Interaction of Cisplatin and Carboplatin with Sodium Thiosulfate: Reaction Rates and Protein Binding

F. Elferink, W. J. F. van der Vijgh, I. Klein, and H. M. Pinedo

Toxicity of cisplatin can be decreased by concomitant administration of sodium thiosulfate, which perhaps chemically inactivates this platinum compound. We studied the disappearance of cisplatin and carboplatin in aqueous solutions of thiosulfate at 37 °C by means of liquid chromatography. At initial concentrations that were similar to therapeutic concentrations in plasma, both drugs disappeared, with half-lives of 66 and 537 min for cisplatin and carboplatin, respectively. At higher thiosulfate concentrations, as found in urine, the respective half-lives were 3.7 and 33.8 min. These values suggest that direct chemical interaction in the plasma compartment has limited therapeutic consequences, whereas the anti-toxic effect of thiosulfate might be explained by the rapid inactivation of cisplatin in the kidneys. Reaction products of cisplatin and thiosulfate bound instantaneously and mainly reversibly to plasma proteins. Protein-bound cisplatin was not released by added thiosulfate—which may explain why thiosulfate, to be effective, must be given in advance of and during cisplatin administration.

Additional Keyphrases: pharmacokinetics, "rescue" chemotherapy, chromatography, reversed-phase, cancer

Cisplatin (cis-diaminedichloroplatinum(II), Figure 1) is successfully used against solid tumors. Its dose-limiting side effect is nephrotoxicity (1). Carboplatin, (diammine(1,1-cyclobutanedicarboxylato)platinum(II), Figure 1) is one of the most promising second-generation platinum compounds, with myelosuppression as the dose-limiting side effect (2). Several animal studies showed decreased nephrotoxicity (3–5) and bone-marrow toxicity (3) when sodium thiosulfate was administered intravenously concurrently with interperitoneal administration of cisplatin. In patients treated with cisplatin intraperitoneally or intravenously, sodium thiosulfate permitted a three- or twofold increase of the dose, respectively (6–8). At moderately increased intraperitoneal doses of cisplatin, thiosulfate prevented renal toxicity, but did not hinder its antitumor activity (9).

Direct chemical interaction between cisplatin and thiosulfate was suggested to be the mechanism of the observed "rescue" (3–5). Thiosulfate is known to be a strong nucleophile, which reacts with cisplatin to form reaction products containing one to four monoor bidentate-bound thiosulfate ligands (10–12). Because ligand-exchange reactions of platinum complexes are rather slow, it is important to know whether cisplatin and carboplatin react with thiosulfate in the therapeutic concentration range with sufficiently high reaction rates to have therapeutic significance. The main object of the present study was to investigate whether the kinetics of the reaction between thiosulfate and the platinum(II) complexes support the proposed rescue mechanism. We also studied the influence of thiosulfate on binding of cisplatin by plasma protein, because cisplatin, in contrast to carboplatin, is highly bound to plasma proteins (1, 2).

Materials and Methods

Apparatus

The liquid chromatography system consisted of a Model 6000A pump (Waters, Etten-Leur, The Netherlands), a Valco manual sample-injection valve with a 10-μL loop (Chrompack, Middelburg, The Netherlands), a home-packed 15 × 0.46 cm column of Spherisorb SS ODS2 (ATS Chromatography, Waddinxveen, The Netherlands), a LDC UV-III Model 1203 214-nm detector (Charles Goffin, De Bilt, The Netherlands), and a Model BD100 recorder (Kipp & Zonen, Delft, The Netherlands). Platinum concentrations were measured with a Model 5000 atomic absorption spectrophotometer, equipped with an AS40 autosampler and a HGA 500 graphite furnace (Perkin-Elmer, Gouda, The Netherlands).

