Kinetic Study on the Reactions of Platinum Drugs with Glutathione

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ABSTRACT

The binding of platinum (Pt) drugs (oxaliplatin, carboplatin, and cisplatin) to glutathione (GSH, 6.75 mM) was investigated at 37°C in Hepes (100 mM, pH ~7.4) or Tris-NO₃ (60 mM, pH 7.4) buffer and NaCl (4.62, 6.63, or 7.82 mM). The conditions were chosen to mimic passage of clinical concentrations of the drugs (135 µM) through the cytosol. The reactions were monitored by UV-absorption spectroscopy, high-performance liquid chromatography (HPLC), and atomic absorption spectroscopy. The initial rates, detected by UV absorbance, were similar for oxaliplatin and cisplatin reacting with GSH and were more than 5-fold faster than for carboplatin reacting with GSH. The Pt contents in HPLC eluates corresponding to unbound drug decreased exponentially with time, confirming that the reactions were first order in [Pt drug] and allowing determination of the pseudo first-order rate constants (k₁). The second-order rate constants (k₂) were calculated as k₁ divided by [GSH]. The k₂ value for oxaliplatin reacting with GSH was ~3.8 × 10⁻² M⁻¹ s⁻¹, for cisplatin reacting with GSH ~2.7 × 10⁻² M⁻¹ s⁻¹, and for carboplatin reacting with GSH ~1.2 × 10⁻³ M⁻¹ s⁻¹ (~32-fold slower than that of oxaliplatin and ~23-fold slower than that of cisplatin). These results demonstrate an influence of ligands surrounding the Pt coordination sphere on the reactivity of Pt²⁺ with GSH.

The clinically available platinum (Pt)-based compounds (oxaliplatin, carboplatin, and cisplatin) exert antitumor activity by binding to cellular DNA and promoting apoptosis (Gelasco and Lippard, 1999). These agents also react with cellular thiols (e.g., GSH and metallothionein), which limit the amount of drug available for binding to DNA (Dedon and Kuchel, 1990a,b; Ishikawa and Ali-Osman, 1993). We recently reported on this reaction using conditions that mimic those found in the cell (Dabrowiak et al., 2002). However, data on oxaliplatin and carboplatin bindings to GSH are lacking. These new cisplatin analogs have different ligand groups occupying the Pt coordination sphere (Fig. 1). They also have different clinical activities and adverse effects than those of cisplatin (Pendyala and Creaven, 1993; Go and Adjei, 1999; Verstraete et al., 2001). Thus, their reactions with GSH may differ from that of cisplatin.

We report here on the reactions of GSH with oxaliplatin and carboplatin and compare the results with that of cisplatin. The reactions are followed by UV, HPLC-UV, and atomic absorption spectroscopy (AAS). Therapeutic concentrations of the drugs (135 µM) and physiologic conditions (pH ~7.4 with Hepes or Tris-NO₃ at 37°C) are used. Our goal is to investigate the effects of ligand groups occupying the Pt coordination sphere on the drugs’ reactions with GSH.

Materials and Methods

Chemicals. Oxaliplatin (mol. wt. 397.3; lyophilized powder containing equal amount of lactose by weight) was purchased from Sanofi-Synthelabo (Bedford, OH); carboplatin (mol. wt. 371.25; lyophilized powder containing equal amount of mannitol by weight) was purchased from Bristol-Myers Squibb Co. (Princeton, NJ); cisplatin (mol. wt. 300.05, 1.0 mg/ml, ~3.3 mM in 154 mM NaCl) was purchased from Pharmaceutical Partners (Los Angeles, CA); GSH, dihexylamine, glacial acetic acid, nitric acid, NaCl, and Tris were purchased from Sigma-Aldrich (St. Louis, MO); Hepes was purchased from Invitrogen (Carlsbad, CA); distilled deionized water (water for injection quality water, dH₂O) was purchased from Meditech (Herndon, VA); and Pt atomic spectroscopy standard (H₃PtCl₆).

