DMPS As a Rescue Agent for the Nephropathy Induced by Mercuric Chloride

RUDOLFS K. ZALUPS, ROBERT M. GELEIN and ELSA CERNICHIA

Division of Basic Medical Sciences (R.K.Z.), Mercer University School of Medicine, Macon, Georgia and Department of Biophysics (R.M.G., E.C.), University of Rochester School of Medicine, Rochester, New York

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ABSTRACT

The effectiveness of 2,3-dimercaptopropane-1-sulfonate (DMPS) as a rescue agent for the acute nephropathy induced by HgCl₂ was studied in uninephrectomized (NPX) and sham-operated (SO) rats. NPX and SO rats that were given a toxic 2.5-µmol/kg dose of HgCl₂ developed severe renal damage within 24 hr after the HgCl₂ was administered. Renal injury was assessed by measuring plasma creatinine, creatinine clearance, fractional excretion of several biological markers, the rate of excretion of cellular enzymes and plasma solutes and severity of morphologically demonstrable necrosis in the pars recta of proximal tubules. When a 10-mg/kg dose of DMPS was given to the NPX and SO rats 1 hr after treatment with the 2.5-µmol/kg dose of HgCl₂, the nephropathy induced by the dose of HgCl₂ was less severe. Moreover, the NPX rats had significantly less severe renal damage than did the SO rats. Renal damage was completely absent from both NPX and SO rats that were given a 100-mg/kg dose of DMPS 1 hr after treatment with the 2.5-µmol/kg dose of HgCl₂. These data indicate that at the 10-mg/kg dose of DMPS, NPX rats are protected against the nephrotoxic effects of a 2.5-µmol/kg dose of HgCl₂ to a greater extent than are SO rats. Moreover, the data show that complete rescue from the 2.5-µmol/kg dose of HgCl₂ is afforded to NPX and SO rats given 100 mg/kg of DMPS within 1 hr after treatment with HgCl₂. This complete rescue is afforded for at least 24 hr. Based on the present findings DMPS may prove to be a useful rescue agent against the acute nephropathy induced by HgCl₂.

The metal complexing agent DMPS removes mercury from the kidney and increases the urinary excretion of mercury (Gabard, 1976a,b; Planas-Bohne, 1981; Hursh et al., 1985). There is evidence that DMPS is secreted by a probenecid-sensitive organic anion transport system localized in the kidney (Stewart and Diamond, 1987, 1988; Klotzbach and Diamond, 1988). Organic anion secretory systems that are sensitive to probenecid are localized in the proximal tubule. The S₁ segment of the proximal tubule is most active in the secretion of the majority of organic anions (Irish and Grantham, 1981). The S₁ and S₂ segments also secrete organic anions, but to a lesser extent. Inorganic mercury accumulates in both the cortex and outer medulla (Zalups and Diamond, 1987; Zalups et al., 1987). Segments of the proximal tubule are located in both regions. Recent unpublished histochemical findings from the authors' laboratory indicate that the proximal tubule may be the principal tubular site in which inorganic mercury accumulates. Therefore, it is possible that the mercury that is removed from the kidney as a result of the actions of DMPS may come from the epithelial cells of the proximal tubule. Although the exact mechanism for how mercury is removed from the kidney by DMPS is not known, it is probable that the mercuric ion complexes with DMPS within the proximal tubular cell as DMPS is being secreted into the tubular lumen. Some evidence for this mechanism comes from a study in which it was shown that the removal of mercury from the kidney and the secretion of DMPS are both probenecid-sensitive (Klotzbach and Diamond, 1988).

Renal damage induced by mercuric chloride (HgCl₂) is localized almost exclusively to the pars recta segment of the proximal tubule situated in the cortex and outer stripe of the outer medulla (Gritzka and Trump, 1968; Ganote et al., 1974; McDowell et al., 1976; Zalups and Diamond, 1987). The pars recta of the proximal tubule is composed of the terminal portion of the S₁ segment and the entire S₂ segment of the proximal tubule, both of which secrete organic anions. If inorganic mercury is removed from these segments of the proximal tubule by some mechanism associated with the secretion of DMPS, then it would seem that DMPS could potentially prevent the necrotic changes from occurring in the proximal tubule after the exposure to a toxic dose of HgCl₂. Protection of renal tubular epithelial cells may also be afforded by the extrarenal complexing of mercury to DMPS.

