Protective Effect of Sodium-2-mercaptoethanesulfonate on the Gastrointestinal Toxicity and Lethality of cis-Diamminedichloroplatinum


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ABSTRACT

cis-Diamminedichloroplatinum (cis-platinum) is an effective and widely used antitumor drug. Patients receiving cis-platinum, however, experience very profound and long lasting gastrointestinal symptoms. The role of intestinal mucosal toxicity in the pathogenesis of these symptoms is unclear. In this study we have investigated the thiol-containing compound mesna (sodium-2-mercaptoethanesulfonate) as a potential antidote to cis-platinum-induced gastrointestinal tract damage. In mice, mesna caused a significant reduction in the gastrointestinal toxicity of cis-platinum assessed by electron microscopy, villus recovery rate, and by disaccharidase estimations. Mesna also significantly reduced serum creatinine levels following cis-platinum administration. Administration of mesna prior (or immediately following) a 67% lethal dose of cis-platinum protected 87-100% of the animals from the lethal effects. The antitumor efficacy of cis-platinum in L1210 leukemia bearing mice was not affected by coadministration of mesna indicating that the protective effect may be tissue specific. In addition this finding indicates that mesna has potential as an agent which may improve the therapeutic index of cis-platinum in clinical practice.

INTRODUCTION

cis-Platinum, a most effective and widely used cytotoxic drug (1), is one of the most emetogenic drugs in clinical practice. Although renal toxicity is the dose limiting factor, the associated emesis, nausea, diarrhea, and anorexia make cis-platinum a most unpleasant drug to receive. The associated renal toxicity has been largely overcome by methods of hydration during therapy (2). The acute emesis, which appears to be mediated partly through central mechanisms (3) and partly through peripheral mechanisms (4), can now be well controlled in many patients, although prolonged gastrointestinal symptoms can persist many days after cis-platinum administration (5). Little is known about the effects of cis-platinum on the small intestinal mucosa and of the relationship between toxicity at this site and prolonged gastrointestinal symptoms. It may be that the protection of intestinal mucosal toxicity could improve the tolerance of cis-platinum in patients. cis-Platinum is thought to be cytotoxic through the formation of a ligand complex with nitrogen atoms in DNA which results in the formation of lethal DNA cross-links. The sulfur atoms of thiol groups (6, 7) have the potential of forming coordination complexes with cis-platinum and therefore provide a competing detoxification reaction. Mesna is a thiol containing compound with this potential which is currently being used clinically to prevent the urotoxicity of oxazaphosphorines by scavenging the acrolein produced by the metabolism of these compounds (8). Mesna is oxidized to dimesna (inactive) in the blood in a metal-dependent reaction and dimesna is selectively taken up by intestinal and renal tubular cells which is then reduced by glutathione reductase in the active thiol (9). On this basis we have investigated mesna as a potential antidote for cis-platinum-induced toxicities.

MATERIALS AND METHODS

Male CBA mice (Bantin & Kingman, Grimston, Hull, United Kingdom) at 6 wk of age were used throughout. The mice were fed on Clarke's rat and mouse chow (Labar) ad libitum and had a ready supply of drinking water. Their bedding consisted of wood shavings and they were kept in a light-dark cycle of 8 a.m.-8 p.m.-8 a.m. The conditions of animal care compiled with the requirements of the United Kingdom Home Office. cis-Platinum (Neoplatin) was obtained from Bristol-Myers Pharmaceuticals, mesna solution (Uromitexan) from WB Pharmaceuticals and colchicine from Sigma Chemicals. The i.p. route of administration was used for cis-platinum and colchicine, thus providing a rapid means of parenteral drug administration. cis-Platinum was dissolved in water for injection and injected in a total volume of 0.25-0.3 ml depending on the mouse weight. Colchicine was dissolved in sterile water and injected in a volume of 0.2 ml/mouse. Mesna was given by gavage so as to provide a high local concentration of drug to the enterocytes. No dilution of mesna was necessary and the maximum gavage volume was 0.12 ml.

