Effects of Mannitol or Furosemide Diuresis on Cis-Dichlorodiammine-platinum(II) Antitumor Activity and Toxicity to Host-renewing Cell Populations in Rats

Martin F. Pera, Jr. and Harold C. Harder

ABSTRACT

Administration of mannitol (300 mg in 3.0 ml 0.45% NaCl solution as a 30-min i.v. infusion) but not furosemide (12.5 mg/kg i.p. with 6.0 ml 0.9% NaCl solution significantly reduced acute (14-day) lethality in normal male F344 rats given cis-dichlorodiammineplatinum(II) (CDDP) (8.5 mg/kg i.v.). Because both diuretics protected rats from CDDP impairment of renal function and because a 30-min i.v. infusion of 3.0 ml 0.45% NaCl solution with CDDP (8.5 mg/kg) resulted in lower blood urea nitrogen levels but did not reduce mortality in F344 rats, it was concluded that prevention of acute lethality with mannitol was not directly related to protection of renal function. Other tissues, particularly gastrointestinal mucosa and bone marrow, were therefore studied. F344 rats given CDDP (6.0 mg/kg i.v., a sublethal dose) and rats given CDDP (8.5 mg/kg i.v.) with mannitol exhibited faster recovery of [methyl-3H]thymidine incorporation into intestinal DNA when compared to rats given CDDP (8.5 mg/kg i.v.) alone or with furosemide. Femoral nucleated cell numbers and spleen, thymus, and body weights recovered more rapidly in F344 rats given CDDP (6.0 mg/kg i.v.) with mannitol compared to animals given the same dose of CDDP alone or with furosemide.

The percentage of increase in life span of male Sprague-Dawley rats inoculated with 2.5 x 10⁶ Shay leukemia cells i.v. and treated 3 days later with CDDP (7.0 mg/kg i.v.) was unchanged by the administration of mannitol or furosemide with CDDP. When mannitol was administered with CDDP (9.5 mg/kg i.v.), a dose of the platinum drug that causes substantial mortality in Sprague-Dawley rats when given alone, tumor-bearing animals given the high dose with mannitol survived longer than those given CDDP (7.0 or 9.5 mg/kg i.v.) alone. In the systems described, it appears that mannitol selectively spared rapidly proliferating host tissues as well as the kidney from CDDP toxicity and thereby improved the therapeutic index of this drug.

INTRODUCTION

In the preceding paper (10), we showed that treatment with furosemide or mannitol afforded significant protection against CDDP-induced impairment of renal function in rats. At the same time, plasma clearance and urinary excretion of platinum were essentially unaltered by diuretic administration. If plasma and urine platinum disposition reflects the disposition of the cytotoxic form(s) of CDDP, then the above findings suggest that diuretic administration should result in decreased renal toxicity of CDDP without alteration of drug toxicity to host renewing cell populations (bone marrow, and gastrointestinal tract) and without alteration in the antitumor activity of the drug. We therefore investigated the effects of diuretics on the gastrointestinal and hematopoietic toxicity of CDDP and found this view to be incorrect. This paper provides evidence that the osmotic diuretic, mannitol, protects rat bone marrow and gastrointestinal cell renewal systems without lowering the antitumor effect of CDDP towards one experimental leukemia.

Ward et al. (14) showed that although furosemide protected rat kidneys against the functional impairment caused by CDDP, the diuretic did not decrease the acute lethal toxicity of the platinum compound. We confirmed this finding, but we also observed that coadministration of mannitol allowed rats to survive a lethal dose of CDDP (Ref. 9 and this publication). The acute lethal toxicity of CDDP involves damage to several systems, including the kidney, proliferating cells of the gastrointestinal mucosa, and hematopoietic and lymphoid tissues (7, 13). Since furosemide and mannitol protect renal function from CDDP toxicity to a similar degree (10), it seemed possible that the enhanced survival of mannitol-treated rats was due to an interaction at sites of CDDP toxicity other than the kidney.

Although gastrointestinal toxicity due to inhibition of crypt cell proliferative activity is not a major clinical problem with CDDP, we noted that the acute toxic response to CDDP in rats resembles the gastrointestinal radiation syndrome in many respects (1). We considered, therefore, that survival of mannitol-treated rats might relate to decreased toxicity in this organ system. Hagemann et al. (4) have stressed the importance of the relationship of the timing of recovery of proliferative activity to animal survival in the gastrointestinal radiation syndrome. Therefore, we followed the course of inhibition and recovery of DNA synthesis in the small intestine of rats given a sublethal dose of CDDP and compared this pattern to that observed in rats given a lethal dose of the platinum compound either alone or in combination with furosemide or mannitol. We next examined the effects of the 2 diuretics on the toxicity of CDDP to hematopoietic cell populations in rats.