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ABBREVIATIONS: DMPS, 2,3-dimercaptopropane-1-sulfonate; NPX, uninephrectomized; SO, sham-operated; γ-GT, γ-glutamyltransferase; NAG, N-acetyl-β-glucosaminidase; AP, alkaline phosphatase; LDH, lactate dehydrogenase; AST, aspartate aminotransferase.
The aim of the present study is to determine whether a single dose of DMPS can prevent the renal damage induced by a single toxic dose of HgCl₂ by using a number of sensitive indicators of renal damage. The present study is the first to use quantitative structural, biochemical and physiological methods to evaluate the antitotal effects of DMPS on the acute nephropathy induced by HgCl₂. The animals used in this study are NPX and normal (SO) rats. The reason for using NPX rats is as follows: 1) the renal accumulation of mercury and the degree of proximal tubular damage is increased after uninephrectomy and compensatory renal growth (Ramos-Frendo et al., 1979; Houser and Berndt, 1986; Zalups and Diamond, 1987; Zalups et al., 1987) and 2) numerous structural and functional changes occur in the remnant kidney (Arrizurietza de Muchnik et al., 1969; Bricker and Fine, 1981), many of which occur in the proximal tubule. Because numerous metabolic processes are altered in the remnant kidney, it is possible that the renal handling of DMPS may also change. Thus, the second aim of this study is to determine if uninephrectomy and compensatory renal growth affect the actions and handling of DMPS in the kidney.

Materials and Methods

Animals and operative procedures. Male Sprague-Dawley (Harlen Sprague-Dawley, Indianapolis, IN) rats weighing 175 to 200 g were used in the present study. The animals were divided into two surgical groups. One group underwent unilateral nephrectomy and the other group underwent a sham-nephrectomy. The operative procedures are the same as those described previously (Zalups and Diamond, 1987; Zalups et al., 1987).

The NPX and SO rats were housed individually in plastic metabolic cages, where a standard commercial laboratory diet (Ralston Purina Co., St. Louis, MO) and water were provided ad libitum. All the animals were given 11 days to recover from surgery. The 11 days was also given to allow for the completion of the rapid phase of compensatory renal growth in the NPX rats.

Injections and groups. On the morning of the 12th day after surgery, the NPX and SO rats were placed into treatment groups consisting of six animals in each group. There were six groups in total. All six groups of rats received a toxic 2.5-μmol/kg dose of HgCl₂ i.v. (Zalups and Diamond, 1987). Each injection was given into the right femoral vein while the animal was anesthetized lightly with ether. One hour after the dose of HgCl₂ was administered, two groups of NPX and SO rats were given i.p. injections of DMPS (Sigma Chemical Co., St. Louis, MO). One group of NPX and SO rats received a 10-mg/kg dose of DMPS, whereas the other group of NPX and SO rats received a 100-mg/kg dose of DMPS. One group of NPX and SO rats did not receive a dose of DMPS.

In a pilot study it was found that the 100-mg/kg dose of DMPS had no adverse effects on the histology of the kidneys of three NPX and three SO rats after 24 hr.

Both the HgCl₂ and DMPS were dissolved in a solution containing 0.9% NaCl. The injection solutions were designed to deliver 2.0 ml/kg.

Urinary collection and analysis. Urine was collected from all the NPX and SO rats at 17°C for 24 hr before (base line) and 24 hr after (experimental) the injections of HgCl₂ had been given. A 2.0-ml portion of each 24-hr urine sample was analyzed for enzyme-content on the same day as the collection was completed. The remaining portion of each sample was stored at −10°C and analyzed later for osmolality, glucose (Lowry and Passonneau, 1972), total protein (Peace and First, 1979), albumin (Doumas et al., 1971; Gustafsson, 1976), α-amino nitrogen (Klein and Standaert, 1976), creatinine (Lancer Creatinine II Rapid Stat Diagnostic Kit, Lancer Division of Sherwood Medical, St. Louis, MO) and total mercury (both inorganic and organic) (Magos and Clarkson, 1972). The reason for measuring the rates of excretion of α-amino nitrogen and glucose was to examine the transport capacity of the proximal nephron. The analysis of α-amino nitrogen is used as a substitute for the analysis of amino acids.

The enzymes γ-GT (Szasz, 1969; Dierickx, 1981) and NAG (Lockwood and Bosman, 1979) were quantified in samples of urine that were diluted with water that was deionized and distilled. All other urinary enzymes were quantified in urine that had been dialyzed. One-milliliter samples of urine were placed in dialysis bags (Spectropor 1) and dialyzed for 4 hr at 4°C against 1.0 l of deionized water. The dialyzed samples of urine were analyzed for AP (Wright et al., 1972), LDH (Leathwood et al., 1972) and AST (Sigma Kit No. 58–10, Sigma Chemical Co.).

Collection and handling of blood and renal tissue. On the morning of the 13th day after surgery, all the NPX and SO rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.). Once anesthesia was achieved, a 2.0-ml sample of blood was obtained from the inferior vena cava of each animal. After blood was drawn, the total renal mass was removed from each rat.

The samples of blood were spun down for 5 min in a centrifuge set at 1500 × g. The plasma portion of the blood was removed for quantitation of glucose, α-amino nitrogen, creatinine and osmolality.

The kidneys from the NPX and SO were weighed after they were removed. The left kidney from each animal was sliced in half along the transverse plain. One half of each kidney was used for determining the content of total mercury in the renal tissue. The content of mercury in the renal tissue was quantitated by cold vapor atomic absorption spectrophotometry (Magos and Clarkson, 1972). A 4-mm slice of tissue was obtained from the other half of each kidney for histopathological analysis.