ABBREVIATIONS: GSH, glutathione; HPLC, high-performance liquid chromatography; tᵣᵣ, retention time; k₁, pseudo first-order rate constant; k₂, second-order rate constant; Pt, platinum; AAS, atomic absorption spectroscopy; dH₂O, distilled deionized water; Pt-SG, Pt-sulfur product.
1 mg/ml in 10% HCl) was purchased from PerkinElmer Instruments (Norwalk, CT).

**Solutions.** Carboplatin and oxaliplatin were dissolved in dH₂O (10 mg of drug per milliliter = 27 and 12.6 mM, respectively) immediately before use. Hepes buffer was made in dH₂O and the pH was adjusted to ~7.4 with 50% NaOH. Tris-NO₃ buffer was made in dH₂O and the pH was adjusted to ~7.4 with nitric acid. Dihexylammonium acetate (2.5 mM) HPLC solvent A was prepared in the hood by the addition of 590 l of 4.24 M dihexylamine and 144 l of 17.4 M glacial acetic acid to each liter of dH₂O; the pH was adjusted to ~7.0 by small additions of dihexylamine or acetic acid, and the solution was continuously stirred. The GSH solution was prepared and stored as described previously (Dabrowiak et al., 2002).

**Chemical Reactions.** All reactions were carried out at 37 ± 0.1°C. The oxaliplatin and carboplatin reactions contained 135 μM oxaliplatin or carboplatin, 6.75 mM GSH, 100 mM Hepes (or 60 mM Tris-NO₃), and 4.62 mM NaCl (pH ~7.4). The cisplatin reaction contained 135 μM cisplatin, 6.75 mM GSH, 100 mM Hepes (~pH 7.4) and final [NaCl] of 7.82 or 6.63 mM (mostly from the cisplatin formulation). Because the rate of disulfide formation is relatively slow, the concentration of GSH was assumed not to change much over the time course of the GSH-Pt drug reactions. This assumption was also verified by HPLC, which showed a large excess of GSH at the end of each incubation period. Thus, the 50-fold excess of [GSH] over [Pt drug] assured the reaction was pseudo zero-order with GSH. Because the reaction is first order in Pt drug, it is pseudo first-order overall (Dabrowiak et al., 2002). A control mixture containing the same additions without GSH was incubated along with each reaction. For each experiment, one reaction mixture (10 ml) was prepared and analyzed simultaneously by UV absorbance and HPLC-UV-AAS, as described below.

**UV Absorbance.** UV absorbance (at 260 nm) as a function of time (from 0 to 10 h) for oxaliplatin, carboplatin, and cisplatin reactions with GSH (with the absorbance changes of GSH alone subtracted) are shown in Fig. 2. The absorbance changes in Fig. 2 thus reflect only the Pt-sulfur bond (product) formation. The reaction mixtures (at 37°C) and their initial rates (calculated by linear fit of the data from 0.5 to 2.0 h) are shown in Table 1.

**HPLC-UV-AAS.** Analysis was performed on a Beckman reversed-phase HPLC system exactly as described previously (Hagrman et al., 2003). UV detection at 260 nm was used. For each time point, 50 μl of the reaction were loaded on the HPLC. Eluates containing Pt were collected. The Pt contents were determined on the AAS as described previously (Dabrowiak et al., 2002).

**Kinetics.** The kinetics of oxaliplatin, carboplatin, and cisplatin bindings to GSH were analyzed as described previously (Dabrowiak et al., 2002). Briefly stated, concentrations of drug and GSH were calculated from the measured data, and fitted to the second-order rate expression (logarithm of a quotient of concentrations = second-order rate constant × time). Although the fit to a linear function of time was satisfactory, the reactions are not proven to be second order. Thus, the derived rate constants tabulated in Table 2 are effective rate constants, useful for purposes of comparison.

**Results**

**UV Absorbance.** UV absorbance (at 260 nm) as a function of time (from 0 to 10 h) for oxaliplatin, carboplatin, and cisplatin reactions with GSH (with the absorbance changes of GSH alone subtracted) are shown in Fig. 2. The absorbance changes in Fig. 2 thus reflect only the Pt-sulfur bond (product) formation. The reaction mixtures (at 37°C) and their initial rates (calculated by linear fit of the data from 0.5 to 2.0 h) are shown in Table 1.

