Modification of the Bone Marrow Toxicity of cis-Diamminedichloroplatinum(II) in Mice by Diethyldithiocarbamate

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ABSTRACT

It has been shown that cis-platinum-induced nephrotoxicity in rats can be inhibited by diethyldithiocarbamate (DDC). We report here the bone marrow protective properties of DDC in hybrid (C57BL x BALB/c) mice exposed to single and fractionated doses of cis-platinum. Relatively nontoxic doses of DDC afford maximum protection, using that dose of cis-platinum that would result in the death of 50% of the mice within 9 days as an end point (dose-limiting gut toxicity in mice), when injected 0.5 to 2 hr following i.p. cis-platinum treatment. Survivals of colony-forming units in spleen, nucleated bone marrow cells, and peripheral white blood cell were used to assess the bone marrow protective properties of DDC following both single and fractionated doses of cis-platinum. A dose modification factor of 3.2 (based on colony-forming units in spleen survival) was obtained when DDC (1000 mg/kg) was injected into mice 0.5 hr after graded doses of cis-platinum. When fractionated doses of cis-platinum were used (6 mg/kg on Days 0, 10, 20, and 30), the survival of colony-forming units in spleen was markedly enhanced if the animals were rescued with DDC 0.5 hr following each cis-platinum dose. When bone marrow cellularity was measured immediately before and 2 days after each dose of cis-platinum, a similar pattern of depression and recovery was noted whether DDC was present or not; however, the depression was less marked in mice rescued with DDC. When peripheral white blood cell counts were monitored, the nadir and recovery were similar in the presence or absence of DDC; however, recovery occurred sooner in the animals that had received DDC. Our data support the ability of DDC to modify the bone marrow toxicity of cis-platinum in normal mice. Experiments are in progress in tumor-bearing animals exploring the differential protection afforded by DDC between bone marrow and tumor.

INTRODUCTION

The ability to protect normal tissues from the toxic effects of chemotherapeutic agents without concomitant protection of tumor tissue has much appeal in clinical oncology. The protective agent WR-2721 has been shown to convey dose-modifying properties for various chemotherapeutic agents, including cis-platinum, in many normal tissues. This modification has been attained with no apparent loss of the antitumor activity of the agents (27, 28). However, in Phase I clinical trials of WR-2721 with alkylating agent chemotherapy and with radiation therapy, potential dose-limiting toxicities have been noted including hypotension, somnolence, and allergic reactions (3, 14). In contrast, DDC, a chelating drug that has been used in a large population of patients, appears to have minimal toxicity. Doses as high as 4 gm/day have been used in cases of acute nickel-carbonyl poisoning with no apparent acute or long-term toxicity (24). Additional information from patients using Antabuse (of which DDC is a metabolic reduction product) shows that the drug appears to have minimal toxicity (13).

We have recently shown, using 35S-labeled DDC in tumor-bearing mice, that preferential uptake of this drug is noted in kidney, lung, and to a lesser extent bone marrow compared to tumor tissue (10). In addition, we have reported that DDC, in nontoxic concentrations, can significantly increase the lethal dose that would result in the death of 50% within 30 days of mice given total body irradiation and can also reduce the radiation sensitivity of bone marrow stem cells (10).

In view of the broad-based application of cis-platinum in clinical oncology, our laboratory has investigated the ability of DDC to modify the nephrotoxicity and the bone marrow toxicity of cis-platinum. It has been shown previously that cis-platinum-induced nephrotoxicity in rats can be inhibited by DDC (5) without affecting the tumor response of the chemotherapeutic agent (4, 11). We have confirmed, in mice, that DDC markedly reduces the elevated serum blood urea nitrogen noted after high-dose cis-platinum treatment. We have explored more fully the ability of DDC to protect the bone marrow of mice from single and fractionated doses of cis-platinum in the hope that our data will provide a basis for the introduction of high-dose cis-platinum treatment with DDC rescue. To be clinically relevant, it is vitally important to establish that concomitant protection of the tumor by DDC does not occur greater than that protection afforded normal tissues. We report here the results of our studies on bone marrow protection in normal mice; our data in tumor-bearing animals using clinically appropriate cis-platinum doses will be the subject of a future publication.

MATERIALS AND METHODS

Experimental Animals. The mice used were C57BL x BALB/c F1 mice bred and maintained in a specific pathogen-free colony. They were handled aseptically in laminar flow hoods for all experimental manipulations.

