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# Modulation of Plasma Cyst(e)ine by Cisplatin*

WALLY E. WUNG and STEPHEN B. HOWELL†

*Department of Medicine and The Cancer Center, University of California, San Diego, La Jolla, CA 92093, U.S.A.*

**Abstract**—*Some types of human malignant cells are cyst(e)ine auxotrophs. In 8 cancer patients given 10 courses of treatment cisplatin caused a mean (±S.D.) 83 ± 17% decrease in plasma cyst(e)ine when measured before and 6 hr after drug infusion. The magnitude of the decrease correlated with peak plasma cisplatin concentration, and serial measurements demonstrated that in some patients plasma cyst(e)ine was still decreasing at 6 hr. The mean 6-hr plasma cyst(e)ine was low enough (9 ± 9 μM) to prevent the proliferation of human promyelocytic (HL-60) cells in culture.*

## INTRODUCTION

*cis*-DICHLORODIAMMINEPLATINUM(II) (DDP) is an important chemotherapeutic agent which has activity against a variety of human neoplasms [1]. Its cytotoxicity has been attributed to its ability to react with nucleophilic sites on DNA, forming interstrand and DNA–protein cross-links [2]. DDP can also react with nucleophilic sites on smaller molecules to form soluble complexes that are no longer cytotoxic. A number of sulfur-containing compounds, including thiourea, thiosulfate and diethyldithiocarbamate, reduce DDP cytotoxicity [3–6], and cyst(e)ine blocks the binding of DDP to purified maleate dehydrogenase [7] and calf thymus DNA [8].

In mammalian cells cyst(e)ine can be synthesized from methionine via the trans-sulfuration pathway [9]. Methionine is converted first through a series of steps to cystathionine, and then in the final step cystathionine is cleaved by γ cystathionase (E.C. 4.2.1.15) (CSE) to cyst(e)ine, α-ketobutyrate and ammonia [10]. Prior studies have demonstrated that many malignancies of both human and murine origin are deficient in CSE and behave as cyst(e)ine auxotrophs, while non-malignant Epstein–Barr virus-transformed lymphoid cell lines contain the enzyme and are cyst(e)ine prototrophs [11, 12]. CSE activity was also found to vary with the extent of leukemic infiltration of human marrow and lymphomatous replacement of the AKR mouse thymus, being much lower when the large numbers of malignant cells were present [13, 14]. The decreased CSE activity is due to a reduced amount of enzyme protein rather than to the presence of an inhibitor, or structural or kinetic differences [11, 14, 15]. Although CSE deficiency is probably not unique to malignant cells [15], these observations have fostered attempts to use depletion of plasma cyst(e)ine by enzymatic techniques as a means to develop a therapeutic program based on enzymatic depletion of plasma cyst(e)ine [12]. The fact the L-selenocysteine, a compound that blocks the transport of cyst(e)ine, produced responses in murine tumors and in man has given further impetus to this approach [16, 17].

The present study demonstrates that the treatment of cancer patients with DDP causes a marked reduction in plasma cyst(e)ine, that DDP reacts directly with cyst(e)ine and that plasma cyst(e)ine concentrations are reduced into a range that is low enough to impair the proliferation of human promyelocytic leukemia cells (HL-60) *in vitro*.

## MATERIALS AND METHODS

Eight patients received treatment with 10 courses of DDP 90–180 mg/m$^2$ instilled into the peritoneal cavity for 4 hr. Patients receiving DDP at a dose greater than 90 mg/m$^2$ also received

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†To whom requests for reprints should be addressed at: Department of Medicine T-012, University of California, San Diego, La Jolla, CA 92093, U.S.A.

sodium thiosulfate intravenously for 12 hr after the start of DDP exposure. The details of this treatment program have been described elsewhere [18].

Plasma amino acids were measured in heparinized samples immediately before and at 6 hr after the start of DDP exposure on 10 courses of treatment, and at more frequent intervals on 3 courses. Plasma samples were deproteinated with perchloric acid, which oxidized all of the cysteine to cystine, and the sum of the cysteine and cystine concentrations (cyst(e)ine concentration) were quantitated using a Beckman 119CL amino acid analyzer. The concentration of the other amino acids shown to block the reaction of DDP with DNA [8] (asparagine, glutamine, methionine, histidine and lysine) were also measured on the same samples.

