Effect of Diethylthiocarbamate on cis-Diaminedichloroplatinum(II)-induced Cytotoxicity, DNA Cross-Linking, and \(\gamma\)-Glutamyl Transpeptidase Inhibition

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ABSTRACT

Diethylthiocarbamate (DDTC) has been shown to protect against the toxicity of cis-diaminedichloroplatinum(II) (DDP) without inhibition of antitumor effect. We report here that DDTC is unreactive toward DDP complexes in which both chlorides have been replaced by guanine residues but removes platinum from a variety of other ligands, and that this difference in reactivity may provide the basis for the selective protection observed with DDTC. Platinum-DNA complexes were unreactive toward DDTC (10 mM, >4 h) when the platinum:base ratio \(r < 0.02\). DDTC did not react with the 1:2 complex of DDP:guanosine but reacted rapidly with the 1:1 complex and with the 1:2 complexes of DDP:adenosine. Reaction of DDP with DDTC was second order with a rate constant \(k = 4.4 \text{ M}^{-1} \text{s}^{-1}\) at 37°C, corresponding to a \(k_0 = 150\) min at [DDTC] = 1 mM. Treatment of L1210 cells with DDTC (0.5–10 mM) after exposure to DDP indicated that DDTC had no effect on cell kill if DDTC treatment was delayed for 1 h after DDP. The effect of DDTC on DDP-induced DNA interstrand cross-links was also examined in L1210 cells. Interstrand cross-links were decreased by 50% when cells were treated with DDTC immediately after DDP; no change in DNA interstrand cross-links was observed when DDTC treatment occurred 3 h after DDP. A modified alkaline elution procedure was used to evaluate the effects of high concentrations of DDTC, thiourea, and cyanide caused extensive reversal of cross-links at concentrations as low as 10 and 1 mM, respectively. Both commercial and rat kidney brush border preparations of \(\gamma\)-glutamyl transpeptidase were inhibited by exposure to 2 mM DDP; exposure of the inhibited enzyme to DDTC (1 or 10 mM) resulted in significant restoration of enzyme activity. These data indicate that DDTC has unique chemical specificity in its reactions with platinum complexes and that this specificity is ideal for application as a chemoprotective drug against cis-platinum toxicity.

INTRODUCTION

DDP\(^{1}\) is believed to generate tumoricidal lesions by formation of bifunctional adducts with DNA (1). In contrast, the mechanistic basis for DDP's toxicity to the kidney and other organ systems is not known. We previously hypothesized that nephrotoxicity and gastrointestinal toxicity may be a consequence of platinum binding and inactivation of thiol-containing enzymes, and that the ability of DDTC to inhibit DDP toxicity results from DDTC's ability to remove platinum from these protein thiol groups (2). Evidence in support of this enzymatic basis for DDP toxicity has appeared (3, 4). A number of sulfur nucleophiles has been studied as inhibitors of DDP-induced nephrotoxicity (5–10); however, selective protection of normal tissue without inhibition of antitumor effect has been difficult to achieve. Thiourea reacts rapidly with platinum complexes and has been shown to reverse platinum:DNA cross-links in solution (11) and inhibit platinum:DNA cross-links in cultured cells (12, 13). We and others (14–21) have shown that DDTC provides protection against renal, gastrointestinal, and bone marrow toxicity induced by DDP without concomitant inhibition of DDP's antitumor effect. We describe here the results of our studies on the reaction of DDTC with a number of platinum complexes, which support the hypothesis that DDTC selectively reverses platinum:thiol complexes without reversal of platinum:DNA cross-links.

MATERIALS AND METHODS

Chemicals. cis-Platinum was obtained through the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, or from Bristol Laboratories. Sodium diethylthiocarbamate, salmon sperm DNA, \(\gamma\)-glutamyl \(\beta\)-nitroanilide, guanosine, adenosine, cytidine, Tris, sodium dodecyl sulfate, and \(\gamma\)-glutamyl transpeptidase were obtained from Sigma Chemical Co. HPLC solvents, sodium cyanide, thiourea, and EDTA were obtained from Biber Chemical Company. Noble agar was obtained from Difco Laboratories, and proteinase K and tetrapropyl ammonium hydroxide were obtained from Merck and RSA, Inc., respectively. Radiolabeled thymidine and Aquasol were purchased from New England Nuclear. RPMI Medium 1640 and horse serum were obtained from Gibco.