![Fig. 1. Chemical structures of the Pt compounds.](image)

![Fig. 2. UV absorbance changes associated with Pt drugs binding to GSH. Absorbance at 260 nm is shown as a function of time. The reaction mixtures and the initial rates (from linear fits to the data for 0.5–2.0 h) are shown in Table 1.](image)
2.0 h) are shown in Table 1. The initial rate of change of absorbance for the reaction of GSH with oxaliplatin was ~32% slower than that with cisplatin. By contrast, the initial rate of absorbance change for the reaction of GSH with carboplatin was ~5.7-fold slower than that with oxaliplatin and ~8.3-fold slower than that with cisplatin (Table 1).

For oxaliplatin reaction with GSH, the rate of change of absorbance from 10 to 40 h was very small (0.0001 per min, 
$r > 0.95$) compared with the initial rate (Table 1). By contrast, for carboplatin reaction with GSH, the rate of change of absorbance from 10 to 40 h (0.0003 per min, $r > 0.97$) was the same as the initial rate (Table 1). For cisplatin reaction with GSH, the rate of absorbance change from 10 to 40 h (0.0004 per min, $r > 0.94$) was much slower than the initial rate (Table 1).

**Oxaliplatin Reaction with GSH as Monitored by HPLC-UV-AAS.** We first investigated the reaction of oxaliplatin (135 μM) with GSH (6.75 mM) in 100 mM Hepes buffer (pH 7.4) and 4.62 mM NaCl. The control mixture contained the same additions minus GSH. A representative chromatogram for the control mixture (at min 1.5) is shown in Fig. 3A, and for the reaction mixture (at min 63) in Fig. 3B. The areas of the HPLC peaks with $t_R$ of ~10 min and the Pt contents (determined by AAS) of the eluates decreased with time; these corresponded to unbound oxaliplatin. On the other hand, the areas of peaks with $t_R$ = 24 to 26 min and the Pt contents of the eluates increased with time. These peaks were partly due to Pt-sulfur products (Pt-SG) and partly to the solvent. Eluates for other $t_R$ were free of Pt. The results of two independent experiments (experiments I and II, described below) are shown in Fig. 4. The $k_f$ value for oxaliplatin reaction with GSH was determined from the decay of Pt content (determined by AAS) in the eluates corresponding to unbound oxaliplatin (Table 2).

**Experiment I.** For the control mixture, recovery of Pt from the eluate with $7 < t_R < 16$ min (corresponding to unbound oxaliplatin) was 93% at hour 0 and 86% at hour 7.2; eluates with $23 < t_R < 31$ min (corresponding to Pt-SG) remained free of Pt from hour 0 to hour 7.2. For the reaction mixture, unbound oxaliplatin declined to ~10% of the original value by 1.07 h. By ~2.08 h, ~97% of the original Pt occurred in eluates with $23 < t_R < 27$ min and ~3% in eluates with $27 < t_R < 31$. In this experiment, there were not enough data points for $t < 3$ h to determine the oxaliplatin decay rate accurately. Nevertheless, Fig. 4A (squares) shows exponential decay ($r > 0.99$) of the areas of peaks with $t_R$ of ~10 min. From the exponential fit, $k_f = 2.58 \times 10^{-4}$ s$^{-1}$. The areas of peaks with $t_R$ ~24–26 min (Fig. 4A, circles), after subtraction of the control, were fit to a rate constant of $1.4 \times 10^{-4}$ s$^{-1}$ (the average rate constant for a series of reactions).

**Experiment II.** For the control condition, recovery of Pt from the eluate corresponding to unbound oxaliplatin ($9 < t_R < 13$ min) was 85% at min 1.5 (Fig. 3A) and 76% at hour 6.1. The lower Pt recovery in this experiment was due to the shorter eluate collection period (see experiment I). For the reaction mixture, the Pt contents (Fig. 4B, diamonds) and the areas (Fig. 4B, squares) of the peaks corresponding to unbound oxaliplatin decayed exponentially ($r > 0.99$) with similar rates. The curves of natural logarithm of the data in Fig. 4B (diamonds and squares) versus time were linear with slopes of $0.0154 \pm 0.0010$ and $0.0170 \pm 0.0010$ min$^{-1}$, respectively. The $k_f$ value for oxaliplatin reaction with GSH was $0.0154 \div 0.0150 = 2.6 \times 10^{-4}$ s$^{-1}$. The $k_f$ value was thus $k_f = 6.75$ mM (Table 2). The Pt contents (Fig. 4C, diamonds) and the areas (Fig. 4C, squares) of the peaks corresponding to Pt-SG increased with similar rates (see linear fits in Fig. 4C). Because these peaks arise partly from species other than Pt-SG product, the peak area from the control mixture has been subtracted.

