Protective Effect of Piperacillin against the Nephrotoxicity of Cisplatin in Rats

TOSHIRO HAYASHI,¹* YASUO WATANABE,¹ KATSUHIKO KUMANO,¹ RIEKO KITAYAMA,¹
TETSURO MURATANI,¹ TAKASHI YASUDA,¹ ISAMU SAIKAWA,¹ JUNICHI KATAHIRA,²
TEPEI KUMADA,² AND KIYACHIRO SHIMIZU²

Research Laboratory, Toyama Chemical Co., Ltd., 2-4-1 Shimookai, Toyama 930,¹
and Department of Internal Medicine, Tokyo Women’s Medical College, 10 Kawadacho, Shinjuku-ku, Tokyo 162,² Japan

Received 25 July 1988/Accepted 3 January 1989

The protective effect of piperacillin against the nephrotoxicity of cisplatin was compared with that of fosfomycin in Fischer 344 rats. Blood urea nitrogen, serum creatinine, and morphological changes were evaluated as the renal toxicological parameters. Rats receiving 2 mg of cisplatin per kg of body weight for 5 days showed significant (P < 0.01 by multiple-comparison test) elevation of blood urea nitrogen and serum creatinine concentrations compared with rats receiving saline alone and also exhibited development of cell lesions in the pars recta of the tubes in the outer stripe of the outer medulla. However, piperacillin (250 and 1,000 mg/kg) significantly (P < 0.01 by multiple-comparison test) reduced these toxicological parameters in comparison with results for cisplatin alone. The protective effect of piperacillin was superior to that of fosfomycin, although platinum levels in the kidney were higher with the combination of cisplatin and piperacillin than with cisplatin plus fosfomycin. Although the nephrotoxicity of cisplatin was also reduced when cisplatin was administered concomitantly with sodium chloride in mole-equivalents to 250 and 1,000 mg of piperacillin per kg, its protective effect was less than that of the corresponding piperacillin dose. These results suggest that piperacillin may have a role as a protective agent against the nephrotoxicity of cisplatin.

Cisplatin (cis-diaminedichloroplatinum) is an antitumor chemotherapeutic agent that shows clinical and experimental efficacy against various kinds of tumors (14, 15, 24, 28, 29). Unfortunately, clinical use of cisplatin is commonly accompanied by hematopoietic, auditory, gastrointestinal, and renal toxicity. In particular, nephrotoxicity is dose limiting in humans (8). With use of animal models, many attempts have been made to prevent cisplatin nephrotoxicity with candidate protective agents. For example, diuresis (13, 32; C. Merrin, Am. Soc. Clin. Oncol. 17:C-26, 1976), chelating agents (1; D. J. Highy, H. J. Wallace, Jr., and J. G. Bekesi, Proc. Am. Assoc. Cancer Res. 16:131, 1975), radical scavengers (21, 27, 34), thiourea (2), thiosulfate (16), probenecid (26), and fosfomycin (18, 22) have been found to reduce the nephrotoxicity of cisplatin. However, the mechanism of renal pathogenesis of cisplatin and the protective mechanisms of these candidate protective agents against the nephrotoxicity of cisplatin have not been well explained.

Previously, we reported that piperacillin has probenecid-like action (12) and significant protective effects against the nephrotoxicity caused by cephaloridine in rabbits and by gentamicin in rats by inhibiting the transport of these drugs to proximal tubules (11). Since it has been reported that cisplatin, like cephaloridine and gentamicin, injures the proximal tubular cells, we investigated the protective effect of piperacillin against the nephrotoxicity of cisplatin in rats.

MATERIALS AND METHODS

Drugs. Cisplatin (Nippon Kayaku Co., Ltd., Tokyo, Japan), piperacillin (sodium salt; Toyama Chemical Co., Ltd., Tokyo, Japan), and fosfomycin (sodium salt; Meiji Seika Kaisha Ltd., Tokyo, Japan) were commercial preparations. Cisplatin was diluted with sterile 0.9% saline immediately before use. Piperacillin and fosfomycin were dissolved in sterile 0.9% saline.

Protective effect of piperacillin against nephrotoxicity caused by cisplatin in rats. Fischer 344 (F344) male rats weighing 178 to 205 g were randomly assigned to 10 groups of nine rats each and housed individually in cages. They were fed with a commercial rat chow (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and received tap water ad libitum. Dosage regimens and total load of anions and cation are shown in Table 1. The rats were sacrificed 24 h after the last dose. Blood samples were obtained from the inferior vena cava under the ether anesthesia, and bilateral kidneys were removed.