The abbreviations used are: CDDP, cis-dichlorodiammineplatinum(II); [H]dThd, [methyl-3H]thymidine; BUN, blood urea nitrogen; TCA trichloroacetic acid; ILS, increase in life span; PBS, phosphate-buffered saline.

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2 From a dissertation to be presented to the Graduate School of Arts and Sciences, The George Washington University in partial fulfillment of the requirements for the Ph.D. degree.

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topoietic and lymphoid tissue by serial measurement of femoral nucleated cell numbers and spleen and thymus weights because myelosuppression and immunosuppression are important features of the clinical toxicity of CDDP [12].

Finally, if mannitol altered the toxicity of CDDP to rapidly proliferating host tissues, it might also be expected to alter the antitumor activity of the drug. To address this question, we studied effects of mannitol and furosemide diuresis on the survival of Sprague-Dawley rats bearing the Shay leukemia, a rat tumor with moderate sensitivity to CDDP.

MATERIALS AND METHODS

Animals and Drug Treatment. Male F344 rats weighing between 100 and 150 g were used in acute toxicity studies and experiments on gastrointestinal epithelium and bone marrow. For passage of the Shay leukemia, weaning Sprague-Dawley rats (35 to 50 g) were used immediately following arrival from the supplier; in survival studies, we used Sprague-Dawley rats weighing 75 to 100 g. All animals were purchased from Charles River Breeding Laboratories, Inc., Wilmington, Mass. and were maintained in stainless steel cages, 4 to 6 animals per cage. The rats were fed a standard diet (Ralston-Purina Co., St. Louis, Mo.) and allowed access to water ad libitum.

Administration of CDDP, furosemide, and i.v. infusions of mannitol was performed as previously described [10]. CDDP was administered by i.v. bolus injection. Furosemide was injected i.p. at a dose of 12.5 mg/kg in 3.0 ml 0.9% NaCl solution 30 min prior to CDDP; 4 hr later, an additional 3.0 ml 0.9% NaCl solution was administered to reduce the possibility of gross fluid or electrolyte derangement. Mannitol was infused i.v. as a 10% solution in 0.45% NaCl solution for 25 min prior to and 5 min post-CDDP at the rate of 0.10 ml/min.

Chemicals. The sources and preparations of CDDP, furosemide, mannitol, and pentobarbital were as described earlier [10]. [3H]dThd (specific activity, 20 Ci/mmol) was obtained from New England Nuclear, Boston, Mass. and diluted to a concentration of 100 μCi/ml in 0.9% NaCl solution prior to injection. Calf thymus DNA, used as a standard in the DNA assays, was obtained from Worthington Biochemical Corp., Freehold, N. J. PBS (pH 7.2) contained 145 mM NaCl solution, 2.68 mM KCl, 8.10 mM NaH₂PO₄, and 1.47 mM KH₂PO₄. Eagle’s minimal essential medium and Fisher’s medium were obtained from Grand Island Biological Co., Grand Island, N. Y. and used without addition of serum.

Effects of Diuretics on Acute Lethal Toxicity of CDDP. To determine if diuretics had any effect on the acute lethal toxicity of CDDP, we gave groups of 7 to 9 F344 rats injections of CDDP (8.5 mg/kg i.v.), a dose which consistently resulted in about 90% mortality in control animals. Control animals received no treatment other than CDDP. Mannitol and furosemide were administered to separate groups as described previously [10]; and, in addition, 2 other treatments were studied. One group of rats received sodium pentobarbital (30 mg/kg i.p.) 40 min prior to CDDP, and a second group was given an i.v. infusion of 0.45% NaCl solution, performed in the same manner as the mannitol infusion, prior to the i.v. bolus of CDDP. These 2 controls were performed to evaluate the possibility that either the pentobarbital used to sedate rats during the infusion or the infusion of large fluid volumes could account for differences in survival of the rats. All animals were weighed and observed daily for a 14-day period. In some cases, blood samples were drawn from the retroorbital sinus 3 or 4 days following CDDP injection for analysis of BUN, as described earlier [10], to determine the effect of the treatments on renal function.