Reagents

Cisplatin was a gift of Bristol Myers, Brussels, Belgium, and carboplatin was obtained from Johnson Matthey, Reading, Berkshire, U.K. Water was demineralized and doubly distilled. Sodium thiosulfate (Ph. Eur. grade) was from Brocacef, Maarsen, The Netherlands; NaCl, 9 g/L sterile solution, was from Lansberg, Uden, The Netherlands. Hexadecyltrimethylammonium hydroxide, 0.5 mol/L solution, was purchased from Kodak (Tramidoce, Weesp, The Netherlands). Sodium sulfate and sulfuric acid were of analytical quality.

Chromatography

The reversed-phase column was loaded to saturation with hexadecyltrimethylammonium sulfate, 10–2 mol/L (13). The mobile phase contained 10–4 mol of hexadecyltrimethylammonium monohydroxide per liter and was acidified by pH 3 with sulfuric acid to keep the hydrolyzed platinum species in the protonated form during separation (14). Sodium sulfate, 0.1 mol/L, was added to decrease the high retention of thiosulfate on this system. The flow rate was 1.5 mL/min. To measure platinum in the column effluent, we collected 1-min fractions and determined their platinum concentrations by atomic absorption spectrophotometry.
Procedures

Rate of reaction. The 10-mL incubation solutions contained either cisplatin (2 \times 10^{-3} to 2 \times 10^{-4} mol/L) and sodium thiosulfate (2 \times 10^{-3} to 5 \times 10^{-2} mol/L) in 0.15 mol/L NaCl solution (pH 6.4) or carboplatin (2 \times 10^{-5} to 2 \times 10^{-4} mol/L) and sodium thiosulfate (2 \times 10^{-3} to 5 \times 10^{-2} mol/L) in water (pH 6.1). These are the concentrations present in patients under therapeutic conditions (Table 1). Cisplatin and carboplatin were added to the incubation mixture at time zero from freshly prepared 1 mmol/L stock solutions. All solutions were incubated in the dark in a water bath, at 37 \pm 0.5^\circ C. We withdrew and analyzed 0.1-mL samples at regular intervals after the start of the incubation.

Protein binding. Plasma from healthy volunteers was incubated at 37 \textdegree C, with gentle agitation, with: (a) cisplatin, (b) cisplatin and thiosulfate, and (c) reaction products of cisplatin and thiosulfate. Cisplatin and sodium thiosulfate were added from 1.6 \times 10^{-3} and 1.6 \times 10^{-3} mol/L stock solutions (250 \mu L added to 20 mL of plasma). Reaction products of cisplatin and thiosulfate were obtained by incubating equal volumes of both stock solutions at 37 \textdegree C for 24 h; 1 mL of this solution was added to 40 mL of plasma. The experiments were performed in duplicate. We ultrafiltered 1-mL plasma samples, using the MPS-1 system provided with YMT-filters (cutoff 30 000 Da; Amicon, Oosterhout, The Netherlands). We dialyzed 4-mL plasma samples in Spectra- por membrane tubing (cutoff 12 000–14 000 Da; Lab-Center, Breda, The Netherlands) by swirling these at 4 \textdegree C for 22 h in 1 L of a solution containing, per liter, 0.15 mol of NaCl and 2 \cdot 10^{-2} mol of sodium thiosulfate. Platinum concentrations were determined in the plasma ultrafiltrate and in the dialyzed plasma by atomic absorption spectrometry as described previously (16).

Results

Rate of Reaction

Chromatograms of solutions of cisplatin and carboplatin incubated with sodium thiosulfate are shown in Figure 2. Cisplatin (peak 2) disappeared rapidly, but new platinum-containing peaks (no. 4 and 5) appeared rapidly. After 24 h cisplatin could no longer be detected, whereas thiosulfate (peak 3) was still abundantly present. Carboplatin (peak 6) disappeared more slowly than cisplatin and was still detectable after 24 h of incubation. New platinum-containing peaks (no. 7 and 8) appeared. Peak 8 may represent the same reaction product as peak 5 from Figure 2a, but it is formed more slowly.