Blood urea nitrogen (BUN) and serum creatinine (Scr) were determined as previously described (11). One kidney was bisected longitudinally and fixed in 10% Formalin solution. After being embedded in paraffin, the section was stained with hematoxylin and eosin. Histopathological sections of kidneys were independently evaluated by a pathologist who was unaware of the regimen used. The pathologist estimated the percentage of renal tubular cell lesions according to criteria previously reported (11). In the other kidney, the platinum concentration was determined by spectrophotometry as described by Kirkland and Yoe (17).

Effect of piperacillin on platinum levels in rat kidneys. F344 rats were randomly assigned to five groups of 10 rats each. They received cisplatin alone and in combination with piperacillin (250 and 1,000 mg/kg of body weight), fosfomycin (250 mg/kg), and sodium chloride (144 mg/kg) (Table 1). Five rats from each group were sacrificed 6 and 24 h after drug administration. Kidney samples were removed for determination of platinum concentration.

Effect of piperacillin on the efficacy of cisplatin against L-1210 cells. To determine whether piperacillin influenced the therapeutic effect of cisplatin, groups of six BDF1 male mice weighing 23 to 25 g were intraperitoneally injected with

* Corresponding author.
10^5 L-1210 leukemia cells. After 24 h, each mouse received three intraperitoneal doses of 3 or 5 mg of cisplatin per kg at 96-h intervals. Piperacillin (100 or 1,000 mg/kg) was administered subcutaneously just before cisplatin. The number of survivors was recorded once daily, and the mice were observed for 30 days.

**Statistical analysis.** Data on BUN and SCr were analyzed by the Bartlett test for uniformity of variance among all groups. However, since the difference was shown as P < 0.05, the Kruskal-Wallis H test and Scheffé multiple-comparison test were applied to determine which pairs of groups were statistically different. Histopathological scores were analyzed by the Kruskal-Wallis H test and Scheffé multiple-comparison test. Comparison of platinum levels in kidney was performed by one-way analysis of variance and the Scheffé multiple-comparison test. In these tests, the differences among groups, for which P was less than 0.05, were considered significant.

**RESULTS**

**Protective effect of piperacillin against the nephrotoxicity of cisplatin in rats.** The protective effect of piperacillin against the nephrotoxicity of cisplatin in rats is shown in Fig. 1. In comparison with results for 0.9% saline alone, a significant (P < 0.01) elevation of BUN and SCr was observed in rats receiving cisplatin alone. However, these toxicological parameters were significantly (P < 0.01) reduced by the combination of cisplatin with piperacillin at doses of both 250 and 1,000 mg/kg. Although these parameters were also reduced when sodium chloride was administered with cisplatin at a dose of 63 or 144 mg/kg, the protective effect was less than with the corresponding piperacillin dose. The protective effect of fosfomycin was evaluated at a dose of 250 mg/kg because lethal toxicity of fosfomycin was seen at a dose of 1,000 mg/kg. After coadministration of cisplatin and fosfomycin, BUN and SCr values were also reduced. However, the protective effect of fosfomycin at 250 mg/kg was inferior to that of piperacillin at 250 mg/kg.

**Histopathological score.** Consecutive administration of cisplatin at 2 mg/kg for 5 days led to development of cell lesions in the pars recta of the tubules in the outer stripe of the outer medulla. This segment showed obvious morphological changes, which involved degeneration and necrosis of proximal tubules together with regeneration. These cell lesions were not observed in glomeruli and distal tubules. However, hyaline cast formation was observed in lumina of distal tubules (Fig. 2A). When cisplatin was administered with piperacillin, these cell lesions were significantly (P < 0.01) reduced in comparison with results for cisplatin alone (Fig. 2B). After administration of cisplatin with sodium chloride at

<table>
<thead>
<tr>
<th>Group</th>
<th>Regimen (mg/kg)</th>
<th>Drug and vehicle</th>
<th>Total load of cation and anions (mmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Drug (mg/kg)</td>
<td>Vehicle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saline (%)</td>
<td>NaCl (mg/kg)</td>
</tr>
<tr>
<td>1</td>
<td>Saline alone</td>
<td>None</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>CIS-P (2) alone</td>
<td>None</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>PIPC (1,000) alone</td>
<td>CIS-P (2)</td>
<td>0.9</td>
</tr>
<tr>
<td>4</td>
<td>NaCl (144)² alone</td>
<td>None</td>
<td>0.9</td>
</tr>
<tr>
<td>5</td>
<td>FOM (250) alone</td>
<td>None</td>
<td>0.9</td>
</tr>
<tr>
<td>6</td>
<td>PIPC (250) + CIS-P (2)</td>
<td>None</td>
<td>0.9</td>
</tr>
<tr>
<td>7</td>
<td>PIPC (1,000) + CIS-P (2)</td>
<td>None</td>
<td>0.9</td>
</tr>
<tr>
<td>8</td>
<td>NaCl (63)³ + CIS-P (2)</td>
<td>None</td>
<td>1.575</td>
</tr>
<tr>
<td>9</td>
<td>NaCl (144) + CIS-P (2)</td>
<td>None</td>
<td>0.9</td>
</tr>
<tr>
<td>10</td>
<td>FOM (250) + CIS-P (2)</td>
<td>FOM (250)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