Effect of Diuretics on the Inhibition and Recovery of DNA Synthesis in the Small Intestine after CDDP. To ascertain whether the effects of mannitol on survival were related to an alteration in the gastrointestinal toxicity of CDDP, we measured the pattern of inhibition and recovery of [3H]dThd incorporation into gastrointestinal mucosa DNA after a sublethal dose of the platinum compound. The observed pattern was then compared to that seen after a lethal dose of CDDP alone, with furosemide, or with mannitol. F344 rats were divided into 4 groups of 16 animals each. One group was given CDDP (6 mg/kg i.v.), the second received CDDP (8.5 mg/kg i.v.), and the other 2 groups were given CDDP (8.5 mg/kg i.v.) with furosemide or mannitol. A group of 6 animals served as Day 0 controls. At 24, 48, 72, and 96 hr after drug injection, 4 rats from each treatment group were used for measurement of [3H]dThd incorporation.

Rats were given injections of [3H]dThd (500 μCi/kg, 20 Ci/mmol), in 0.9% NaCl solution via the lateral tail vein. Fifteen min later, when incorporation was linear (data not shown), the rats were sacrificed by cervical dislocation. A section of small intestine, extending about 12.5 cm from below the pyloric sphincter and including the duodenal loop plus the portion of the jejunal immediately distal to the ligament of Treitz, was excised, rinsed with PBS, and frozen on solid CO₂. Approximately 500 mg of tissue were placed in a glass homogenizer tube with 10 ml of ice-cold 10% TCA (w/v). The tissue was homogenized with 5 to 6 strokes of a Teflon pestle driven at 6000 rpm. The homogenate was poured off, nonhomogenized muscular tissue was discarded, and the homogenate was centrifuged (all centrifugations were run at 500 × g for 5 min at 4°C). The pellet was resuspended, washed with 10 ml ice-cold 10% TCA, and centrifuged; the process was repeated. Then the pellet was suspended in 10 ml of ethanol:ether (3:1, v/v), centrifuged, resuspended in 10 ml 10% TCA, spun down, and then resuspended in 2.5 ml cold 5% TCA (w/v). The pellet was hydrolyzed in 5% TCA for 30 min at 70°C. After cooling and centrifugation of the pellet, a 500-μl aliquot of the supernatant was removed and placed in a glass liquid scintillation vial with 10 ml of Aquasol (New England Nuclear) aqueous scintillation fluid. The dpm/ml of 5% TCA hydrolysate were determined using a Beckman LS255 liquid scintillation counter with the external standard method of quench calibration. Another 200-μl aliquot of the 5% TCA hydrolysate was analyzed for deoxypentose content by the diphenylamine method of Richards [11] using calf thymus DNA as a standard. Results were expressed as the number of dpm/μg DNA. All tissues were analyzed in duplicate.

Effect of Diuretics on CDDP Toxicity to Hematopoietic and Lymphoid Tissue. To evaluate effects of diuretics on...
the extent and duration of CDDP toxicity to hematopoietic tissue, serial measurements of femoral nucleated cell numbers and spleen and thymus weights were made. Since recovery of these parameters is slow, we used a nonlethal dose of CDDP which enabled us to follow animals for 10 days. Male F344 rats received CDDP (6 mg/kg i.v.) alone or in combination with furosemide or mannitol. Twelve rats served as control, and at 1-, 3-, 5-, 7-, and 10-day time points after drug injection, 4 rats from each treatment group were weighed and sacrificed by decapitation. The spleen and thymus of each rat were dissected free of connective tissue, removed, rinsed in PBS, blotted dry on filter paper, and weighed. One femur was removed from each animal, the epiphysis was dissected off, and a 21-gauge needle was inserted into the distal end of the bone. The head of the bone was clipped off with scissors, and were injected into the cavity to express the marrow into a test tube. The syringe was used to gently disperse the cells three 2.5-ml aliquots of Eagle's minimal essential medium diluted to an appropriate concentration and held on ice before counting in a hemacytometer.