To examine mesna effects on the gastrointestinal toxicity of cis-platinum a total of 80 mice were divided into 4 groups of 4 mice each for each day of study and drugs were administered as follows. cis-Platinum was administered at maximally tolerated doses, 10 mg/kg. Mesna were given p.o. at 400 mg/kg at t = -2, 0, and +2 h after cis-platinum unless otherwise stated. This dose of mesna was chosen to accord with potential doses used in humans (10). The groups used were as follows: (a) saline, 0.1 ml i.p.; (b) mesna controls; (c) cis-platinum; and (d) cis-platinum and mesna. Colchicine, a metaphase arrest agent, was administered to mice 5 mg/kg i.p. prior to timed sacrifice (i.e., 30, 60, 90, and 120 min for the 4 mice in each group) in order to allow the later counting of metaphases within intestinal crypt cells (11). Mice were sacrificed by cervical dislocation and a section of ileum was taken (95% portion of small intestine) from each mouse in each group at the timed intervals following colchicine. This procedure was carried out on days 1, 3, 5, 7, and 10 after exposure to the experimental drugs. The ileal samples were washed with saline, laid open on cards, and kept for 4-12 h in Clarke's fixative (75% ethanol-25% acetic acid) prior to storage in 75% ethanol. Samples were then stained using the Feulgen reaction and strips of villi with associated crypts were dissected and placed on a coverslide in 45% acetic acid [modified microdissection technique as described by Ferguson et al. (12)]. These samples were then used at random for the measurement of villus height and crypt height using a calibrated microscope graticule, and counts were done of accumulated metaphases in the crypts. Five measurements of villus height and crypt height were made on each ileal portion giving a mean of 20 villi/crypts/group for comparison. This number of measurements was chosen following previous statistical analysis of the number of measurements required within and between animals. Little variation occurred within animals but the major variance was present between animals. The mean accumulation of metaphases was calculated from 10 separate crypts on each ileal portion for each point, 30, 60, 90, and 120 min after colchicine. Cytos cell production rate per h for each treatment group was calculated from the accumulated metaphases plotted against time.
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The remaining portion of ileum (approximately, 0.5 cm²) was de-
stained in 60% acetic acid overnight and then placed villi upwards on
a microscope slide under a coverslip. Focusing with the microscope
initially on the crypt necks (using a squared area graticule) the number of
crypts in a given area was counted and by refocusing of the micro-
scope a similar count of villi was obtained, therefore allowing the
calculation of the crypt:villus ratio. The product of crypt cell production
rate and crypt:villus ratio gives the villus epithelial cell influx per h
(13). These methods allow a greater understanding than does simple
villus height of the changes occurring in a complex three-dimensional
structure such as the small intestine on exposure to drugs which act on
the proliferative units, i.e., the crypts.

Using a similar technique the effects of different mesna schedules on
gut toxicity were determined. Samples were examined on the same days
after drug exposure as described above. Four mice were taken for each
experimental point. The experimental groups were: (a) saline control;
(b) cis-platinum with mesna, -2, 0, and +2 h after cis-platinum; (c) cis-
platinum and mesna immediately after; and (d) cis-platinum with mesna
2 h after cis-platinum only.

The functional activity of enterocytes was estimated by sucrase and
maltase activity in jejunal portions from each mouse and group (35%
portion of small intestine) using a modified Dahlqvist method (14).

For electron microscopy small portions of jejunum were placed in
2.5% glutaraldehyde and soaked in 1% osmium tetroxide. Samples were
critically point dried from liquid CO₂ and sputter gold coated.

The survival of mice given a lethal dose of cis-platinum, 15 mg/kg
was followed over a 15-day period. Initially 5 mice/group received
either (a) cis-platinum, 15 mg/kg alone i.p.; (b) cis-platinum, 15 mg/kg
with mesna as before, -2, 0, and +2 h after; (c) cis-platinum, 15 mg/
kg with mesna immediately after cis-platinum; or (d) cis-platinum, 15
mg/kg with mesna i.p. immediately after cis-platinum. This experiment
was repeated with 10 mice/group and the results were pooled.