Hydrolysis of DDP: A chloride ion-specific electrode (Orion, Inc.) was placed in a solution of DDP (1.7 or 3.3 mM in 5 mM sodium nitrate), the electrode was connected to a Corning pH meter set on the millivolt scale, and labeled chloride ion was measured every 2 h for 2 h by comparison of the millivolt readings to those obtained from chloride ion standard curves. Determinations were made in triplicate at both 20°C and 37°C.

Kinetics of the Reaction between DDP and DDTC. A solution of 5 mM DDTC and 50 mM Tris (pH 7.4, 37°C) in distilled water was divided into ten 975-\(\mu\)l portions. To each sample were added 25 \(\mu\)l of a freshly prepared DDP solution (0.60 mg in 1.0 ml of distilled water) to give a final DDP concentration of 50 \(\mu\)M. Surplus were removed from the constant temperature bath at intervals between 2 h; CHCl\(_3\) (2.0 ml) was added, the sample was agitated for 1 min, and the CHCl\(_3\) layer was analyzed for platinum by flameless atomic absorption analysis and by formation, extraction, and HPLC analysis of platinum bis(diethylthiocarbamate) (Footnote 4; Ref. 22). The pseudo-first order rate constant was determined from the slope of the plot of \(\ln(H_0 - H_t)/t\), where \(H_0\) and \(H_t\) are the Pt(DDTC) peak heights at 3 h and time \(t\), respectively. The slope was \(6.2 \times 10^{-2} \text{s}^{-1}\) determined by linear regression, \(r^2 = 0.995\).

Kinetics of DDTC Binding to DNA. A solution of salmon sperm DNA was prepared in 20 mM NaClO\(_4\) to give a solution which was 2.5 mM in DNA base, determined by absorbance at 260 nm. A solution of DDP (1 mg/ml in 20 mM NaClO\(_4\)) was prepared, and aliquots were added to the DNA solution in amounts required to produce platinum:base ratios of 0.01, 0.02, 0.05, 0.08, and 0.1, respectively. Final base concentration was adjusted to 2.2 mM by the addition of 20 mM NaClO\(_4\). Aliquots (1 ml) were removed every 15 min for 3 h and every 30 min for an additional 5 h, added to 1 ml of 0.1 M NaCl, and centrifuged for 7 min at 450 \(\times \) g through spin dialysis cones (Amicon; \(M_t\) cutoff, 30,000). The platinum concentration in the ultrafiltrate was determined by flameless atomic absorption analysis and by formation, extraction, and HPLC analysis of platinum bis(diethylthiocarbamate) (Footnote 4; Ref. 22). Excellent agreement was obtained between the two methods.

The effect of phosphate buffer on binding kinetics was examined at a platinum:base ratio of 0.01. The solutions were prepared as described

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: DDP, cis-diaminedichloroplatinum(II); DDTC, sodium diethylthiocarbamate; GGT, \(\gamma\)-glutamyl transpeptidase; HPLC, high-pressure liquid chromatography.


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above, except that the appropriate concentrations of sodium phosphate buffer were used in place of the NaClO₄ solutions.

**Reversal of Platinum:Base and Platinum:DNA Complexes.** Platinum:DNA complexes were prepared at platinum:base ratios 0.01-0.1 using the procedures described above and allowing the reactions to go to completion in the dark. The resulting solutions of the platinum:DNA complexes were equilibrated at 37°C for 15 min, and DDTC was added to give a final concentration of 10 mM. Aliquots were removed at 30-min intervals and extracted with CHCl₃ (1 ml), and the CHCl₃ extract was analyzed for Pt(DDTC)₂ by HPLC as described previously (Footnote 4; Ref. 22).