Effect of CDDP Alone or in Combination with Diuretics on Survival of Rats Bearing Shay Leukemia. To determine whether or not diuretic administration would alter the antitumor effect of CDDP and to determine whether survival of tumor-bearing rats could be improved by the combined use of CDDP with mannitol, we studied the effects of CDDP alone or with diuretics on the survival of rats bearing the Shay leukemia. The tumor was obtained from Vincent King in the laboratory of Dr. W. Maloney, the Sidney Farber Cancer Institute, Boston, Mass. Since inoculation of 2.5 × 10⁶ Shay leukemia cells into inbred F344 rats did not consistently result in 100% mortality, we used the outbred Sprague-Dawley strain in this work. The methods for maintenance of the tumor are similar to those described previously (5). Briefly, a rapidly growing tumor free of necrosis and 15 to 20 mm in diameter, was harvested about 9 days after inoculation of 1 × 10⁶ cells s.c. The tumor area was cleansed with alcohol, and the nodule was dissected free and minced into small fragments (2 to 3 mm), which were suspended in Fisher's media. Four gentle strokes of a loose-fitting Teflon pestle were used to disperse the cells, after which the suspension was filtered through a glass wool column to remove debris. The resulting single-cell suspension was counted in a hemacytometer, then diluted with Fisher's media so as to yield a final concentration of 5 × 10⁶ cells/ml. For routine passage, 1 × 10⁶ cells were inoculated s.c. into male weanling (35 g) Sprague-Dawley rats; for experiments, 2.5 × 10⁶ cells were inoculated i.v. into male Sprague-Dawley rats weighing 75 to 100 g. Drug treatment was administered on Day 3 after tumor inoculation. Diuretic dosage and administration were identical to that described previously for F344 rats; CDDP was administered at a dose of 7.0 or 9.5 mg/kg i.v. Animals were observed daily until Day 45 when any survivors were sacrificed and examined grossly for evidence of disease.

Statistical Analysis. The numbers of survivors in groups given lethal doses of CDDP alone, with diuretics, with pentobarbital, or with 0.45% NaCl solution infusion were compared by χ² contingency table analysis; data on BUN levels, specific incorporation of [³H]dThd into DNA; marrow-nucleated cell numbers; and spleen, thymus, and body weights were analyzed first by single-factor analysis of variance and then by the Student-Neuman-Keuls multiple range test (15). Comparison of survival of tumor-bearing animals was performed using 2 nonparametric procedures; first, the Kruskal-Wallis H test was applied to all groups, then the Mann-Whitney procedure was used for comparing pairs of treatment groups, with the error rate controlled on a per experiment basis (8, 15). Differences among groups were considered significant when the value of p was less than 0.05.

RESULTS

Effects of Diuretics on Acute Lethality of CDDP. Table 1 shows the influence of furosemide, mannitol, pentobarbital, and 0.45% NaCl solution on the acute lethal toxicity of CDDP to F344 rats. Animals dying from toxicity did so 4 to 8 days after drug injection with severe weight loss, ruffled, unkempt fur, watery diarrhea, dehydration, gastric retention, splenic and thymic involution, and enteritis. Survivors began to gain weight on Days 5 to 8, and their weights continued to increase through the remainder of the 14-day period. Treatment with furosemide failed to alter lethality, but mannitol administration resulted in a significant increase in survival compared to CDDP alone or CDDP with furosemide or pentobarbital (p < 0.05 by χ²) and a lower renal toxicity, as judged by BUN levels, compared to CDDP alone (p < 0.05 by multiple-range test). The dose of pentobarbital used for sedation of animals during infusions was without effect on lethality or BUN levels. Infusion of 0.45% NaCl solution resulted in a decreased BUN level (p < 0.05 compared to CDDP alone by multiple-range test), but failed to prevent the death of the animals. Results with mannitol, furosemide, and 0.45% NaCl solution infusion suggest that acute lethality is not related to renal function as measured by BUN.