The rate of disappearance of cisplatin and carboplatin in the presence of sodium thiosulfate, in the therapeutic concentration range of 5 \cdot 10^{-2} to 2 \cdot 10^{-3} mol/L, is shown in Figure 3. The disappearance of cisplatin and carboplatin appears to depend strongly on the thiosulfate concentration. The semilogarithmic plots could be reliably fitted with use of the linear least-squares method (r = 0.96–0.9999). Data on the half-lives of cisplatin and carboplatin (Table 2, mean values of duplicate experiments) indicate that cisplatin can be rapidly inactivated (t_{1/2} \approx 4 min) by thiosulfate under such conditions as are supposed to be present in the kidneys.

Table 1. Therapeutic Concentrations of Cisplatin and Carboplatin

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose, g/m²</th>
<th>Plasma</th>
<th>Peritoneum</th>
<th>Urine</th>
</tr>
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<tbody>
<tr>
<td>Cisplatin*</td>
<td>0.270</td>
<td>2.5 \times 10^{-3}</td>
<td>3 \times 10^{-4}</td>
<td></td>
</tr>
<tr>
<td>Carboplatin#</td>
<td>0.40</td>
<td>2 \times 10^{-3}</td>
<td>5 \times 10^{-4}</td>
<td></td>
</tr>
<tr>
<td>Na2S2O4</td>
<td>4 \times 8</td>
<td>2.6 \times 10^{-3}</td>
<td>2 \times 10^{-3}</td>
<td></td>
</tr>
</tbody>
</table>

\*Administered intraperitoneally over 4 h; results from ref. 6.  
\#Administered intraperitoneally over 4 h; unpublished own results.  
\$Administered intravenously, bolus injection plus infusion over 4 h; results from refs. 7, 15.

Fig. 2. Chromatograms of aqueous solutions of (a): cisplatin 2 \times 10^{-4} mol/L (peak 2), sodium thiosulfate 2 \times 10^{-3} mol/L (peak 3), and NaCl 0.15 mol/L (peak 1); and (b): carboplatin 2 \times 10^{-4} mol/L (peak 6) and sodium thiosulfate 2 \times 10^{-3} mol/L (peak 3) at time zero (left) and after 3 (middle) and 24 h (right) of incubation at 37 \textdegree C All peaks arising during incubation (peaks 4,5,7,8) contained platinum.

Fig. 3. Semilogarithmic degradation curves of (a): cisplatin 2 \times 10^{-4} mol/L and (b): carboplatin 2 \times 10^{-4} mol/L, in solutions of sodium thiosulfate at concentrations of 2 \times 10^{-3} (●), 5 \times 10^{-3} (■), 2 \times 10^{-2} (○), and 5 \times 10^{-2} mol/L (△).
the other hand, half-lives for the disappearance of cisplatin are rather long for concentrations such as those in plasma.

The disappearance rates of cisplatin and its hydrolysis products were also determined in aqueous solutions without sodium chloride. A $10^{-3}$ mol/L stock aqueous solution of cisplatin contained products corresponding to half-hydrolysis at 18.5 h after start of dissolution, as determined by the chromatographic–atomic absorption analysis. This solution was added to a $2 \cdot 10^{-3}$ mol/L solution of thiosulfate, without NaCl, to give a total platinum concentration of $2 \cdot 10^{-4}$ mol/L. Under these circumstances, the peak corresponding to the hydrolyzed products disappeared almost instantaneously, while the cisplatin peak degraded with a half-life of 61.9 min, an interval comparable with those found for solutions containing NaCl (Table 2). NaCl, 0.15 mol/L, did not influence the degradation of $2 \cdot 10^{-4}$ mol/L carboplatin by reaction with $2 \cdot 10^{-3}$ mol/L thiosulfate.