**Carboplatin Reaction with GSH.** The reaction of carboplatin (135 μM) with GSH (6.75 mM) was investigated in 100 mM Hepes or 60 mM Tris-NO$_3$ (pH 7.4) and 4.62 mM NaCl. The control mixture contained the same additions minus GSH. A representative chromatogram for the control mixture at hour 0 is shown in Fig. 5A, and for the reaction mixture at hr 5.2 in Fig. 5B. The areas of the peaks with $t_R$ ~7.5 min,
corresponding to unbound carboplatin, and the Pt contents of these peaks, decreased with time. The areas and Pt contents of peaks with $24 \text{ min} < t_R < 26 \text{ min}$, corresponding to Pt-SG, increased with time. The results of two independent experiments (experiments I and II, described below) are shown in Figs. 6 and 7.

**Experiment I.** For the control mixture, recovery of Pt from the elute with $7 < t_R < 11 \text{ min}$ (corresponding to unbound carboplatin) was 118% at hour 0 and 119% at hour 26 (the high yields reflect an experimental error), remaining essentially constant (Fig. 6A, circles). The areas of the peaks with $t_R \sim 7.5 \text{ min}$ also decreased only slightly over the 26-h period (Fig. 6B, circles), and the eluates with $23 < t_R < 31 \text{ min}$ remained free of Pt. Thus, reaction of carboplatin with buffer was negligible. For the reaction mixture, unbound carboplatin, whether measured by Pt contents (determined by AAS) or peak areas, declined exponentially with reaction time with similar rates (0.0272 and 0.0146/h, $r = 0.992$ and 0.795, respectively) (Fig. 6, A and B, triangles). Because of the much larger $R$, the $k_1$ value (Table 2) for carboplatin reaction with GSH was calculated from the Pt concentrations (Fig. 6A, triangles). Furthermore, the Pt contents (Fig. 6C, circles) and the areas (Fig. 6C, squares) of the peaks corresponding to Pt-SG product ($t_R = 24-26 \text{ min}$) increased with time with similar rates. The linear fit to the Pt contents is shown in Fig. 6C ($r > 0.98$). As indicated by their presence in the HPLC chromatogram of the control mixture, these peaks contain species other than Pt-SG. Therefore, the peak areas from the control mixture have been subtracted from the areas in the reaction mixture.

**Experiment II.** For the control mixture, recovery of Pt from the elute with $6 < t_R < 10 \text{ min}$ was 65% at hour 0 and 54% at hour 28.4 (Fig. 7A, circles). The lower Pt recovery in this experiment was due to the shorter eluate collection period (see experiment I). The areas of peaks corresponding to unbound carboplatin ($t_R \sim 7.5 \text{ min}$) remained relatively stable over the 28.4-h period (Fig. 7B, circles), and the eluates with $23 < t_R < 31 \text{ min}$ also remained free of Pt. For the reaction mixture, unbound carboplatin, whether measured by Pt contents or peak areas, declined exponentially with time ($r > 0.98$ and > 0.97, respectively) with similar rates (0.048 and 0.028/h, respectively) (Fig. 7, A and B, triangles). The $k_1$ value (Table 2) for carboplatin reaction with GSH was calculated from the Pt decay as determined by AAS (Fig. 7A, triangles). Furthermore, the Pt contents (Fig. 7C, circles) and the areas (Fig. 7C, squares) of peaks corresponding to Pt-SG increased with time with similar rates.