Effects of Diuretics on the Inhibition and Recovery of DNA Synthesis in the Small Intestine after CDDP. Observations in the above experiments on rats dying from acute CDDP toxicity suggested that many clinical features of CDDP intoxication were similar to the gastrointestinal radiation syndrome in rats (1). We therefore considered that since survival of rats seemed unrelated to renal function, perhaps the enhanced survival seen with mannitol was the result of protection of the gastrointestinal mucosa. Inhibition and recovery of incorporation of [³H]dThd into DNA in the upper small intestine was followed for 4 days after a dose of CDDP (6.0 mg/kg i.v.) alone, CDDP (8.5 mg/kg i.v.) alone, or CDDP (8.5 mg/kg i.v.) with furosemide or with mannitol. Previous results and the studies on acute lethal toxicity described above indicate that most rats given the low dose of CDDP or the high dose with mannitol will survive, whereas most rats given the high dose of CDDP alone or with furosemide will die 4 to 8 days after injection. The pattern of inhibition and recovery of DNA synthesis is illustrated in Chart 1. Initially, 24 hr after drug treatment, all groups showed an equal reduction in [³H]dThd incorporation, to roughly 10% of Day 0 control levels. By 48 hr, however, the groups began to diverge. Rats given a low
dose of CDDP or the high dose of CDDP with mannitol exhibited levels of [3H]dThd incorporation into DNA (125 and 99% of Day 0 control, respectively), which were significantly higher than incorporation rates in rats given a high dose of CDDP alone or with furosemide (47 and 42% of Day 0 control, respectively; p < 0.05 by the multiple-range test). By 72 hr, the rate of incorporation of [3H]dThd in the low-dose CDDP group and the high-dose mannitol group had markedly exceeded the Day 0 control levels (403 and 288% of Day 0 control, respectively); both were significantly higher than were rats in the high-dose group of CDDP alone. Therefore, to describe recovery fully, we used a sublethal dose of CDDP (6.0 mg/kg i.v.) alone, with furosemide, or with mannitol and followed rats for 10 days. Results are shown in Charts 2 to 4. As was observed with [3H]dThd incorporation in the small intestine, the initial degree of suppression seen at 24 hr in nucleated cell numbers and spleen and thymus weights was not different in the groups. By Day 3, however, the mannitol group exhibited higher nucleated cell counts than did the furosemide group. At this time, spleen and thymus weights were higher with mannitol than with CDDP alone or furosemide (p < 0.05 by the multiple range test). On Days 5 and 7, there was still no recovery of nucleated cell number in the femurs of rats given CDDP alone or CDDP with furosemide. On the other hand, the mannitol group showed clear recovery of nucleated cell numbers with values significantly higher than those of the other 2 groups (p < 0.05 by the multiple range test). Spleen and thymus weights on Days 5 and 7 tended to reflect changes seen in marrow. By Day 10, nucleated cell numbers in the mannitol group had reached control levels, while values in rats receiving CDDP and CDDP with furosemide remained significantly below the mannitol group, although some recovery had begun (p < 0.05 by the multiple range test). At this time, spleen weights had recovered in all groups; however, thymus weights in furosemide-treated rats were still significantly depressed below those of the other groups (p < 0.05 by the multiple range test).

In this experiment, body weights in mannitol-treated rats

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### Table 1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>n</th>
<th>Survivors (14 Day)</th>
<th>BUN (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CDDP</td>
<td>9</td>
<td>1</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt; 188 ± 13.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>CDDP with pentobarbital&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9</td>
<td>0</td>
<td>ND 206 ± 9.1</td>
</tr>
<tr>
<td>1</td>
<td>CDDP with mannitol&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7</td>
<td>7&lt;sup&gt;f&lt;/sup&gt;</td>
<td>ND 63.7 ± 8.7&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>CDDP</td>
<td>9</td>
<td>1</td>
<td>ND</td>
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<tr>
<td>2</td>
<td>CDDP with furosemide</td>
<td>9</td>
<td>1</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>CDDP with mannitol&lt;sup&gt;e&lt;/sup&gt;</td>
<td>9</td>
<td>7&lt;sup&gt;f&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>CDDP</td>
<td>7</td>
<td>0</td>
<td>67.3 ± 17</td>
</tr>
<tr>
<td>3</td>
<td>CDDP with 0.45% NaCl solution</td>
<td>7</td>
<td>0</td>
<td>45.0 ± 3.54&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of rats in both survival and BUN experiment.
<sup>b</sup> BUN levels not determined.
<sup>c</sup> Values are mean ± S.E. when all rats were still alive.
<sup>d</sup> Pentobarbital was administered at a dose of 30 mg/kg i.p. 40 min prior to CDDP.
<sup>e</sup> Significantly different from other groups, p < 0.05 by x<sup>2</sup>.
<sup>f</sup> Significantly less than other group(s) in the experiment, p < 0.05 by multiple range test.

F344 rats were given the indicated drug treatments and then observed for a 14-day period. In some cases, blood samples were drawn from the retroorbital sinus for BUN determination to evaluate renal function. Pentobarbital and 0.45% NaCl solution were administered to see whether the process of sedating the animals or the infusion of large fluid volumes could account for the effects of mannitol on survival.