To determine mesna effects on the creatinine rise following cis-
platinum, 10 mice each received cis-platinum, 15 mg/kg i.p. or cis-
platinum, 15 mg/kg i.p. with mesna, 400 mg/kg at -2, 0, and +2 h, or
placebo saline injection. Mice were anesthetized with ether on day 4
after being given injections and blood was drawn from the inferior vena
cava for creatinine analysis. Creatinine was estimated at random by a
Technicon (AA2) Autoanalyzer.

To test whether mesna influences the therapeutic index of cis-
platinum, 1 x 10⁶ L1210 cells (Institut Jules Bordet, Brussels, Belgium)
were injected into groups of 10 mice and drugs (at doses used in the
initial experiment) were administered following the day as follows:
Group 1, controls, 0.1 ml saline i.p.; Group 2, cis-platinum; Group 3,
cis-platinum and mesna, -2, 0, and +2 h; Group 4, cis-platinum and
mesna immediately after cis-platinum only; Group 5, cis-platinum and
mesna, 400 mg/kg i.p. Survival was assessed by the 30th day and
median survival time was expressed as a percentage of controls.

Statistical analysis of the data for intestinal morphology, villus
epithelial cell influx, and disaccharidase activity was by analysis of
variance using the statistical package GENSTAT. Differences between
means were assessed by paired t test.

RESULTS

When mice were treated with cis-platinum, 15 mg/kg, only
33% survived at 15 days. When mesna was given in conjunction
with cis-platinum a significant reduction in lethality was ob-
erved. Eighty-seven % of those mice receiving either 1 or 3
consecutive doses of mesna in conjunction with cis-platinum
survived, as did all those receiving concurrent i.p. mesna (Fig.
1).

Cis-platinum caused a significant reduction in villus height
on days 3–10, when compared with controls, as shown in Fig.
2. In the groups given mesna together with cis-platinum no
significant difference was noted in villus height compared with
controls and the preservation of villus height in the mesna
groups was significant (P < 0.05) when compared with the cis-
platinum alone on days 5 and 7. The mesna control group
showed no significant variation from saline controls. Changes
in crypt height are related to the proliferative response following
cytotoxic insult in the crypts. A more rapid elongation of crypts
occurred in the mesna-treated group, with return to control size
by day 10 (Fig. 2). Scanning electron microscopy of normal
jejunum and jejunum 4 days after cis-platinum or cis-platinum
with mesna by gavage demonstrated very clearly the protective
effect of mesna against the cis-platinum-induced reduction in
villus height (Fig. 3). Table 1 shows the changes in crypt cell
production rate over a 10-day period. cis-Platinum caused a
severe depression in crypt cell production, evident by 2 h and
maximal between 12 and 24 h, with only a slow recovery towards
compensatory rebound cell production on day 7. A mild reduction in
crypt:villus ratio, due to crypt loss, was evident in the cis-platinum
group but this did not reach statistical significance. In the mesna-treated mice cell production rate was less severely affected than in the cis-platinum alone
performed with 10 mice/group and the results were pooled.

* Unpublished data.
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Fig. 3. Scanning electron micrograph of mouse jejunum following cis-platinum administration. a, normal mouse jejunum stained with 1% osmium tetroxide and coated with gold, x 33; b, mouse jejunum 4 days after cis-platinum 10 mg/kg i.p., x 33; c, mouse jejunum 4 days after cis-platinum, 10 mg/kg i.p. and mesna, 400 mg/kg x 3 p.o.

