CO_2']} = K_a \quad (4)$$

Park et al. [38] have measured K_a from 25 to 175 °C and given pK_a as a power series in T, from which we calculate $pK_a = 6.297$ and $K_a = 5.049 \times 10^{-7}$ M at 37 °C. Thus, the true acid ionization constant of carbonic acid is actually given by Eq. (5)

$$\frac{[H^+][HCO_3^-]}{[H_2CO_3]} = K_a \frac{[CO_2']}{[H_2CO_3]} \approx \frac{K_a}{K_x} = 4.453 \times 10^{-4} \quad (5)$$

so that $pK_a(H_2CO_3) = 3.344$. The ionization constant of bicarbonate is simply Eq. (6)

$$\frac{[H^+][CO_3^{2-}]}{[HCO_3^-]} = K_b = 10^{-10.16} \quad (6)$$

where pK_b was obtained from the review by Eremenko [39], who gives recommended values of K_b over a range of temperatures including 37 °C. By interpolation we derive $K_b = 6.87 \times 10^{-11}$.

Using the values for K_x, K_a, and K_b cited above, one can calculate the relative amounts of all four species present in a carbonate buffer at equilibrium at any desired pH at 37 °C, Fig. 2. Since these relative amounts (fraction of species on the *y*-axis) are, of course, independent of total concentration of carbonate buffer present in solution, the plot allows the determination of the species distribution in a carbonate buffer as a function of pH. At pH 7.4, the main carbonate buffer components in blood and the cytosol are dissolved CO_2, ~2 and ~1 mM, respectively, and hydrogen carbonate, ~23 and ~11 mM, respectively.

Extensive work has shown that metal complexes containing bound hydrogen carbonate or carbonate ligands, which can be collectively referred to as "carbonato complexes", can form via rapid addition of carbon dioxide gas to a metal hydroxo species, Fig. 3a, or by a slower route involving ligand displacement by carbonate or hydrogencarbonate, Fig. 3b [40–44]. Second-order rate constants of 37–590 $M^{-1} s^{-1}$ at 25 °C have been reported for the reaction of dissolved CO_2 gas with hydroxo complexes of cobalt, chromium, iridium, rhodium, and zinc [40], which results in the initial formation of a hydrogen carbonato complex. Depending on the nature of the metal ion, its charge and the pH of the medium, the initially formed hydrogen carbonato complex can lose a proton to give a monodentate carbonato complex or, if the ligand *cis* to the carbonate is easily displaced, the monodentate carbonate ligand can attach the metal ion by loss of the *cis* ligand to form a bidentate carbonato complex. Since all carbonato complexes are in equilibrium with carbon dioxide gas, if the gas is allowed to escape from the system, the originally formed carbonato complexes can decompose to their aqua/hydroxo precursors through the loss of CO_2, Fig. 3a [40–45]. Although some hydrogen carbonato complexes are stable enough to be characterized using X-ray analysis [46], others, cobalt, iridium, and rhodium [40,45] are unstable, and loose CO_2 with rate constants of 0.25–4.40 s^{-1} at 25 °C.

Using [^{1}H, ^{15}N] HSQC NMR, ^{1}H NMR, ^{13}C NMR and UV-Visible spectroscopy, we showed that carboplatin reacts with physiological carbonate buffer, to produce platinum-carbonato and -hydroxo species, Fig. 4 [11,47,48]. Increasing pH at a constant total carbonate concentration, or increasing the total carbonate concentration at a constant pH, leads to an increase in the rate of reaction of carboplatin in carbonate buffer [11]. Since the concentration of carbonate ion, CO_3^{2-}, increases when either total carbonate or pH increases, the attacking nucleophile in the ring opening reaction of carboplatin is carbonate ion which is actually one of the minor components of the carbonate buffer system at pH 7.4, Fig. 2. Thus, carboplatin reacts with carbonate ion ($k_1 = 2.04 \pm 0.81 \times 10^{-6}$ in 24 mM carbonate buffer, pH 7.5 at 37 °C) to produce carbonato carboplatin, *cis*-$[Pt(NH_3)_2(CBDCA\text{-}O)(CO_3)]^{2-}$, which is in proton equilibrium with a hydrogen carbonato species, *cis*-$[Pt(NH_3)_2(CBDCA\text{-}O)(HCO_3)]^-$. The latter complex rapidly decarboxylates to the monohydroxo complex, *cis*-$[Pt(NH_3)_2(CBDCA\text{-}O)(OH)]^-$. Although the latter complex can also be produced by the reaction of carboplatin with OH^-, the values of k_1 measured using [^{1}H, ^{15}N] HSQC NMR