**Effect of Diuretics on CDDP Toxicity to Hematopoietic and Lymphoid Tissue.** The accelerated recovery seen in the small intestine of mannitol-treated rats prompted us to consider whether recovery of other host-renewing cell populations might be altered by mannitol administration. In particular, we wished to examine bone marrow and lymphoid tissue, since myelosuppression and immunosuppression are significant clinical toxicities of CDDP (12). Therefore, we measured numbers of femoral nucleated cells as well as spleen and thymus weights following CDDP administration. Initial studies with lethal doses of CDDP suggested that recovery of these parameters was slower than was recovery of gastrointestinal DNA synthesis, but pointed out significant increases in splenic weights 3 days after CDDP injection with mannitol treatment compared to CDDP alone. Therefore, to enable us to describe recovery fully, we used a sublethal dose of CDDP (6.0 mg/kg i.v.) alone, with furosemide, or with mannitol and followed rats for 10 days. Results are shown in Charts 2 to 4. As was observed with [3H]dThd incorporation in the small intestine, the initial degree of suppression seen at 24 hr in nucleated cell numbers and spleen and thymus weights was not different in the groups. By Day 3, however, the mannitol group exhibited higher nucleated cell counts than did the furosemide group. At this time, spleen and thymus weights were higher with mannitol than with CDDP alone or furosemide (p < 0.05 by the multiple range test). On Days 5 and 7, there was still no recovery of nucleated cell number in the femurs of rats given CDDP alone or CDDP with furosemide. On the other hand, the mannitol group showed clear recovery of nucleated cell numbers with values significantly higher than those of the other 2 groups (p < 0.05 by the multiple range test). Spleen and thymus weights on Days 5 and 7 tended to reflect changes seen in marrow. By Day 10, nucleated cell numbers in the mannitol group had reached control levels, while values in rats receiving CDDP and CDDP with furosemide remained significantly below the mannitol group, although some recovery had begun (p < 0.05 by the multiple range test). At this time, spleen weights had recovered in all groups; however, thymus weights in furosemide-treated rats were still significantly depressed below those of the other groups (p < 0.05 by the multiple range test).

In this experiment, body weights in mannitol-treated rats

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<sup>6</sup> M. F. Pera, Jr. and H. C. Harder, unpublished observations.
Therapeutic Efficacy of CDDP with Diuretics

that could be administered without producing excessive lethal toxicity. In the first experiment (Chart 5A), mannitol had no effect on the antitumor activity of CDDP (88% ILS for both treatment groups relative to untreated control). In the second experiment (Chart 5B), the life span of mannitol-treated rats was slight, but not significantly longer (79% ILS) than that of rats treated with CDDP alone or CDDP with furosemide (58% ILS for both these 2 groups). The improved ILS seen with mannitol here may have been due to the occurrence of several early deaths probably attributable to drug toxicity in groups given CDDP alone or CDDP with furosemide.

If mannitol prevented the acute lethal toxicity of CDDP without altering the antitumor activity, then it might be

recovered faster than did other treatment groups (Chart 4). This is in disagreement with results from a similar study described in the accompanying paper (10), where no differences in weight recovery were observed. Possible reasons for the discrepancy were discussed earlier (10).

Effect of CDDP Alone or in Combination with Diuretics on Survival of Sprague-Dawley Rats Bearing Shay Leukemia. If administration of mannitol reduced the toxicity of CDDP to rapidly proliferating host tissues, then the question arose as to whether this protective effect extended to tumor cells as well. To evaluate effects of mannitol on the survival of tumor-bearing rats, we used the Shay leukemia because the intermediate sensitivity of this tumor to CDDP allowed us to determine whether survival would be enhanced or decreased. In preliminary studies, we found that the Walker 256 carcinosarcoma was easily cured by low doses of CDDP in male F344 rats and was, therefore, not useful for these studies. Since the Shay leukemia did not grow reproducibly in the inbred F344 rat (data not shown), we used the outbred Sprague-Dawley strain of rats in these studies.

In the first 2 experiments, we administered a dose of CDDP (7.0 mg/kg i.v.) alone or with diuretics 3 days after i.v. inoculation of 2.5 × 10⁶ leukemia cells. This dose was chosen to approximate the maximum dose of CDDP alone

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M. F. Pera, Jr. and H. C. Harder

Mally lethal dose of COOP (9.5 mg/kg i.v.) with mannitol to the survival of tumor-bearing rats given COOP (9.5 mg/kg i.v.) alone, or a lower dose of COOP alone (7.0 mg/kg). In both experiments (Chart 6), rats given COOP (9.5 mg/kg i.v.) with mannitol achieved a significantly higher ILS [113 and 128% of untreated controls, compared to 87 and 86% for COOP (7.0 mg/kg i.v.) alone, and 20 and 14% for COOP (9.5 mg/kg i.v.) alone]. Thus, the reduction in the toxicity of COOP to the host without a simultaneous reduction in antitumor activity allowed us to administer a larger dose of COOP and thereby achieve longer survival. In other words, mannitol raised the therapeutic index of COOP. It should be noted, however, that the modest improvement in survival of this group was accompanied by the occurrence of a few early deaths probably attributable to drug toxicity.