Table 1 Effect of mesna on crypt cell production rate, crypt-villus ratio, and villus influx in mice treated with cis-platinum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1, saline controls</td>
<td>6.42</td>
<td>7.07</td>
<td>6.54</td>
<td>6.60</td>
<td>6.10</td>
</tr>
<tr>
<td>CCPR</td>
<td>4.98</td>
<td>4.88</td>
<td>4.94</td>
<td>5.63</td>
<td>5.03</td>
</tr>
<tr>
<td>Villus influx</td>
<td>31.97</td>
<td>34.53</td>
<td>32.32</td>
<td>37.17</td>
<td>30.70</td>
</tr>
<tr>
<td>Group 2, cis-platinum</td>
<td>0.24</td>
<td>7.07</td>
<td>5.68</td>
<td>11.13</td>
<td>12.10</td>
</tr>
<tr>
<td>CCPR</td>
<td>3.96</td>
<td>3.16</td>
<td>3.05</td>
<td>4.48</td>
<td>5.02</td>
</tr>
<tr>
<td>Villus influx</td>
<td>0.96*</td>
<td>23.34*</td>
<td>17.31*</td>
<td>39.81</td>
<td>60.77*</td>
</tr>
<tr>
<td>Group 3, cis-platinum/mesna</td>
<td>1.51</td>
<td>16.13</td>
<td>9.54</td>
<td>8.79</td>
<td>6.13</td>
</tr>
<tr>
<td>CCPR</td>
<td>5.12</td>
<td>4.71</td>
<td>4.66</td>
<td>5.22</td>
<td>5.24</td>
</tr>
<tr>
<td>Villus influx</td>
<td>7.74*</td>
<td>75.90*</td>
<td>44.46*</td>
<td>45.87</td>
<td>32.11*</td>
</tr>
</tbody>
</table>

* CCPR, crypt cell production rate (cells per h); CVR, crypt-villus ratio; villus influx, cells per h influxing per villus. * Significant difference between cis-platinum and cis-platinum/mesna by analysis of variance at P < 0.001.

Fig. 4. Effect of 3 schedules of mesna on villus influx 1–10 days after cis-platinum (CP.), 10 mg/kg i.p. Four mice/group/experimental time point were used. Experimental details are given in “Materials and Methods.” Each point is the mean of 4 mice. Bar, SE; immed., immediately.

Table 2 Effect of mesna on sucrase activity in jejunal samples from mice treated with cis-platinum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1, controls</td>
<td>4.02</td>
<td>4.61</td>
<td>5.57</td>
<td>6.08</td>
<td>5.71</td>
</tr>
<tr>
<td>Group 2, cis-platinum</td>
<td>4.15</td>
<td>1.18*</td>
<td>2.68*</td>
<td>4.50</td>
<td>3.45*</td>
</tr>
<tr>
<td>Group 3, cis-platinum/mesna</td>
<td>4.42</td>
<td>2.19*</td>
<td>4.26*</td>
<td>3.06*</td>
<td>5.47*</td>
</tr>
</tbody>
</table>

* Significant difference compared to control by analysis of variance at P < 0.05. * Significant difference between cis-platinum and cis-platinum/mesna by analysis of variance at P < 0.05.

The effect on villus influx by cis-platinum or the cis-platinum/mesna combination are shown in Table 1. These data demonstrate that mesna administration resulted in a much higher rate of crypt recovery following cis-platinum administration. These experiments were repeated with similar results. The effect of different mesna-dosing regimens on cis-platinum effects on villus influx are shown in Fig. 4. When given immediately after cis-platinum, mesna had a similar effect as that of the three consecutive mesna doses; however, when given 2 h after cis-platinum, no protection was observed and values were similar to those observed after cis-platinum alone.

The effect of cis-platinum on gastrointestinal tract disaccharidase activity and the effect of mesna on these changes are shown in Tables 2 and 3. cis-Platinum caused a significant depletion in both maltase and sucrase activity which was re-
EFFECT OF MESNA ON cis-PLATINUM TOXICITY

Table 3 Effect of mesna on maltase activity in jejunal samples from mice following cis-platinum administration