DDC. The sodium salt of DDC (Sigma Chemical Co.) was dissolved in 0.9% NaCl solution (saline) before each experiment and injected i.p. (0.1 ml/10 g body weight) at different times and concentrations as noted below.

cis-Platinum. cis-Diamminedichloroplatinum(II) as its sodium salt (Platinol) was kindly supplied by Bristol-Myers Co. The drug, in 10-mg vials, was made up in sterile water and injected i.p. (0.1 ml/10 g body weight) to obtain the required dose of cis-platinum (mg/kg) or i.v. during spleen colony assay.

1 Supported in part by Grant CA 15083 to the Mayo Clinic Comprehensive Cancer Center and by the Mayo Foundation.
2 To whom requests for reprints should be addressed.
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3 The abbreviations used are: DDC, diethyldithiocarbamate; LD90, that dose of cis-platinum that would result in the death of 50% of the mice within 9 days; CPU, colony-forming units in spleen; MeDDC, methyl diethyldithiocarbamate.
4 R. G. Evans, unpublished data.
Spleen Colony Assay for Mouse Bone Marrow Cells (CFU). The methods of Till and McCulloch (25) and Ainsworth and Larsen (1) were used to obtain bone marrow stem cell (CFU) survival curves without DDC or with DDC (1000 mg/kg) injected 0.5 hr after cis-platinum exposure. Total body irradiation (950 rads) was used to prepare the recipient mice; no endogenous spleen colonies were noted at this dose. The donor mice were treated with graded doses of i.v. cis-platinum and survival of bone marrow stem cells (removed 2 hr following cis-platinum treatment) was determined by spleen colony assay. Surviving fractions were based on injecting bone marrow cells from one to 3 treated mice into 10 recipient mice/data point and determining the number of spleen colonies 8 days later. Control data were obtained by injecting 0.9% saline i.p. in the same volume-to-animal-weight ratio as used for the 'no DDC' (cis-platinum injections only) manipulations. In experiments designed to determine the survival of CFU as a function of fractionated cis-platinum treatment, cis-platinum (6 mg/kg) was used for each dose fraction either in the absence of DDC or with DDC (1000 mg/kg) injected 0.5 hr after each cis-platinum dose. Two mice served as marrow donors at each data point.

Bone Marrow Cellularity and Peripheral WBC Count. Bone marrow, from the femur of each of 5 mice treated in the same manner, was obtained by pushing approximately 3 ml of 5% Hanks’ balanced salt solution through the end of each femur. This bone marrow cell suspension was diluted 1 to 20 with 2.8% acetic acid using a Unopette. and the number of nucleated cells per femur was determined from counts obtained with a hemacytometer. Viability was indicated by trypan blue staining. Control bone marrows were counted at all points with 5 mice serving as donors at each point. Surviving fractions were obtained, at each point, by counting the number of cells per femur and expressing this number as a fraction of the average number of cells in the control bone marrows.

To obtain blood for the determination of peripheral WBC count, a heparinized capillary tube was used to obtain 40-μl samples from the retroorbital venous plexus. The blood was then added to a known amount of Isoton, and following the addition of 6 drops of Zap-Oglobin II, the cell count was determined with a Coulter Counter. Mice were bled every other day, and 7 mice were used for each group. All mice were treated on day 0 but different sets of mice were bled between Days 0 and 10, 10 and 20 (having received a second dose of cis-platinum on Day 10), and between Days 20 and 30 (this latest set having a third treatment on Day 20). Three sets of mice were used for bleeding due to problems encountered with multiple samples from the same eye. Surviving fractions were obtained, at each point, by counting the number of WBC per blood sample (7 mice at each point) and expressing this number as a fraction of the number of WBC in the blood from control animals.

RESULTS

Toxicity of cis-Platinum in F1 Mice with and without DDC. The data in Table 1 indicate that the LD50, as computed by probit analysis (21), is approximately 11 mg/kg and that all animals destined to die do so within 9 days. This is consistent with our hypothesis that injury to the gut is dose limiting following cis-platinum injection in mice. No deaths have been noted in numerous experiments using 6 mg/kg single dose of cis-platinum. When DDC, at a concentration of 1000 mg/kg, is injected into mice 0.5 hr following graded doses of cis-platinum, an LD50 of approximately 24 mg/kg is obtained (Table 2). This dose-modification factor of 2.2 (ratio of LD50 with and without DDC) is not associated with any change in the pattern of death.