The reaction of carboplatin (135 $\mu$M) with GSH (6.75 mM) was also investigated in 60 mM Tris-NO$_3$ (pH 7.4) and 4.62 mM NaCl. Results of two experiments are shown in Fig. 8, A and B. The Pt contents of the peaks with $t_R \sim 7.5 \text{ min}$ are shown as a function of time for the reaction (squares) and control (circles) mixtures, with exponential fits (same control reaction used in both experiments). The data for the reaction mixtures are plotted on a logarithmic scale in Fig. 8C. The exponential parameter for the control reaction is appreciable
(\(k_1 = 0.0063 \text{ h}^{-1}, r > 0.98\)), indicating significant reaction with the Tris buffer. Thus, the rate constants for the reaction with GSH were obtained by subtracting 0.0063 h\(^{-1}\) from the exponential parameters for the reaction mixture, 0.0298 h\(^{-1}\) (\(r > 0.93\)) for Fig. 8A and 0.0275 h\(^{-1}\) (\(r > 0.98\)) for Fig. 8B. The resulting \(k_1\) and \(k_2\) values are shown in Table 2.

**Cisplatin Reaction with GSH.** For comparison, the reaction of cisplatin with GSH was investigated in Hepes buffer. The results of two independent experiments are shown in Fig. 9. For experiment I (Fig. 9, A and B), the mixture contained cisplatin (135 \(\mu\)M) and GSH (6.75 mM) in 100 mM Hepes (pH 7.4) and final [NaCl] of 7.84 mM. The control mixture contained the same additions minus GSH. For the control mixture, recovery of Pt from eluate corresponding to Pt-SG (23 < \(t_R\) < 31 min) remained free of Pt. For the reaction mixture, unbound cisplatin declined exponentially (Fig. 9A, squares) with the \(k_1\) value shown in Table 2. Figure 9B shows the increase of peak areas (\(t_R \sim 24–26\) min, squares) and Pt contents (23 < \(t_R\) < 31 min, triangles) corresponding to Pt-SG. Both are apparently leveling off after 3 h.

For Experiment II (Fig. 9, C and D), the mixture contained cisplatin (135 \(\mu\)M) and GSH (6.75 mM) in 100 mM Hepes buffer (pH 7.4) and final [NaCl] of 6.62 mM. The control mixture contained the same additions minus GSH. For the control mixture, recovery of Pt from eluate corresponding to unbound cisplatin (3 < \(t_R\) < 7 min) was 72% at hour 0 and 54% at hour 5.2 (Fig. 9C, circles). For the reaction mixture, unbound cisplatin declined exponentially (Fig. 9C, squares) with the \(k_1\) value shown in Table 2. Figure 9D shows the same increment rates for areas of peaks with \(t_R \sim 24–26\) min (squares) and Pt contents of eluates with 23 < \(t_R\) 31 min (triangles), corresponding to Pt-SG.

**Discussion**

We recently showed that the amount of Pt-DNA adducts increases ~8-fold when cellular thiols are blocked with N-ethylmaleimide (Sadowitz et al., 2002). This finding demonstrates the capacity of cellular thiols to trap Pt\(^{2+}\). Thus, thiol groups (e.g., those on GSH, metallothionein, and histones) affect the amount of Pt\(^{2+}\) available for binding to cellular DNA and may influence the outcome of tumor treatment with Pt-based compounds (Mistry et al., 1991).

GSH is the most abundant cellular nonprotein thiol, and its reaction with cisplatin has been extensively investigated (Berners-Price and Kuchel, 1990a,b; Ishikawa and Ali-Osman, 1993; Dubrowiak et al., 2002). However, the kinetics of GSH binding to oxaliplatin and carboplatin has not been previously studied. Because these newly developed cisplatin analogs have different ligand groups occupying the Pt coordination sphere (Fig. 1), their reaction rates with GSH are expected to differ from that of cisplatin. Moreover, these agents have different clinical activity and toxicity profiles, which reflect their reactive properties (Pendyala and Creaven, 1993; Go and Adjei, 1999; Verstraete et al., 2001).

The results in Table 2 confirm different reaction rates of the three Pt drugs. However, it remains unknown whether the \(k_2\) values shown in Table 2 can account for the different clinical activities and toxicities of these compounds.