---

Abbreviations: CIS-P, cisplatin; PIPC, piperacillin; FOM, fosfomycin.

² Drugs and saline were administered intravenously in a volume of 4 ml/kg for 5 days.
³ Total volume of fluid in all cases was 8 ml/kg.
⁴ Sodium chloride mole-equivalent to the total sodium content in 4 ml of 0.9% saline containing 1,000 mg of piperacillin per kg.
⁵ Sodium chloride mole-equivalent to the total sodium content in 4 ml of 0.9% saline containing 250 mg of piperacillin per kg.
63 and 144 mg/kg, histopathological scores were also reduced. However, marked improvement after treatments with fosfomycin was not observed (Table 2).

Effect of piperacillin on levels of platinum in the kidney. Platinum concentrations in the kidney at 6 and 24 h after the first administration and 24 h after 5-day administration are shown in Fig. 3. Platinum concentrations at 6 h after coadministration of cisplatin and piperacillin (1,000 mg/kg) and at both 6 and 24 h after treatment with cisplatin plus NaCl (144 mg/kg) were lower than after treatment with cisplatin alone (Fig. 3A). On the other hand, although platinum concentrations after coadministration of cisplatin and piperacillin for 5 days were slightly lower than after treatment with cisplatin alone, a significant ($P < 0.05$) reduction was observed after administration of cisplatin plus fosfomycin (Fig. 3B).

Effect of piperacillin on the efficacy of cisplatin against L-1210 cells. Mean survival of the non-drug-treated control animals was 8.3 ± 0.4 (standard error) days. When cisplatin was administered three times at 96-h intervals at doses of 3 and 5 mg/kg, mean survival was 16.8 ± 2.8 and >25.3 days, respectively. The combination of cisplatin plus piperacillin at both 100 and 1,000 mg/kg had no apparent effect on the efficacy of cisplatin against L-1210 leukemia cells in mice (Table 3).

DISCUSSION

It has been reported that male F344 rats have high sensitivity to the nephrotoxic effects of cisplatin (30); this nephrotoxicity can be verified by elevation of BUN levels and destruction of proximal tubular cells (31). Therefore, we used F344 rats in this study to elucidate the protective effect of piperacillin against the nephrotoxicity of cisplatin. Nephrotoxicity was induced with good reproducibility at an intravenous dose of 2 mg of cisplatin per kg alone for 5 consecutive days, which is a well-accepted duration of treatment in clinical practice. Moreover, histopathological sections demonstrated typical cell lesions caused by cisplatin in the pars recta of the tubules in the outer stripe of the outer medulla, as reported previously (4, 10, 30). Since it has been reported that probenecid protects against the nephrotoxicity of cisplatin (26) and that piperacillin has probenecidlike action (12), we investigated the protective effect of piperacillin at a cisplatin-piperacillin ratio of 1:125 to 1:500. This dose ratio is approximately equal to that in clinical use (about 1:40 to 1:160), since cisplatin is commonly used at 15 to 20 mg/m² of body surface area (about 0.5 mg/kg) and piperacillin is usually used at 1 to 4 g per patient. The results of this study demonstrate that nephrotoxicity due to cisplatin in rats was significantly ($P < 0.01$) reduced by the combination of cisplatin plus piperacillin and that this combination did not affect the antitumor activity of cisplatin in mice. Moreover, since it has been reported that fosfomycin is protective against cisplatin-induced nephrotoxicity (18, 22), we compared the protective effects of fosfomycin and piperacillin. As judged by the extent of reduction in renal toxicological parameters, the protective activity of piperacillin was superior to that of fosfomycin.