Timing of DDC and cis-Platinum Treatment. The data in Table 3 indicate that, using survival by 9 days as an end point, maximum protection is afforded by DDC when it is added after cis-platinum treatment. However, a more detailed study would be necessary to show a specific point of maximum protection.

Toxicity of cis-platinum (i.p.) in C57BL × BALB/c F1 mice

<table>
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<th>Dose of cis-platinum (mg/kg)</th>
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</tr>
</thead>
<tbody>
<tr>
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<td>0/20</td>
</tr>
<tr>
<td>8</td>
<td>7/20</td>
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<td>11/20</td>
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<tr>
<td>16</td>
<td>15/20</td>
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<td>20</td>
<td>18/20</td>
</tr>
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</table>

Table 1

Dose of cis-platinum and cis-platinum (20 mg/kg) treatment

| cis-Platinum alone (20 mg/kg) | 19/20 |
| DDC 2 hr before cis-platinum | 19/20 |
| DDC 0.5 hr before cis-platinum | 15/20 |
| DDC 0.5 hr after cis-platinum | 0/20  |
| DDC 2 hr after cis-platinum | 0/20  |

Table 2

Time course of cis-platinum rescue

<table>
<thead>
<tr>
<th>Dose of DDC (mg/kg i.p.)</th>
<th>Deathsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>19/20</td>
</tr>
<tr>
<td>250</td>
<td>15/20</td>
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<tr>
<td>500</td>
<td>9/30</td>
</tr>
<tr>
<td>1000</td>
<td>1/20</td>
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Table 3

Dose necessary to rescue 50% of mice = 375 (300 to 470)b

Table 4

Ability of DDC to Rescue F1 Mice. The data in Table 4, using death within 9 days as an end point, indicate that the ability of DDC to rescue animals from cis-platinum toxicity is a function of the dose of DDC used and that the dose necessary to rescue 50% of the animals is DDC (375 mg/kg). However, in order to obtain the maximum protection, DDC (1000 mg/kg) was found to be necessary and was used in subsequent experiments.

CFU Survival for Mouse Bone Marrow Cells with and without DDC. Bone marrow survival curves without and with DDC injected 0.5 hr following graded doses of cis-platinum were obtained as described under "Materials and Methods." In the control group, 2 CFU/104 bone marrow cells were found. The data points in each of the survival curves in Chart 1 were obtained from 3 separate experiments. We could not distinguish between CFU survival obtained 2 hr following cis-platinum treatment and that obtained from marrow collected 4 hr after treatment. A dose modification factor of 3.2 was obtained from the ratio of the D0s...
of the survival curves with and without the presence of DDC. There was no significant difference in the survival of CFU₀ between the DDC alone and the control group; therefore, data have been combined and plotted as no DDC.

Survival of CFU₀ as a Function of Fractionated cis-Platinum Treatment. In an attempt to simulate the clinical setting, a regimen of 6 mg/kg every 10 days was chosen. The ability of the bone marrow stem cells to recover from this fractionated regimen was monitored after each dose. The data in Chart 2 indicate that DDC added at 0.5 hr after each dose of cis-platinum markedly decreases the depression of CFU₀ as monitored 2 hr after each dose of cis-platinum and immediately before subsequent doses. The data demonstrate, particularly after the first 3 doses of cis-platinum, a substantially higher survival of CFU₀ in the groups of mice receiving DDC.

Bone Marrow Cellularity after cis-Platinum Treatment with and without DDC. The data in Chart 3 shows the surviving fraction (treated marrow compared to control) of nucleated cells in the bone marrow 2 days following and immediately prior to each of 3 doses of cis-platinum. Note that administration of DDC 0.5 hr after each dose of cis-platinum modifies the depression of bone marrow cellularity by cis-platinum, particularly after the first 2 fractions.

Survival of Peripheral WBC after cis-Platinum Treatment with and without DDC. The data in Chart 4 show a pattern of depression and recovery of WBC similar to that seen with bone marrow cellularity. Note that following each dose of cis-platinum, the recovery is delayed less when DDC is added 0.5 hr after each cis-platinum dose.