DDP was measured using a modification of a high-pressure liquid chromatographic assay reported by Bannister *et al.* [19] which quantitates only those species of DDP that are still capable of reacting with a nucleophilic site on another molecule. Blood was drawn into ice-cold heparinized tubes, the plasma separated at 4°C and then the protein removed by centrifugation through CF 25 A filter cones (Amicon Corp., Lexington, MA). Reactive forms of DDP were immediately derived by addition of excess diethyldithiocarbamate and promptly subjected to chromatographic separation and quantification.

Chromatographic separation of cysteine from the DDP–cysteine complex was accomplished using Whatman No. 1 filter paper and ethanol acetic acid (water:ethanol:acetic acid = 50:50:0.1 × v/v) as the solvent.

## RESULTS

Plasma amino acids were measured immediately before and 6 hr after the start of DDP exposure on 10 courses of treatment (Fig. 1a). The mean (±S.D.) cyst(e)ine concentration prior to treatment was 50 ± 29 μM and that at 6 hr after treatment was 9 ± 9 μM ($P < 0.01$, $t$ test for paired observations). Figure 1(a) shows the reduction in plasma cyst(e)ine for each individual case; the average (± S.D.) decrease was 83 ± 17%. DDP treatment did not change the plasma concentration of any of the other amino acids reportedly capable of blocking the binding of DDP to DNA [8] (asparagine, glutamine, methionine, histidine and lysine).

Serial measurements of plasma cyst(e)ine were made to define the time course of the DDP-induced effect in 3 patients (Fig. 1b). In 2 of the 3 cases plasma cyst(e)ine was still falling at 6 hr, so that the percentage decrease measured at 6 hr

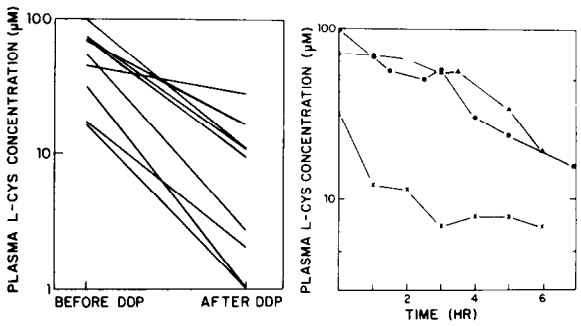


*Fig. 1. (a) Plasma cyst(e)ine concentrations were measured immediately before and 6 hr after infusion of DDP on 10 courses of treatment. (b) Time course of the decrease in plasma cyst(e)ine concentration in 3 patients following infusion of DDP. Plasma cyst(e)ine was measured using a Beckman 119 CL amino acid analyzer after oxidation of cysteine to cystine. DDP doses ranged from 90 to 180 mg/m² and were administered by the i.p. route.*

probably underestimated the nadir cyst(e)ine concentration. Detailed measurements of the non-protein-bound reactive plasma DDP concentrations [18] were available for 7 of the 10 courses presented in Fig. 1(a). The peak DDP concentration occurred at 1 hr and averaged 9 μM, and the mean area under the plasma elimination curve was 10 μM·hr. The magnitude of the decrease in plasma cyst(e)ine correlated with the peak plasma DDP concentration ($r = 0.70$, $P < 0.05$) but not with the area under the DDP elimination curve.

Although previous studies have demonstrated that cysteine can prevent the binding of DDP to macromolecules, we sought direct evidence for a chemical reaction between DDP and cysteine. Graded concentrations of DDP were reacted with 83 μM [$^{14}$C]-cysteine for 4 hr at 37°C, and then the reactants and products were separated by paper chromatography. Two ninhydrin-reactive spots were demonstrated, one at the origin which co-migrated with pure DDP–[$^{14}$C]-cysteine complex, and one with an $R_f$ of 0.7 which co-migrated with [$^{14}$C]-cysteine. As the concentration of DDP was raised from 0 to 330 μM the predominant radioactive peak shifted from an $R_f$ of 0.7 to the origin consistent with the formation of DDP–[$^{14}$C]-cysteine complex. Little complex was formed at DDP concentrations of <3.3 μM (DDP:cysteine molar ratio <0.4); at a DDP concentration of 330 μM (molar ratio of 4.0) most of the radioactivity was associated with the spot at the origin. The formation of the DDP–cysteine complex was time dependent with a rate constant of 0.069/hr at 3.3 μM DDP and 0.17/hr at 330 μM DDP. Elemental analysis of pure DDP–cysteine complex re-crystalized from water suggested a chemical structure consistent with $Pt(cysteine)_2 \cdot 4H_2O$. These results establish that DDP does