In this study, we measure the rates of GSH reactions with Pt drugs, as close to physiological and clinical conditions as possible.
Various cellular thiols can react with Pt drugs. In this study, we focused on the abundant cytosolic GSH, which is emphasized in the literature as a significant contributor to Pt drug resistance. Other thiols (e.g., those on metallothionein and histones), however, may be equally (or even more) important than GSH in trapping Pt$^{2+}$ and in contributing to Pt drug resistance.

The $k_2$ value for cisplatin binding to GSH ($\sim 0.027$ M$^{-1}$ s$^{-1}$; Table 2) is too small to fully account for the effect of N-ethylmaleimide on DNA platination by cisplatin. Because the rate of transport through the cell membrane is high, cisplatin concentration in the cell is maintained relatively constant at the value outside the cell ($\sim 5$–20 $\mu$M) (Ishikawa and Ali-Osman, 1993; Souid et al., 2003). Thus, the rate of cellular GSH reaction with cisplatin is at most $0.54 \times 10^{-6}$ s$^{-1}$, $0.027$ M$^{-1}$ (0.027 M$^{-1}$ s$^{-1} \times 20 \times 10^{-6}$ M) and $t_{1/2}$ (time to use up 50% of cellular GSH) $\sim 357$ h ($20/0.54 \times 10^{-6}$ s$^{-1}$). We recently showed that the rate constant for cisplatin binding to metallothionein is $\sim 28$-fold larger than that to GSH (Hagman et al., 2003). Because cellular concentration of metallothionein is also in the millimolar range (Hagman et al., 2003), the time to deplete 50% of cellular metallothionein is $\sim 12.7$ h ($\sim 357 \div 28$). Therefore, other thiols, such as those on the histones, are likely to impact cellular disposition of the Pt drugs.

The UV absorbance data (Fig. 2) reflect formation of Pt-sulfur bonds (Pt-SG), because the absorption changes for GSH alone are subtracted. It is clear that the absorbance profiles for the three drugs studied are different (Fig. 2), reflecting the diversity of Pt-SG products. The relatively slow rate of Pt-SG formation in the carboplatin reaction is constant over 40 h (0.0003/min, $r > 0.97$; see Results and Table 1), whereas the rate of Pt-SG formation in the oxaliplatin reaction is relatively fast in the first 2 h (0.0017/min, $r > 0.99$; Table 1) but slows down significantly thereafter (0.0001/min, $r > 0.95$). The slow rate of oxaliplatin reaction with GSH after 2 h matches the HPLC-UV-AAS data, showing depletion of the unbound oxaliplatin in $\sim 2$ h (see Results, experiment I, and Fig. 4, A and B). The reaction stops almost completely when the free oxaliplatin is depleted.

By contrast, Pt-SG formation continues in the cisplatin reaction from 0 to 40 h, despite near total depletion of free cisplatin in $\sim 5$ h (Fig. 9, A–C, and Results). Thus, GSH reacts with free cisplatin as well as with its Pt-SG products, and the absorbance changes in the cisplatin reaction (Fig. 2) reflect GSH reacting with both free cisplatin and Pt-SG. The faster initial rate constant is for cisplatin reaction with GSH (Table 1).

Despite the diversity in the Pt-SG products among the three drugs, the data in Fig. 2 and Table 1 clearly show that oxaliplatin and cisplatin react with GSH at a comparable rate, and carboplatin reacts at a much slower rate. This is consistent with the HPLC-AAS data (Table 2). We find for the effective second-order rate constants of the initial reaction: $k_2 = 0.038$ M$^{-1}$ s$^{-1}$ for oxaliplatin, $k_2 = 0.027$ M$^{-1}$ s$^{-1}$ for cisplatin, and $k_2 = 0.0012$ M$^{-1}$ s$^{-1}$ for carboplatin. The $k_2$ value for cisplatin reaction with GSH is similar to that found in our previous investigation of this reaction in 100 mM Tris-NO₃ (pH $\sim$ 7.4), 0.013 M$^{-1}$ s$^{-1}$ (Dabrowiak et al., 2002).