The platinum:bis-nucleoside complexes were prepared by reacting 80 µmol of adenosine, guanosine, or cytidine and 20 µmol of DDP in 200 µl of medium (20% horse serum) for 1 h at 37°C. The samples were then washed with EDTA, and the DNA was eluted as described above. Plating efficiencies with the 35% internal standard DNA retention as the end point of reaction was monitored by HPLC as described below. The platinum:guanosine monoadduct was prepared by reaction of guanosine with excess DDP; the reaction was monitored by HPLC and terminated when monoadduct formation was maximal. Adducts were isolated by preparative HPLC and characterized by UV, nuclear magnetic resonance, and platinum atomic absorption spectroscopy, and by comparison of HPLC retention times using conditions identical to those reported by Eastman (23). The bisadenosine complexes were obtained as an inseparable mixture of two isomers, and subsequent reactions were carried out on the isomeric mixture. The kinetics of platinum:base reversal was measured by adding DDTC to these solutions to a final concentration of 10 mM at 37°C. Aliquots were removed, and the platinum:base complex was quantified by HPLC.

**Cell Treatments.** Exponentially growing L1210 cells in RPMI Medium 1640 containing 10% horse serum were labeled for 24-48 h with [3H]thymidine (0.01 µCi/ml). The cells were washed twice with RPMI Medium 1640 and then resuspended in this medium to give a final density of 5-8 × 10⁶ cells/ml after addition of drug solution. The cells were equilibrated at 37°C for 15-30 min and then treated with DDP for 1 h. Cells were then washed 3 times with supplemented medium, resuspended in the same medium, and treated with DDTC for 1 h at the concentrations and times indicated. Again cells were washed 3 times in supplemented medium and assayed for viability by the method of Chu (24). Between 10⁶ and 10⁷ cells were suspended in 3 ml of medium containing 0.01% Noble agar, and colonies were counted after 12-15 days of incubation.

**DNA Interstrand Cross-Linking.** The alkaline elution assay was performed as previously described (25). L1210 cells were labeled and exposed to DDP with or without DDTC as described above. Experimental cells were subjected to 300 rads of X-ray at 4°C, and [3H]-labeled internal standard L1210 cells received 100 rads of X-ray at 4°C. Equal numbers (2 × 10⁶) of treated and control cells were mixed, deposited on polyvinyl chloride filters, and lysed with a solution of 2% sodium dodecyl sulfate, 0.3% glycine, 20 mM EDTA, and proteinase K (0.5 mg/ml). The filters were then eluted with a solution of 0.1 M tetrapropylammonium hydroxide and 0.02 M EDTA at pH 12.1 with an elution rate of 2 ml/h. A total of ten 3-ml fractions was collected, and the radioactivity of the eluted fractions and filter was determined by scintillation counting in Aquasol. Cross-linking was expressed in rotequiv of reaction was monitored by HPLC as described below. The platinum:base complex was quantified by HPLC.

**RESULTS**

**DDP Reaction Kinetics.** Rate constants for the hydrolysis of DDP in 5 mM aqueous NaNO₂ were 1.7 ± 0.3 × 10⁻³ min⁻¹ and 6.4 ± 0.8 × 10⁻³ min⁻¹ at 20°C and 37°C, respectively, determined by measuring chloride release from the platinum complex. This corresponds to a half-life of almost 2 h at 37°C for the replacement of the first chloride ligand by a water molecule. These rate constants are in reasonable agreement with reported values (28) and were determined to provide a base-line value for the experiments to be described. The second-order rate constant for the reaction of DDP with DDTC was 4.4 m⁻¹ min⁻¹ at 37°C, over 4 orders of magnitude greater than the rate constant for reaction of DDP with water. When [DDTC] = 1 mM, however, the reaction rates between DDP and water or DDTC are comparable (t₅₀, 110 and 150 min, respectively, at 37°C). Thus, DDTC is essentially unreactive toward DDP at maximal plasma concentrations of approximately 1 mM observed after a protective dose in vivo, suggesting that the mechanism of DDTC protection does not involve direct interception and inactivation of the parent drug.

The rate constant (6.2-6.9 × 10⁻³ min⁻¹) for reaction of DDP with salmon sperm DNA (pH 6.8, 37°C) was essentially identical to the rate of DDP hydrolysis and was independent of the platinum:base ratio over the range 0.01-0.1. Reaction of salmon sperm DNA with the diaquo complex of DDP was complete in minutes. These results are consistent with DDP hydrolysis as the rate-determining step in DNA binding as reported previously (29).