The mechanism of renal pathogenesis and the process by which cisplatin accumulates in the kidneys have not been
well explained (5, 9, 19, 33). However, it has been postulated that the selective and long-lasting accumulation of platinum in tubular cells is closely related to the tubular toxicity of cisplatin in animals (3, 20). Therefore, we measured platinum levels in the kidney and found no marked relationship between protective effect and platinum concentration (Fig. 1 and 3B). With respect to the relationship between protective effect and accumulation of platinum in the kidney, Pera et al. concluded that although furosemide and mannitol afforded protection against nephrotoxicity, platinum contents in the kidneys of rats receiving cisplatin with mannitol were similar to those in rats receiving cisplatin alone but lower than the levels in furosemide-treated rats (23). In addition, Daley-Yates and McBrien detected cisplatin and three metabolized platinum compounds in urine (6). Since platinum concentration in the kidney does not necessarily correlate with the extent of nephrotoxicity, it remains to be elucidated where and in what form platinum complexes (cisplatin itself and its metabolite) exist in the tubular cells.

We also examined the influence of the sodium ion contained in the sodium salt of piperacillin by concomitant administration of cisplatin with the equivalent number of moles of sodium chloride found in 250 and 1,000 mg of piperacillin per kg, respectively. Although BUN levels were significantly (P < 0.01) reduced, cisplatin plus sodium chloride showed a lesser protective effect than did the combination of cisplatin and piperacillin. If sodium ion reduces cisplatin-induced nephrotoxicity, then fosfomycin should show a greater reduction because fosfomycin at a dose of 250 mg/kg (Na, 2.74 mmol/kg) contains 6 and 1.5 times as much sodium ion as do piperacillin doses of 250 mg/kg (Na, 0.46 mmol/kg) and 1,000 mg/kg (Na, 1.85 mmol/kg), respectively. However, the protective effect of piperacillin was superior to that of fosfomycin at the comparable dose of 250 mg/kg. On

### TABLE 2. Histological toxicity scores in rats given cisplatin alone and in combination with piperacillin, fosfomycin, or sodium chloride

<table>
<thead>
<tr>
<th>Renal morphology</th>
<th>Toxicity scorea after dosing with (mg/kg):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CIS-P (2) alone</td>
</tr>
<tr>
<td>Tubular degeneration</td>
<td>30</td>
</tr>
<tr>
<td>Tubular necrosis</td>
<td>16</td>
</tr>
<tr>
<td>Hyaline cast formation</td>
<td>8</td>
</tr>
<tr>
<td>Cell infiltration in interstitium</td>
<td>14</td>
</tr>
</tbody>
</table>

* Total scores for groups of eight rats each. Drug abbreviation are as given in Table 1, footnote a.
the other hand, Littest (20) reported that preparation of cisplatin in vehicles containing 4.5% sodium chloride significantly protected animals against the toxicity observed when the drug was prepared in distilled water or in isotonic saline. In this respect, it has been suggested that cisplatin is transformed to an aquated form in the presence of low concentrations of chloride ion and that the aquated products are responsible for the toxicity in tubular cells (7, 25). Therefore, the effect of NaCl in reducing nephrotoxicity may be due to the chloride ion rather than the sodium ion, and the protective effect of piperacillin may be due to the piperacillin itself as an anion rather than to the sodium ion. However, the protective effect of piperacillin remains to be clarified.

In this study with animal models, we have confirmed that piperacillin has a significant protective effect against cisplatin-induced nephrotoxicity without altering the antitumor activity of cisplatin. The potential role of piperacillin as a protective agent against the nephrotoxicity of cisplatin requires clinical confirmation.

TABLE 3. Effects of piperacillin on the efficacy of cisplatin against L-1210 leukemia cells in mice

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Survival (days, mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>Piperacillin</td>
</tr>
<tr>
<td>100</td>
<td>8.3 ± 0.4</td>
</tr>
<tr>
<td>1,000</td>
<td>8.2 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>7.7 ± 0.2</td>
</tr>
<tr>
<td>3, 100</td>
<td>16.8 ± 2.8</td>
</tr>
<tr>
<td>3, 1,000</td>
<td>&gt;18.2*</td>
</tr>
<tr>
<td>5</td>
<td>&gt;25.5</td>
</tr>
<tr>
<td>5, 100</td>
<td>&gt;25.5</td>
</tr>
<tr>
<td>5, 1,000</td>
<td>&gt;25.5</td>
</tr>
</tbody>
</table>

* Groups of six mice each received 10^7 L-1210 cells on day 0. Cisplatin was administered on days 1, 4, and 7. Mice received piperacillin (or 0.9% saline) just before cisplatin.

ACKNOWLEDGMENTS

We thank D. V. M. Yasuhiro Kawamura for coding and reading of histological sections, Masashi Noguchi for statistical analysis, and Mineko Nagasuwa and Seiko Inagaki for technical assistance.

LITERATURE CITED