show that reaction with hydroxide is less important than the reaction of the drug with carbonate. Once the ring is opened, other compounds form, including *cis*-$[Pt(NH_3)_2(CO_3)(OH)]^-$, which can also be produced by the reaction of cisplatin in a carbonate buffer [48,49].

Complete cell culture medium (culture medium plus fetal bovine serum) is similar in composition to blood; thus, studies performed in RPMI culture medium offer insight into the behavior of carboplatin *in vivo*. Such studies confirmed that carbonate is one of the most important nucleophiles in culture medium for the activation of carboplatin [11]. The pseudo-first-order rate constant for the disappearance of the [^1H, ^{15}N] HSQC NMR peak for ^{15}N-labeled carboplatin in complete cell culture medium is approximately 1.5 times larger than that obtained for the disappearance of carboplatin in medium without carbonate. This disappearance is due to nucleophiles reacting with carboplatin, resulting in species that are ring-opened or have the CBDCA ligand completely displaced from the Pt^{2+} ion. Chaney and coworkers also recognized the importance of $NaHCO_3$ as an activating agent by showing that carbonate present in tissue culture medium can displace the malonate chelate ring of a Pt^{2+} antitumor agent structurally related to carboplatin [50].

The mechanistic implications of the activation of carboplatin in carbonate buffer have also been investigated. Aging carboplatin in carbonate buffer, pH 8.4–8.6, produces species that are more toxic to human neuroblastoma (SK-N-SH) and Namalwa-luc Burkitt's lymphoma (BL) cancer cells, and proximal renal tubule (HK-2) cells. We also showed that Jurkat cells that have been made resistant to cisplatin by continual exposure to the drug have reduced capacity to bind/take up platinum when in contact with solutions containing significant concentrations of carboplatin–carbonate reaction products [47]. This is probably because the resistant cells were selected for their ability to survive higher concentrations of cisplatin, and their survival is associated with mechanisms to prevent platinum from entering cells and/or rapidly removing platinum from cells by an efflux mechanism. Since some of the products shown on the right side of Fig. 4 are the same as those identified in the cisplatin system [48,49], and the cells were made resistant to these species, it is not surprising that the resistant cells exhibit reduced uptake of the platinum compounds in carbonate aged carboplatin.

Osella and coworkers also studied the activation of carboplatin by carbonate, using a DNA-biosensor with a sensing element capable of immobilizing DNA on the surface of a screen-printed electrode [51]. In these studies, calf thymus DNA was incubated with carboplatin in either 24 mM phosphate or carbonate buffers for various times, the biosensor was dipped into a solution containing platinum, and the amount of Pt binding to the guanine bases on DNA measured using square wave voltammetry (SWV). The study showed that in carbonate buffer, carboplatin forms species that can bind to the guanine bases of DNA. The ability of carbonate buffer to produce carboplatin species that can bind to and unwind supercoiled DNA has also been reported. When carboplatin is aged in a carbonate buffer and then added to a 24 mM HEPES (pH 7.4) buffered solution containing pBR322 DNA, the platinum species in the medium bind to and unwind supercoiled Form I DNA [52]. Since Form I DNA co-migrates with nicked circular Form II DNA at some value of r, where r = [drug]/[DNA-bp], the carbonate aged carboplatin species in the HEPES buffer system act like cisplatin in that they appear to produce the classic 1,2-intrastrand crosslink on DNA [8]. However, when the carbonate aged carboplatin species are reacted with DNA in a two buffer system, 24 mM HEPES plus 24 mM carbonate (pH 7.4) the mobilities of Forms I and II pBR322 DNA do not change, indicating that the carbonate that is present in the buffer either blocks the binding of the carbonate aged carboplatin species to DNA or that binding without a change in DNA supercoiling occurs.