The presence of phosphate buffer decreases the rate of platinum binding to DNA in a concentration-dependent manner (Table 1); 100 mM phosphate decreases the rate of binding approximately 3.5-fold. The rate of binding at a constant phosphate buffer concentration is also pH dependent, with a slower rate at increased pH above 6.8. These data are consistent with the reactive platinum species having the structure...
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**Table 1** Effect of phosphate concentration and pH on the rate of cis-platinum binding to salmon sperm DNA (platinum:base ratio r = 0.01) at 37°C

<table>
<thead>
<tr>
<th>PO4 (mM)</th>
<th>pH</th>
<th>10^3 × k (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6.8</td>
<td>5.2</td>
</tr>
<tr>
<td>20</td>
<td>6.8</td>
<td>4.3</td>
</tr>
<tr>
<td>50</td>
<td>6.8</td>
<td>3.3</td>
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<tr>
<td>100</td>
<td>6.8</td>
<td>1.9</td>
</tr>
<tr>
<td>50</td>
<td>6.5</td>
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</tr>
<tr>
<td>50</td>
<td>7.0</td>
<td>2.4</td>
</tr>
<tr>
<td>50</td>
<td>7.5</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*Pseudo-first-order rate constant k determined from the slope of ln (DDT/ DDP) versus t derived from linear regression; r² > 0.98 for all experiments.

Pt(NH₃)₂(H₂O)(OH)⁺; the pKₐ of the underlined hydrogen is 7.2, and as this proton dissociates, the easily replaced water molecule becomes a far less reactive hydroxide ligand. Phosphate presumably competes with DNA for the reactive platinum species to form a stabilized phosphate complex.

Effect of DDTC on Platinum:DNA and Platinum:Purine Complexes. Platinum:DNA complexes were prepared by reaction of salmon sperm DNA with DDP at different platinum:base ratios. These complexes were then reacted with DDTC (10 mM, 37°C), and the platinum was removed and quantified as the DDTC complex by HPLC analysis. For platinum:base ratios < 0.05, < 1% of the platinum bound to DNA was removed after 2 h of DDTC treatment (data not shown). This suggests that DDTC should not reverse the cytotoxic lesion in tumor cells, where the platinum:base ratio is < 0.01 (27). Reaction of DDTC with the DDP:guanosine mono- and bisadducts and with a mixture of the DDP:adenosine bisadducts was also examined (Fig. 1). The bisadduct with guanosine was stable to DDTC (10 mM, 37°C), showing no evidence of reaction after 4 h. In contrast, the DDP:guanosine monoadduct and the two DDP:adenosine bisadducts reacted rapidly with DDTC, showing pseudo-first-order kinetics with half-times of approximately 2, 30, and 100 min, respectively. These data suggest that replacement of both chloride ligands by guanosine residues confers unique stability to DDTC for the platinum complex; the absence of significant platinum:DNA reversal at low platinum:base ratios is consistent with the observations of Eastman that guanine is the initial platinum coordination site in DNA (23).

Effect of DDP with or without DDTC on L1210 Cell Survival. In order to assure that DDTC was used at concentrations which had no direct effect on cell survival, L1210 cells were exposed for 1 h to DDTC alone at a concentration ranging from 0.5–5 mM, and cell survival was evaluated by the soft agar cloning assay. Cell survival was >90% at DDTC concentrations ≤ 2 mM, so DDTC was used at a concentration of 0.5–1 mM in subsequent experiments.

The effect of DDP on L1210 survival was assessed using a 1-h exposure at DDTC concentrations of 1–20 μM; this concentration range gave dose-response curves with reproducible D₀ values (the concentration of DDTC that reduces survival to 37% as derived from the exponential portion of the survival curve). The effect of a 1-h DDTC exposure beginning 1.5, 2, and 4 h after the start of a 1-h DDP exposure is summarized in Table 2; these times corresponding to an interval of ½, 1, and 3 h, respectively, between the end of DDP treatment and initial DDTC exposure demonstrate that DDTC has no effect on DDP cytotoxicity under these conditions. These data indicate that a delay of at least 1 h between DDP and DDTC administration in vitro should be sufficient to prevent the inhibition of DDP-induced cytotoxicity.