DISCUSSION

The data described above show that the administration of mannitol with COOP to male F344 rats was accompanied by a decreased acute lethality and a faster recovery of rapidly proliferating host systems in the gastrointestinal mucosa and hematopoietic and lymphoid organs. It seems unlikely that the prevention of acute lethal toxicity resulted solely from a reduction of kidney toxicity due to COOP. Earlier, possible to achieve improved survival by the use of this diuretic. We tested this possibility in 2 experiments by comparing the survival of tumor-bearing rats given a normally lethal dose of CDDP (9.5 mg/kg i.v.) with mannitol to the survival of tumor-bearing rats given CDDP (9.5 mg/kg i.v.) alone, or a lower dose of CDDP alone (7.0 mg/kg). In both experiments (Chart 6), rats given CDDP (9.5 mg/kg i.v.) with mannitol achieved a significantly higher ILS [113 and 128% of untreated controls, compared to 87 and 86% for CDDP (7.0 mg/kg i.v.) alone, and 20 and 14% for CDDP (9.5 mg/kg i.v.) alone]. Thus, the reduction in the toxicity of CDDP to the host without a simultaneous reduction in antitumor activity allowed us to administer a larger dose of CDDP and thereby achieve longer survival. In other words, mannitol raised the therapeutic index of CDDP. It should be noted, however, that the modest improvement in survival of this group was accompanied by the occurrence of a few early deaths probably attributable to drug toxicity.

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furosemide and mannitol were shown to protect renal function to an equivalent degree. In this study, furosemide failed to prevent the lethal toxicity of CDDP, and in addition, administration of 0.45% NaCl solution i.v. was also shown to reduce BUN elevations without altering lethality of high doses of CDDP. These findings indicate that protection of renal function alone does not provide protection against the acute lethal toxic effect of the platinum drug.

Examination of recovery of DNA synthesis in the gastrointestinal mucosa revealed a similar pattern of recovery in rats given a sublethal dose of CDDP and rats given a normally lethal dose with mannitol. Recovery in rats given a lethal dose of CDDP alone or with furosemide was delayed by about 24 hr compared to the other groups. The significance of this lag in recovery lies in the fact that until cell production in the crypts of Lieberkühn is sufficient to replace cells lost from the villi, the animal must suffer the physiological consequences of denudation of the mucosa, such as dehydration. If the period of inhibition of cell production is too long, the physiological deficit may result in death even though, biochemically speaking, recovery may have begun. Thus, when Hagemann et al. (4) studied intestinal DNA synthesis in mice after single X-ray exposures, they found that the timing of recovery rather than the magnitude of the recovery response was the critical determinant of survival in the gastrointestinal radiation syndrome. Mice given a dose of 1000 rads, a dose insufficient to cause gastrointestinal radiation death, showed a marked increase in mucosal [3H]dThd incorporation above control levels by 3 days after exposure. Mice given 1200 rads, a dose causing gastrointestinal lethality in most mice, did not markedly exceed control levels of [3H]dThd incorporation until 4 days after exposure; hence, biochemical recovery came too late to prevent the death of the mice. The critical time interval described by these authors is similar to that shown here. Thus, DNA synthesis in rats expected to survive acute toxicity (animals given a sublethal dose of CDDP, or a lethal dose with mannitol) markedly exceeded the Day 0 control incorporation levels by Day 3; rats expected to die (those receiving a lethal dose of CDDP alone or with furosemide) did not substantially exceed Day 0 control incorporation levels until 4 days after drug administration. Although it is difficult to relate the death of rats after CDDP intoxication to failure of any one organ system, it is likely that faster recovery of the gastrointestinal mucosa in mannitol-treated rats plays a major role in their survival after high doses of the drug.