4.3. Interaction of carboplatin with amino acids

Since sulfur in a thiolate or thioether is considered a soft base and Pt^{2+} is a soft acid [53], investigators have focused on the ability of cysteine and methionine, or peptides and proteins containing these amino acids, to react with carboplatin [20,54–60]. Sadler and coworkers showed that the reaction of carboplatin with methionine is rapid, $k = 2.7 \times 10^{-3}\ M^{-1}\ s^{-1}$ at 25 °C, and results in the formation of a stable ring-opened species, *cis*-[Pt(CBDCA-*O*)$(NH_3)_2$(L-HMet-*S*)], which has a long half-life [55]. These authors suggested that this reaction may play a role in the biological activity of carboplatin. From this, the rate constant for the reaction of carboplatin with 0.1 mM L-methionine, the concentration of L-methionine in RPMI culture medium [50], is approximately $2.7 \times 10^{-7}\ s^{-1}$. While a thioether group can readily displace the bidentate chelate ring of carboplatin, the resulting platinum–methionine adduct is thermodynamically stable, which could make transfer of the platinum to a target via breakage of the Pt–S bond difficult [63]. For example, a *cis*-$(NH_3)_2Pt^{2+}$ moiety which is bound to the terminal Met1 of ubiquitin cannot be transferred to a potential DNA target, 5′-GMP [61]. However, for certain structures containing both a thioether group and a DNA base, the initially formed Pt–S adduct can rearrange to the Pt-base adduct [62]. This suggests that reaction of carboplatin with a thioether could be a means of activating the drug *in vivo*. Sheldrick and coworkers showed that a thioether group in a tripeptide can displace the bidentate dicarboxylate ligand of carboplatin and labilize the ammonia molecule *trans* to the thioether-ultimately leading to the replacement of all of the ligands of carboplatin with donors from the peptide [58]. Interestingly, although carboplatin reacts relatively fast with methionine, the reaction of drug with cysteine is slow [55]; Sadler and coworkers suggested that hydrolysis may be the rate-limiting step in this reaction [55].

4.4. Interaction with GSH and MT

The two most abundant cellular thiols are glutathione (GSH) and metallothionein (MT) [63]. Hagrman et al. showed that the pseudo-first-order rate constant for reaction of carboplatin with 6.75 mM GSH in HEPES buffer containing 4.62 mM NaCl, 37 °C, is $k_1 \approx 9.5 \pm 2.8 \times 10^{-6}\ s^{-1}$ [60]; cells typically contain 2 mM glutathione. The second-order rate constant for the reaction of carboplatin with the fully loaded Cd^{2+}/Zn^{2+} form of MT in HEPES buffer containing NaCl, 37 °C, is $0.0121 \pm 0.0039\ M^{-1}\ s^{-1}$ [63]. From these data, the pseudo first order rate constant for the reaction of carboplatin with a physiologically relevant concentration of MT (~2 mM) is, $k_1 \sim 8 \times 10^{-6}\ s^{-1}$. Interestingly, Holler and coworkers showed that the rate of reaction of carboplatin with calf thymus DNA is significantly increased in the presence of L-methionine (thioether), thiourea, and glutathione (thiol), and similar results were obtained using cytosolic extracts from MCF-7 breast cancer cells. This suggests that the product of reaction of carboplatin with these sulfur nucleophiles, most likely a Pt^{2+}–S adduct, can bind to DNA. These observations are consistent with Shi et al. who showed that the Pt^{2+} product resulting from the reduction of a Pt^{4+} complex with GSH is competent to bind to DNA. A similar conclusion was reached by Volckova et al. who showed that while, adduct formation is reduced, some cisplatin can bind to DNA in the presence of GSH. [64,65].

5. Conclusions

In this short review, we discussed the chemistry of carboplatin, from its remarkable stability in the ready-to-use infusion solution used in the clinic, to the activation by carbonate and other nucleo-

philes that makes it more reactive toward substances present in the cell. Understanding the chemistry of a frequently used drug like carboplatin is critical for the development of new and improved platinum-based anticancer agents.

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