**Protein Binding**

Figure 4 illustrates the effect of sodium thiosulfate on the binding of cisplatin to plasma proteins in vitro. Human plasma incubated with only cisplatin at a concentration of $2 \cdot 10^{-5}$ mol/L contained ultrafilterable platinum species, 11% of the total platinum concentration, 24 h after the start of incubation. We added 2 mmol of sodium thiosulfate per liter and continued the incubation for another 24 h. This increased the ultrafilterable platinum species from the original 11% to 15% within 4 h, with no substantial change thereafter. In contrast, the simultaneous addition of cisplatin and sodium thiosulfate to plasma resulted in a protein binding of platinum species of only 57% after 24 h, suggesting that the reaction products of cisplatin and thiosulfate were bound to a lesser extent than was cisplatin itself. This was confirmed by incubating human plasma with a solution containing only reaction products. An immediate protein binding was observed, amounting to 40%, which had increased to 50% by 24 h.

We investigated the reversibility of this binding in a separate experiment. Plasma samples incubated with the reaction products between cisplatin and thiosulfate were ultrafiltered or dialyzed in 1 L of solution containing 0.15 mol of NaCl and $2 \cdot 10^{-3}$ mol of sodium thiosulfate. Sodium thiosulfate was added to the dialysis fluid to prevent the degradation of the formed platinum–thiosulfate reaction products. The volumes inside the bags did not change during dialysis. As Figure 5 shows, initially only 4.5% of the reaction products was bound irreversibly to plasma components with a relative molecular mass $>14,000$; this percentage increased to 20% after 26 h.

**Discussion**

Anti-tumor and toxic properties of platinum complexes are thought to result from ligand-exchange reactions with human macromolecules (17). Reactions of platinum complexes are relatively slow, owing to the covalent bond character of the complex-formed ligands (12, 17). Both the stability of the Pt–ligand bond and the concentration and nucleophilic strength of the reactant determine the rate of reaction. In principle, the reaction between platinum complexes and thiosulfate has a second-order rate constant (18). Owing to the relatively high concentration of thiosulfate—in comparison with that of the platinum compound used—in the incubation experiments, we observed a pseudo-first-order behavior, as shown by the straight lines of Figure 3.

The presence of chloride appeared not to influence the rate of reaction of the platinum(II) complexes with thiosulfate in the concentration range we used. This accords with the much lower nucleophilic strength of chloride as compared with thiosulfate (12, 18). The pH of the incubation

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**Table 2. Half-Lives of Degradation (min)**

<table>
<thead>
<tr>
<th>Thiosulfate concn, mol/L</th>
<th>Cisplatin, mol/L</th>
<th>Carboplatin, mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2 \cdot 10^{-3}$</td>
<td>$2 \cdot 10^{-6}$</td>
<td>$2 \cdot 10^{-4}$</td>
</tr>
<tr>
<td>5 $\times$ 10$^{-3}$</td>
<td>60.1</td>
<td>555</td>
</tr>
<tr>
<td>5 $\times$ 10$^{-2}$</td>
<td>47.5</td>
<td>537</td>
</tr>
<tr>
<td>5 $\times$ 10$^{-1}$</td>
<td>12.0</td>
<td>33.8</td>
</tr>
<tr>
<td>5 $\times$ 10$^{-2}$</td>
<td>3.7</td>
<td>28.0</td>
</tr>
</tbody>
</table>

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Fig. 4. In vitro binding of platinum to human plasma proteins at 37 °C. Platinum concentrations in plasma ultrafiltrate are expressed as percentage of the total platinum concentration in plasma. Incubation of plasma with: cisplatin $2 \cdot 10^{-5}$ mol/L (○); sodium thiosulfate $2 \cdot 10^{-3}$ mol/L after pre-incubation with cisplatin $2 \cdot 10^{-5}$ mol/L, for 24 h (△); cisplatin $2 \cdot 10^{-5}$ mol/L + sodium thiosulfate $2 \cdot 10^{-3}$ mol/L (◇); and reaction products of cisplatin and sodium thiosulfate (□).

Fig. 5. Percentage of cisplatin–thiosulfate reaction products bound to plasma proteins as measured after ultrafiltration (●) and after dialysis (●).
solutions (6.4 and 6.1) differed from physiological pH by only 1 unit. The rate of the reaction between intact cisplatin and thiosulfate will not be influenced by this difference, because the concentrations of the reactants will not change in this pH range.