<table>
<thead>
<tr>
<th>Group</th>
<th>Maltase activity (μmol hydrolyzed/min/g tissue)</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>52.49</td>
<td>50.50</td>
<td>50.53</td>
<td>54.09</td>
<td>55.37</td>
<td></td>
</tr>
<tr>
<td>cis-platinum</td>
<td>49.31</td>
<td>41.57</td>
<td>28.88</td>
<td>45.49</td>
<td>49.53</td>
<td></td>
</tr>
<tr>
<td>cis-platinum/mesna</td>
<td>54.56</td>
<td>37.17</td>
<td>39.53</td>
<td>47.32</td>
<td>56.44</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Median survival (days)</th>
<th>% median survival (treated/control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>cis-platinum</td>
<td>11</td>
<td>157</td>
</tr>
<tr>
<td>cis-platinum/mesna</td>
<td>10</td>
<td>143</td>
</tr>
<tr>
<td>cis-platinum/mesna</td>
<td>11.5</td>
<td>164</td>
</tr>
<tr>
<td>cis-platinum/mesna</td>
<td>10.5</td>
<td>150</td>
</tr>
</tbody>
</table>

Table 4 Effect of mesna on the cytotoxicity of cis-platinum to mouse L1210 leukemia

<table>
<thead>
<tr>
<th>Group</th>
<th>Median survival (days)</th>
<th>% median survival (treated/control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>cis-platinum</td>
<td>11</td>
<td>157</td>
</tr>
<tr>
<td>cis-platinum/mesna</td>
<td>10</td>
<td>143</td>
</tr>
<tr>
<td>cis-platinum/mesna</td>
<td>11.5</td>
<td>164</td>
</tr>
<tr>
<td>cis-platinum/mesna</td>
<td>10.5</td>
<td>150</td>
</tr>
</tbody>
</table>

DISCUSSION

cis-Platinum has profound effects on the kinetics, morphology, and function of the mouse small intestine over a 10-day period. In humans, renal damage is the dose limiting toxicity but for the patient it is the gastrointestinal symptomatology which predominates as the main toxic problem. Although the acute emesis associated with cis-platinum is thought to be mediated centrally (3), the pathogenesis of the prolonged nausea and anorexia, often up to 7–10 days, is unclear. It may be that gut mucosal toxicity is an important factor. The results presented demonstrate a significant protective role of mesna on the survival from a lethal dose of cis-platinum and on the intestinal toxicity which the latter produces. Significant protection against creatinine elevation following cis-platinum was also measured. In the case of intestinal damage mesna lessened the degree of cis-platinum-induced kinetic, architectural, and functional derangement. The above observations explain why the rate of overshoot in crypt cell production was enhanced following cis-platinum when mesna was coadministered and why homeostasis was achieved more rapidly. The mesna control group did not differ from the saline control group and suggests that mesna is interacting with cis-platinum in a protective fashion for the crypt cells rather than stimulating a crypt hyperproliferative response. Clearly the scheduling of mesna is important and coadministration with cis-platinum appears mandatory because dosing with mesna 2 h after cis-platinum did not appear to afford any protection. The mechanism of action of mesna is not certain although it appears that a platinum/mesna complex is produced intracellularly, limiting the cis-platinum available to react with cellular target molecules. The findings of Ormstad et al. (9) suggest that mesna is oxidized in the bloodstream and is selectively taken up in tissues, namely, enterocytes and renal tubular cells. Reduction to mesna by glutathione reductase liberates the derivative thiol which protects the bladder against cytotoxic metabolites of cyclophosphamide and ifosfamide (8). The data presented here indicate that mesna has intriguing potential as a cis-platinum antidote. Whether the protective effect of mesna on cis-platinum lethality is due to protection against renal damage or gastrointestinal tract toxicity is unclear. Although cis-platinum did increase serum creatinine levels which were reversed by mesna, these increases did not appear significant enough to result in lethality; indeed, histological examination of the kidneys following the doses of platinum used in experiments here did not show significant renal necrosis (data not shown). Initial experiments using L1210 leukemia cells indicate that mesna could be used without compromising the antitumor activity of cis-platinum; however, this area warrants further study. Whether the protection of intestinal mucosal toxicity will improve gastrointestinal symptomatology following cis-platinum administration is at present uncertain but represents an interesting possibility.

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