Effect of DDTC with or without DDTC on DNA Interstrand Cross-Links. The effect of DDTC on DDP-induced DNA interstrand cross-links was evaluated by the alkaline elution technique; all cells received 300 R to introduce single-strand breaks (25). L1210 cells were treated with DDP (20 μM, 1 h) with or without DDTC (1 mM, 1 h) 3 h later. The effect of DDTC alone was also evaluated in cells receiving no DDP. Alkaline elution was carried out 6 h after DDP treatment. DDTC alone had no effect on the DNA elution profile; similarly, DDTC treatment 3 h after DDP had no effect on the extent of DDP-induced DNA interstrand cross-linking. The effect of DDTC immediately and 3 h after DDP treatment and washing of cells was evaluated by alkaline elution at different times. The results are expressed in rad-equivalent cross-links (Fig. 2). Although exposure to DDTC 3 h after DDP had no effect on interstrand cross-linking, a significant reduction in cross-links was observed when cells were exposed to DDTC immediately after DDP treatment.

The alkaline elution technique was modified in order to examine the effect of toxic concentrations of DDTC and other agents on platinum:DNA interstrand cross-links formed after an 8–12 h incubation period following cell treatment with DDP. Rad-equivalent cross-links were determined as described for the normal alkaline elution procedure; reversal is expressed in percentage and determined from the decrease in cross-links resulting from exposure of DNA to drug on the filter compared with controls (Table 3). DDTC was unable to reverse interstrand cross-links even after exposure of the DNA to 0.5 mM DDTC for 4 h. In contrast, exposure of DNA to thiourea caused significant removal of platinum interstrand cross-links; Table 2 Effect of 1-h DDTC treatment on L1210 cell survival after cis-platinum exposure (1–20 μM, 1 h)

<table>
<thead>
<tr>
<th>DDTC (μM)</th>
<th>Interval (h)</th>
<th>D₀ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>3.6 ± 0.7*</td>
</tr>
<tr>
<td>0.5</td>
<td>−2.0</td>
<td>8.4 ± 0.4*</td>
</tr>
<tr>
<td>0.5</td>
<td>1.5</td>
<td>4.4 ± 0.5</td>
</tr>
<tr>
<td>1.0</td>
<td>1.5</td>
<td>5.1 ± 0.3</td>
</tr>
<tr>
<td>0.5</td>
<td>2.0</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>0.5</td>
<td>4.0</td>
<td>4.1 ± 0.8</td>
</tr>
</tbody>
</table>

*Time between the beginning of DDP and DDTC exposure. The interval between the end of DDP and the beginning of DDTC treatment is 1 h less than this value.

*Mean ± SD.

*Significantly different from control (P < 0.01, the Student t test); other values not significantly different (P > 0.1).
DISCUSSION

DDP is a relatively unreactive species chemically; even with a powerful nucleophile like DDTC, the reaction half-life is approximately 15 min and 2.5 h at DDTC concentrations of 10 and 1 mM, respectively (37°C). The rate of platinum binding to DNA which we and others (29) have observed, the rate of DDP-induced inactivation of GGT, and the ability of chloride ion to interfere with DNA binding and enzyme inhibition are consistent with DDP hydrolysis as the rate-limiting step in these reactions. In contrast, the reaction of DDP with DDTC is 40,000-fold faster than with water, confirming that DDTC reacts with DDP by direct substitution rather than by initial rate-limiting hydrolysis. It is important to note, however, that the concentration differences between water (55 μM) and pharmacological DDTC (1 mM) in vivo result in nearly equivalent this property of thiourea has been reported previously (11). Cyanide is a ligand with high affinity for platinum, and its ability to reverse DNA interstrand cross-links was used to demonstrate the feasibility of the DNA filter method.

Effect of DDP with or without DDTC on γ-Glutamyl Transpeptidase Activity. GGT was incubated with 2 mM DDP at 37°C, and enzyme activity was assayed at various times (Fig. 3). DDP clearly inhibits enzyme activity in a time-dependent fashion. DDTC restores activity to the platinum-inhibited enzyme, with the higher DDTC concentration providing more rapid and complete recovery. GGT activity was also measured in F344 rat kidney brush border membranes. Incubation with DDP (2 mM) shows first-order loss of enzyme activity (rate constant k = 4.2 × 10−3 min−1). Addition of chloride ion decreases the concentration of aquated platinum species and retards the rate of enzyme inhibition. In contrast, exposure of enzyme to aquated DDP accelerates the initial rate of enzyme inhibition.