The rate of reaction of carboplatin with sodium thiosulfate was 10-fold less than that of cisplatin, which is in agreement with the higher stability of its dicarboxylic acid ligand (19). The relatively long half-lives of carboplatin (+9 h), at the concentrations found in plasma and peritoneum, do not suggest a possible protection against its myelotoxicity by chemical interaction with thiosulfate.

Induction of nephrotoxicity by cisplatin is assumed to be a fast process involving reaction with protein-bound sulfhydryl groups in the renal tubules (17, 20). This renal damage is mainly caused during administration and in the first hours thereafter (21). The high concentration of thiosulfate found in urine during thiosulfate administration (15) suggests that there are relatively high concentrations of thiosulfate in kidney tissue. At these concentrations the reaction rate between cisplatin and thiosulfate is high (k = 0.17 min⁻¹, t½ = 4 min). Consequently, competition between thiosulfate and endogenous thiol groups for cisplatin may decrease tubular damage.

On the other hand, the half-lives of free platinum in plasma of patients [t½β = 10 min, t½β = 40 min (16)] are shorter than the half-life of cisplatin degradation by reaction with thiosulfate in vitro (1 h). Although some cisplatin will be degraded in plasma or peritoneum, the increased dose of cisplatin will result in an increase of the area under the curve for plasma concentration of intact cisplatin vs time (8). Furthermore, thiosulfate distribution is limited to the extracellular fluid, as determined in dogs (22). This indicates that thiosulfate will not react inside the cell with cisplatin or its hydrolyzed derivatives, which are assumed to exert the anti-tumor activity. For the same reason, dissociation of Pt(II)–DNA adducts by thiosulfate, as observed by Filipski et al. (23), is not expected to occur in vitro.

On the other hand, the half-lives of free platinum in plasma of patients [t½β = 10 min, t½β = 30 min (16)] are shorter than the half-life of cisplatin degradation by reaction with thiosulfate in vitro (1 h). Although some cisplatin will be degraded in plasma or peritoneum, the increased dose of cisplatin will result in an increase of the area under the curve for plasma concentration of intact cisplatin vs time (8). Furthermore, thiosulfate distribution is limited to the extracellular fluid, as determined in dogs (22). This indicates that thiosulfate will not react inside the cell with cisplatin or its hydrolyzed derivatives, which are assumed to exert the anti-tumor activity. For the same reason, dissociation of Pt(II)–DNA adducts by thiosulfate, as observed by Filipski et al. (23), is not expected to occur in vitro.

The results of our chemical experiments support the limited data gathered from patient studies so far. Howell et al. (6–8) clearly showed that systemic administration of thiosulfate inhibited nephrotoxicity after high doses of intraperitoneally and intravenously administered cisplatin. Lack of anti-tumor effect (as observed in the study with intravenous cisplatin) may be explained by the extensive pretreatment and the small number of selected patients (8). In contrast to these negative results, responses were observed in patients treated intraperitoneally with cisplatin at 90–270 mg/m² (6, 7). Recently, an objective response was observed in four out of six patients with peritoneal carcinoid, who received cisplatin, 90–135 mg/m², intraperitoneally with simultaneous sodium thiosulfate intravenously (9). Nephrotoxicity was completely prevented.

The reversible protein binding of the platinum–thiosulfate complexes—in contrast to the irreversible protein binding of cisplatin (17, 18)—may explain the protecting capacity of sodium thiosulfate. This study also showed that sodium thiosulfate is not able to reverse platinum–protein binding to any major extent, which explains why renal tubular proteins can only be protected when thiosulfate is present during and shortly after cisplatin administration, as observed in mice (3).

We concluded that the short half-lives of cisplatin at high thiosulfate concentrations, together with the decreased and partly reversible protein binding of the reaction products, may explain the observed protection against nephrotoxicity. The low concentrations of thiosulfate in the plasma, peritoneum, and intracellular compartments will cause only a limited loss of administered cisplatin, which is amply compensated by higher cisplatin doses.

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References