DISCUSSION

The response of carcinomas of the ovary, bladder, and testicle, and to a lesser extent carcinomas of the lung and bone, to cis-platinum makes this drug an excellent candidate for investigations that could lead to the introduction of doses higher than those conventionally used. A group of patients has been reported that, having failed to respond to conventional doses of cis-platinum, responded to higher doses (20). Methods to overcome the nephrotoxicity of high-dose cis-platinum treatment such as administration in hypertonic saline (3%), mannitol diuresis, and continuous infusion have been quite successful (16). Moreover, the inhibition of cis-platinum-induced nephrotoxicity by DDC, demonstrated in rats (5), could permit the clinical use of higher doses of cis-platinum which may constitute a myelotoxic threat (8). An improved therapeutic index of cis-platinum is therefore a possibility, akin to the situation when high-dose methotrexate with citrovorum rescue was introduced. Of clinical relevance is
The nadirs are similar without or with DDC, but the count recovery tends to occur earlier in those mice given DDC 0.5 hr after cis-platinum (Cis-P) administration. This observation is consistent with the hypothesis that DDC is able to chelate cis-platinum that has become bound to sulfhydryl groups in the membrane of kidney tubules, thus blocking its entry and subsequent nephrotoxic effect. Gale et al. (12) also showed DDC to be an effective antagonist of renal proximal tubular damage caused by cis-platinum but found that the degree of protection was highest when the DDC was given prior to cis-platinum and was somewhat reduced in effectiveness when delayed 2 hr after cis-platinum injection. One must not overinterpret, however, the finding that the half-life of the unbound cis-platinum is 17 min, as it is entirely possible that the DDC, in addition to blocking the passage of cis-platinum into the cells of the kidney and bone marrow, may also release protein-bound cis-platinum from the plasma. We have considered the possibility that the reduced effect of cis-platinum on cells of the bone marrow in the presence of DDC is a result of less drug reaching the systemic circulation due to its chelation by DDC in the peritoneal cavity. However, we think that this possibility is unlikely as the cis-platinum moves very quickly into the blood (maximum levels within 15 min) following i.p. injection. Moreover, in the experiments investigating the survival of CFU, with and without DDC, the cis-platinum was given via the tail vein.

Elimination of DDC has been studied in both dogs and rats. Metabolism appears to occur through 3 main pathways, reduction of DDC to diethylamine and carbon disulfide (later exhaled through the lung) and through 2 urinary metabolites (inorganic sulphate and S-glucuronide) (6). It has also been postulated (23) that DCC can be methylated to form MeDDC as an intermediate in the formation of the inorganic sulphate. The elimination half-lives of DDC and MeDDC have been measured in rats following i.p. injection of the drug and have values 8 and 76 min, respectively (23). The values in dogs following i.v. injection are very comparable, being 3.4 and 49 min for DDC and MeDDC, respectively (6).

The dose modification factor of 2.2 is seen with no apparent modification by DDC of the pattern of death. The observation that giving DDC 0.5 hr after cis-platinum was as effective, at least as judged by the number of mice dying by 9 days, as giving it 2 hr after cis-platinum prompted us to investigate the half-life of cis-platinum in the plasma of our mice. Protein-bound platinum constitutes more than 90% of the total platinum present 2 hr after cis-platinum administration, and the drug in this form lacks cytotoxic activity (7, 18), whereas unbound platinum appears to represent biologically active platinum in plasma (15). Preliminary data using both an atomic absorption method (17) and a chromatographic method (2) yield a t_{1/2} of 17 ± 3 (S.D.) min for unbound cis-platinum.6 The finding that DCC is still able to rescue mice when injected 2 hr after cis-platinum, i.e., 6 half-lives later, is difficult to understand in terms of chelation of the active cis-platinum remaining in the plasma. An alternative explanation would be that DDC, injected 2 hr after cis-platinum, is able to circumvent the entry of cis-platinum into the cells of kidney, gut, and bone marrow. Borch and Pleasant (5) have hypothesized that DDC is able to chelate cis-platinum that has become bound to sulfhydryl groups in the membrane of kidney tubules, thus blocking its entry and subsequent nephrotoxic effect. Gale et al. (12) also showed DDC to be an effective antagonist of renal proximal tubular damage caused by cis-platinum but found that the degree of protection was highest when the DDC was given prior to cis-platinum and was somewhat reduced in effectiveness when delayed 2 hr after cis-platinum injection. One must not overinterpret, however, the finding that the half-life of the unbound cis-platinum is 17 min, as it is entirely possible that the DDC, in addition to blocking the passage of cis-platinum into the cells of the kidney and bone marrow, may also release protein-bound cis-platinum from the plasma. We have considered the possibility that the reduced effect of cis-platinum on cells of the bone marrow in the presence of DDC is a result of less drug reaching the systemic circulation due to its chelation by DDC in the peritoneal cavity. However, we think that this possibility is unlikely as the cis-platinum moves very quickly into the blood (maximum levels within 15 min) following i.p. injection. Moreover, in the experiments investigating the survival of CFU, with and without DDC, the cis-platinum was given via the tail vein.