We also found evidence for a more rapid recovery of hematopoietic and lymphoid tissue in rats given mannitol with CDDP. Femoral nucleated cell numbers and spleen and thymus weights initially were depressed to an equivalent degree in rats given CDDP alone, with furosemide, or with mannitol as was the case with gastrointestinal DNA synthesis. However, recovery was slow in rats receiving CDDP alone or CDDP with furosemide, particularly with respect to femoral nucleated cell counts and thymus weights. Mannitol-treated rats, on the other hand, began recovery before the other groups. The prolonged suppression of nucleated cell counts seen with a single, nonlethal dose of CDDP alone or CDDP with furosemide is similar to the effect of busulfan (2) on rat marrow cell numbers. Dunn and Elson (2) showed that the prolonged marrow suppression seen with busulfan was associated with slow recovery of the stem cell compartment. Investigation of the effect of high doses of CDDP, alone or with mannitol, on the hematopoietic stem cell might help elucidate the basis for the accelerated marrow repopulation seen with this diuretic.

Although mannitol afforded protection of rapidly proliferating host tissues, experiments with the Shay leukemia in Sprague-Dawley rats indicated that protection was not extended to tumor cells. Thus, the ILS of tumor-bearing rats given CDDP was not altered by coadministration of mannitol at maximally tolerated doses of the platinum drug. When normally toxic doses of CDDP were combined with mannitol, improved survival was obtained compared to CDDP alone. The life span of the animals was enhanced only by several days without risking excessive toxicity, owing to the relative insensitivity of the tumor to the drug compared to the host. Nevertheless, the effect was reproducible. Although the toxicity experiments were performed in F344 rats, Sprague-Dawley rats die with a similar pattern of toxicity (Ref. 7 and our own observations), and it is likely that the protective effect of mannitol has a similar basis in both strains of rats. We urge caution in extending these observations beyond the systems described here, however. Preliminary work with mice bearing L1210 leukemia suggests that mannitol administration may reduce the antitumor activity of CDDP as well as the host toxicity in this system. Further observations are required to determine the generality of the results described here for rats to other species.

The mechanism whereby mannitol afforded protection of rat marrow and gastrointestinal mucosa is not clear at present. It is not inconceivable that mannitol and CDDP might interact directly, as described in vitro by Eshaque et al. (3). Several alternative possibilities are suggested by the observation that mannitol administration as described here induced a hyperosmotic state in the rats. Alterations in fluid and electrolyte balance between extracellular and intracellular compartments might alter the disposition of CDDP or its metabolites within cells, or administration of hyperosmotic solute might alter the perfusions of various tissues (6) so as to modify their exposure to active metabolites. Distribution studies reported in the preceding paper showed that mannitol did not alter the cumulative uptake of platinum into small intestine and spleen, except after 2 min, at early time points after CDDP administration (10). Therefore, the mechanism by which mannitol results in a more rapid recovery of the gastrointestinal mucosal DNA synthesis and spleen weight is probably unrelated to an interference with total platinum uptake by these tissues. However, with regard to clinical studies of CDDP pharmacology, it should be pointed out that we are unable to predict the interaction of CDDP and mannitol simply on the basis of plasma, tissue, and urine platinum measurements. Improved assays for cytotoxic forms of CDDP are being developed to determine if the disposition of active drug to tissues is changed during mannitol administration. However, it may be that the interaction of CDDP and mannitol is not a result of an alteration in the disposition of the platinum drug or its metabolites. It is possible that mannitol might have other effects on cellular metabolism which
would render target tissues less sensitive to a pharmacologically equivalent dose of CDDP or that the physiological environment of the mannitol-treated animals was more supportive of recovery. Elucidation of the basis of the protective effect of mannitol might lead to further improvement of chemotherapy with CDDP.

Finally, there are a few implications of this work in terms of the clinical use of CDDP. From the accompanying report and the work of other investigators, it seems established now that induction of diuresis prior to CDDP administration will partially protect animals and humans from the impairment of renal function caused by this drug (10, 12, 14). It is equally clear from this paper that diuretic administration can affect the action and toxicity of CDDP at sites other than the kidney and that not all diuretics interact in an identical manner with CDDP. If the situation in humans is similar to that described above in the rat, then obviously there would be an advantage to the use of mannitol. Mannitol diuresis, as described here, involves the rapid infusion of the diuretic in high concentrations, as opposed to prolonged infusions of mannitol in low concentrations usually used in the clinic. Additional animal studies and careful clinical pharmacological testing using improved assays for cytotoxic forms of CDDP and quantitative evaluation of toxicity and therapeutic response are needed to compare the various modes of administration of this promising antitumor drug.

REFERENCES

Effects of Mannitol or Furosemide Diuresis on Cis-Dichlorodiammineplatinum(II) Antitumor Activity and Toxicity to Host-renewing Cell Populations in Rats

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