Membrane suspensions were incubated with DDP (2 mM) for 3 h and then incubated with 0–10 mM DDTC (Fig. 4). Restoration of enzyme activity is observed after treatment with 1 or 10 mM DDTC; recovery is more rapid at the higher DDTC concentration. Enzyme activity after treatment with 0.1 mM DDP is not significantly different from that observed when the enzyme was resuspended in drug-free medium. These results demonstrate that DDP inhibition of a model renal enzyme can be reversed by pharmacological concentrations of DDTC which provide chemoprotection in vivo (14).
half-lives under these conditions.

The reaction of DDTC with platinum:nucleoside and platinum:DNA complexes was examined to determine whether DDTC's selective chemoprotection might be based upon a fundamental difference in reactivity among different platinum complexes. Both the initial (30) and predominant (31) cytotoxic platinum:DNA lesions involve replacement of chloride by guanine bases in the same (32) or opposite strand bound at the N-7 position. Our observation that DDTC is unreactive toward platinum:bisguanine complexes prepared from both the nucleoside and from DNA may explain its failure to inhibit DDP's antitumor response.

Thiourea is another ligand with high affinity for platinum complexes. It is effective as a chemoprotector against DDP nephrotoxicity in animal models, but it also inhibits tumor response to DDP in vivo (5) and enhances survival of DDP-treated tumor cells in vitro (>1 log unit even after a 2-h interval between DDP and thiourea treatment) (13). In contrast, DDTC had no effect on L1210 cell survival when an interval of at least 1 h between DDP and DDTC treatment was used. The effects of thiourea and DDTC on platinum:DNA interstrand cross-linking in L1210 cells are also quite different. Thiourea does not reverse preformed cross-links in cells; it presumably enhances survival by inhibiting subsequent cross-linking of platinum:DNA monoadducts when cells are treated with thiourea after DDP exposure (13). Interstrand cross-links continue to accumulate after DDTC treatment, however, and the extent of cross-linking at later times is unaffected if DDTC treatment is sufficiently delayed. Thiourea (1 M) has been shown to reverse lethal platinum:DNA cross-links in a noncellular system (11). Using a modification of the alkaline elution assay, we observe that thiourea at concentrations as low as 10 mm reverses interstrand cross-links, whereas 500 mm DDTC has no effect on platinum:DNA interstrand cross-links. Thus, in spite of their similarities in affinity for platinum complexes, DDTC and thiourea behave very differently in both their chemical reactions and their effects on DDP-treated cells.

γ-Glutamyl transpeptidase is an enzyme of considerable importance in the renal tubule; it is concentrated in proximal tubule brush border and contains a sulfhydryl group essential for enzymatic activity. This enzyme was chosen as a model to evaluate DDP-induced inhibition and DDTC-induced recovery of enzyme activity. First-order inhibition of the enzyme by DDP was observed at a rate consistent with initial DDP hydrolysis as the rate-limiting step. Enzyme activity was restored to the inhibited complex by treatment with DDTC at concentrations which have been observed in vivo after pharmacological doses (14). These results do not implicate GGT as the primary target in DDP-induced nephrotoxicity; however, they do provide a model for DDTC reversal of cis-platinum-induced inhibition of an important biochemical process. DDTC is known to inhibit other enzymes containing essential sulfhydryl groups (3, 4), and a similar effect of DDTC on DDP-induced cross-linking and inactivation of human α2-macroglobulin has also been described (33).

The data presented in this paper provide a mechanistic basis for the selectivity of DDTC to inhibit DDP toxicity in vivo without inhibition of antitumor effect (14). DDTC provides effective chemoprotection even when given after DDP; this is a unique property of platinum drug chemoprotectors and is consistent with a mechanism involving reversal of toxic platinum lesions. The effect of structural differences on reactivity of platinum complexes can be subtle; DDTC reacts readily with platinum: bisadenine complexes but is essentially unreactive toward the corresponding guanine complexes. The platinum:DDTC complex is thermodynamically stable and chemically and biologically unreactive; thus, platinum removed from sites of potential toxicity is essentially inactivated. We are currently evaluating the potential of DDTC as a DDP chemoprotector in the clinic; the results will be described in a forthcoming publication.

REFERENCES

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