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The dose modification factor of 2.2, noted in Chart 1, for the protection of CFU, by DDC is potentially clinically important and agrees well with the data noted in mice by Wasserman et al. (26) for cis-platinum and WR-2721. The data in Chart 1 were obtained with increasing single doses of cis-platinum, and in an attempt to simulate the clinical situation, the survival of CFU, was also investigated as a function of fractionated doses of cis-platinum as illustrated by the data in Chart 2. There appears to be continued killing of CFU, with each subsequent dose of cis-platinum with little or no recovery between dose fractions. Jenkins et al. (19) also noted a dose-dependent reduction in CFU, in mice when 4 equal doses of cis-platinum (6 or 8 mg/kg) were given on an every-week basis. These authors point out that although the range of doses used in their study are roughly equivalent to the range of doses recommended clinically, the quantitative results probably do not apply directly to humans since the stem cell kinetics would be different from the situation in the mouse. However, we do agree with these authors that data of the type we have generated does have significance in predicting comparative hematopoietic effects of cis-platinum in humans. Dumenil et al. (9) using cis-platinum regimens in mice that are clinically appropriate have noted acute hematopoietic toxicity as determined by the survival of pluripotential CFU, These authors note that 10 days following each injection of cis-platinum, the number of CFU, fluctuated around 50% of control, after which time the number of CFU increased slowly to reach normal values 18 months after the sixth cis-platinum injection.
However, even at this time, although the number of CFU₅ was back to normal, the proliferative capacity of the CFU₅ from treated mice was lower than that of CFU₅ from untreated mice of the same age. Other data, reported by Nowroussian and Schmidt (22), using single doses of cis-platinum (12 mg/kg) showed that the survival of CFU₅ dropped rapidly to 5% of that of the control by the first day after drug administration and that the CFU₅ compartment size increased slowly to reach close to normal within 36 months. These observations are particularly intriguing since the WBC count had fully recovered by 5 days, at which time the survival of CFU₅ was still only 10% of control (22). These observations are somewhat surprising in that they suggest that WBC counts are not a true reflection of the recovery status of the bone marrow.

Although our studies do suggest a potential role for DDC in the abrogation of the toxic effects of cis-platinum on bone marrow as reflected in improved survival of CFU₅ nucleated bone marrow cells, and peripheral WBC by the administration of DDC after each dose of cis-platinum, our results leave some questions unanswered. Stem cells represent a very small fraction of the bone marrow population, and it is pertinent to ask whether DDC protects all the stem cells to the same extent? Although DDC appears to modulate the depression of nucleated bone marrow cells (made up of several histological cell types in various stages of maturation), their recovery prior to each subsequent cis-platinum dose (Chart 3) are not that dissimilar. The ability of the CFU₅ population to recover, in the presence of DDC, following the first 3 fractions of cis-platinum but not after the final one (Chart 2), begs one to ask if there is exhaustion or limited proliferation of the stem cells after a given number of cycles of cis-platinum. Perhaps the question could be more appropriately stated as: is the bone marrow once bathed in cis-platinum more susceptible during the second, third, etc., cycles of cis-platinum or is there complete recovery between fractions?

Having established the bone marrow protective properties of DDC, we need to investigate whether concomitant protection of tumor occurs greater than that noted for bone marrow. Experiments are in progress in tumor-bearing animals, using clinically appropriate cis-platinum regimens, and although preliminary data indicate some tumor protection by DDC it is significantly less than that noted for protection of the bone marrow. Data from these experiments, when complete, will be the subject of a future publication.

REFERENCES

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