Fig. 6. Carboplatin reaction with GSH in Hepes buffer as monitored by HPLC-UV-AAS (experiment I). The reaction mixture (at 37°C) contained carboplatin (135 $\mu$M) and GSH (6.75 mM) in NaCl (4.62 mM) and Hepes (100 mM, pH 7.4). The control mixture contained the same additions without GSH. At the end of each incubation period, 50 $\mu$L was injected on the HPLC. Eluates with $t_k < 11$ min (unbound carboplatin) were collected and the Pt contents determined on the AAS (A, triangles, reaction; circles, control). The HPLC peak areas corresponding to Pt-SG products are shown in C (squares). C (circles) shows the Pt contents for eluates with $t_k < 11$ min (A, triangles). The result is shown in Table 2.

Possible. The formulations given to patients are used throughout the study. GSH concentration in the reaction mixtures is 6.75 mM, which mimics that in the cell (Souid et al., 2001). Drug concentration is 135 $\mu$M, which mimics that achieved clinically (Oguri et al., 1988; Grolleau et al., 2001; Souid et al., 2003). The reactions are thus zero order in GSH, first order in Pt drug, and pseudo first order overall. The temperature is kept at 37°C with an isothermal water bath. The pH is kept at 7.4 with 100 mM Hepes, used as a buffer due to its minimal reactivity with cisplatin as observed by NMR (Prenzler and McFadyen, 1997). Our own control experiments support this observation. Nevertheless, as shown in Table 2, the Tris buffer produced similar results.
The results presented here demonstrate an influence of ligands occupying the Pt coordination sphere on the drug reaction with GSH. The five-membered ring of oxaliplatin is strained, and the strain is relieved by the thiolate attack on Pt(II) (forming mono adducts), which explains the 1.4-fold faster rate of GSH reaction with oxaliplatin than that with cisplatin (Table 2), monitored by HPLC-AAS. Steric hindrance seems to explain the slow reactivity of carboplatin with GSH (Neidle et al., 1980). The six-membered ring formed between the Pt and the bidentate ligand (1,1-cyclobutanedicarboxylato) of carboplatin puckers in solution to produce a "boat" configuration. Adopting this configuration forces the cyclobutane ring to reside in an axial position of the Pt coordination sphere, which blocks the nucleophile from reacting with the Pt(II) by an S$_2$2 mechanism.

It is well known that Pt drugs react in water to produce the chloro-aquo and diaquo forms. When chloride content in the medium is reduced (e.g., to the concentrations used in this study, 4.62–7.82 mM), Pt drugs begin to aquate. However, the time required to produce the aquated forms is several hours (Miller and House, 1990). Because the $t_{1/2}$ for the reaction of GSH with unaged cisplatin is ~62 min and GSH with unaged oxaliplatin ~44 min (Table 2), the major species reacting with GSH are the dichloro forms of the drugs (Dab-
rowiak et al., 2002). In contrast, because the $t_{1/2}$ for the reaction of GSH with unaged carboplatin is 24.5 h (Table 2), all forms of this drug are expected to react with GSH.

As stated above, the products (Pt-SG) are likely to be different for the three Pt compounds. The initial product for each Pt drug is a mono-adduct, but subsequent GSH reactions are more likely to occur with cisplatin than with oxaliplatin.

The data clearly show that the initial reactions of GSH with oxaliplatin and cisplatin are of similar rate. Carboplatin, on the other hand, reacts with GSH at a much slower rate (10% of that of oxaliplatin and cisplatin) (Table 2).

The cytotoxicity of Pt drugs is proportional to the number of cellular DNA-Pt adducts (unpublished data). The amount of drug available to bind to DNA is limited by cellular thiols such as GSH and metallothionein, so Pt agents that react less with cellular thiols (like carboplatin) may be more effective in tumors overexpressing GSH or metallothionein. Our data demonstrate that, under physiological conditions, the Pt drugs react with cellular GSH at a relatively slow rate. This is consistent with reports showing poor correlations between cellular GSH levels and cytotoxicities of cisplatin, oxaliplatin, and carboplatin (Pendyala et al., 1995, Arnould et al., 2003).

Tumors may overproduce GSH to induce resistance (Mis-
try et al., 1991). We show here that a large difference in rates can be observed when ligands occupying the Pt coordination sphere are changed. This observation may impact the design of new Pt drugs with poor reactivity toward cellular thiols.

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This work is dedicated to the honor of Professor Harvey S. Penefsky.

References


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