react directly with cysteine but indicate that the reaction rate is fairly low and that relatively little DDP–cysteine complex is formed at the concentrations of DDP found in the plasma of the patients included in this study (<10 μm).

In order to assess the biologic significance of the DDP-induced decrease in plasma cyst(e)ine, human promyelocytic leukemia cells (HL-60) [20], which contain a relatively low level of CSE protein [15], were cultured in cyst(e)ine-free media with dialyzed calf serum and graded concentrations of cyst(e)ine. Figure 2 demonstrates that the proliferation rate of this malignant cell line was very sensitive to cysteine concentration, and that at concentrations <20 μM there was cell death. Thus a mean 41-μM decrease in plasma cysteine concentration, from an average of 50 μM before treatment to an average of 9 μM 6 hr after treatment, was of a sufficient magnitude to make the difference between rapid proliferation and death of HL-60 cells *in vitro*.

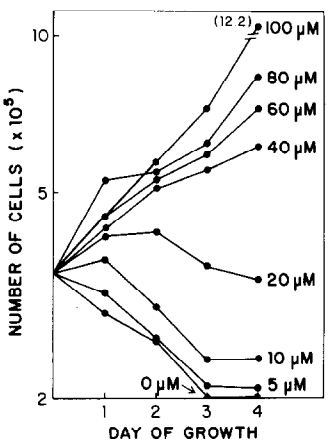


*Fig. 2. The growth rate of human promyelocytic leukemia cells (HL-60) in cystine-free media supplemented with graded concentrations of cysteine from 0 to 100 μM.*

## DISCUSSION

Our data demonstrate a mechanism, in addition to the formation of adducts with macromolecules, by which DDP can inhibit cell proliferation. To the extent that malignant cells are cysteine auxotrophs and the progenitor cells of dose-limiting normal tissues are cysteine prototrophs, this mechanism may be highly selective. Recent data indicate that while CSE deficiency is common in human malignancies, some types of tumor cells contain readily quantitated amounts of CSE [15], and thus the importance of plasma cysteine depletion may vary from patient to patient. Since cysteine can react directly with DDP and can block its binding to macromolecules [7, 8], the plasma concentration of cysteine and other thiol-containing compounds at the time of treatment may be an important determinant of the amount of active DDP that reaches both normal and malignant tissues *in vivo*.

The mechanism by which DDP reduces plasma cyst(e)ine remains uncertain. Although DDP can react directly with cysteine, the reaction rate is slow, and relatively little complex was formed even after 4 hr over the range of DDP concentrations observed in the plasma of these patients. However, DDP causes functional renal tubular abnormalities [21], so that renal cysteine wasting and other mechanisms related to the production of cysteine [22] may be involved. It is important to point out that the DDP-induced depletion of plasma cysteine may have a variety of biochemical effects that influence the sensitivity of cells to other chemotherapeutic agents. Depletion of cysteine may influence levels of other intracellular thiols, such as glutathione [23], that block the activity of cyclophosphamide [24] and melphalan [25]. In addition, depletion of essential amino acids and cysteine [26] cause a rapid and extensive decrease in *de novo* purine synthesis that may contribute to cell death.

These patients were treated as part of a phase I study of cisplatin given by the i.p. route and some patients also received infusions of sodium thiosulfate [18]. While the use of thiosulfate did not affect the decrease in plasma cyst(e)ine, the presence of high concentrations of DDP in the peritoneal cavity may have acted like a heat sink for cyst(e)ine, and it remains to be demonstrated that i.v. administration of DDP produces similar changes in plasma cyst(e)ine.

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