Histopathological analysis. Mercuric chloride-induced cellular and tubular necrosis in the proximal segment of proximal tubules in the cortex and outer stripe of the outer medulla was evaluated on a qualitative and semiquantitative basis. Quantitation of damage to the proximal segment of proximal tubules was done by using rank scores. Necrosis was ranked on a scale of 0 to 4 in severity. The definition of each rank score is as follows: 0, no necrosis; 1, less than 25% of the proximal tubules displaying signs of cellular necrosis; 2, 26 to 50% of the proximal tubules displaying signs of cellular necrosis; 3, greater than 50% of the proximal tubules displaying signs of cellular necrosis; and 4, greater than 50% of the proximal tubules displaying signs of complete tubular necrosis. An average rank score was assigned to each animal after counting the number of proximal tubules that were affected by necrosis in at least four randomly selected low power (200×) fields of the outer stripe of the outer medulla and the cortico-medullary junction. Epithelial cells that had undergone necrosis were identified by their bright eosinophilic cytoplasm and dark pyknotic nuclei. Tubular necrosis was characterized by the death and shedding of all the epithelial cells in an affected tubule.

Calculations and statistical analysis. The rates of excretion of cellular enzymes are expressed as units of enzyme excreted in 24 hr per gram of kidney (1 U = 1 nmol of substrate consumed in 1 min). The rates of excretion of other urinary solutes are expressed as milligrams of solute excreted in 24 hr per gram of kidney.

Creatinine clearance and the fractional excretion of glucose, α-amino nitrogen, water and osmotic particles were also determined according to standard methods.

Each set of urinary excretion data was analyzed statistically using a 2 × 3 analysis of variance for one repeated measure. The repeated measure being before and after HgCl₂ was administered. Other sets of data, such as plasma creatinine, creatinine clearance and fractional excretion of plasma solutes, were analyzed statistically using a 2 × 3 analysis of variance with no repeated measures. When significant F values (P < .05) were computed within the analysis of variance, statistical differences (P < .05) between sets of all logical combinations of any two means were analyzed within the analysis of variance were evaluated statistically using the "protected-t" multiple comparison test. The histopathological data were analyzed statistically with the Mann-Whitney U test.
Results

Animal and renal growth after surgery. There was no significant difference in body weight between any of the groups of NPX and SO rats 12 days after surgery (table 1).

Compensatory renal growth had occurred in the NPX rats during the 12 days after uninephrectomy. The left remnant kidneys in the NPX rats were on average 32 to 38% greater in mass than the left kidneys in corresponding SO rats (table 1). Although compensatory renal growth had occurred, the total renal mass in the NPX rats was significantly less than that in the SO rats.

Histopathology. Severe damage occurred in the kidneys of the NPX and SO rats during the 24 hr after the 2.5-μmol/kg dose of HgCl$_2$ was administered. Cellular and tubular necrosis was localized exclusively in the pars recta segment of proximal tubules located in the outer stripe of the outer medulla and in the medullary rays of the cortex. On a qualitative basis, there did not appear to be a difference in the severity of damage to the pars recta of proximal tubules between the NPX and SO rats. This was confirmed by the semiquantitative rank-score method for analyzing the sections of renal tissue (table 2).

Cellular and tubular necrosis in the pars recta segment of proximal tubules also occurred in the kidneys of the NPX and SO rats given the 2.5-μmol/kg dose of HgCl$_2$ and the 10 mg/kg dose of DMPS. The degree of necrosis in the kidneys from the SO rats given the dose of HgCl$_2$ plus the 10-mg/kg dose of DMPS appeared to be slightly less than that in the kidneys from the SO rats given the HgCl$_2$ alone. Statistical evaluation of the semiquantitative data revealed that there was no statistically significant difference in the severity of necrosis between the two groups of SO rats (table 2). By contrast, the level of damage to the pars recta of proximal tubules appeared to be considerably less in the kidneys of the NPX rats given the HgCl$_2$ plus DMPS, when compared with that in the kidneys of the NPX rats given only the 2.5-μmol/kg dose of HgCl$_2$. Semiquantitative analysis supported this observation (table 2).

No signs of cellular or tubular necrosis were found in the kidneys of the NPX and SO rats treated with the 2.5-μmol/kg dose of HgCl$_2$ and the 100-mg/kg dose of DMPS. The renal tissue from both groups of rats appeared normal.

Urinary excretion of cellular enzymes. A fairly consistent pattern developed for the excretion of the cellular enzymes (fig. 1). With the rare exception, the base-line level for the rate of excretion of LDH, AST, γ-GT, AP or NAG between the six groups of rats was not significantly different.

When the 2.5-μmol/kg dose of HgCl$_2$ was administered alone, the rates of excretion of all the cellular enzymes increased tremendously above base-line levels in both the NPX and SO rats. The rates of excretion of LDH, γ-GT and AP were slightly, but significantly, less in the NPX rats than in the SO rats. In contrast, the rates of excretion of AST and NAG were significantly greater in the NPX rats than in the SO rats.

Both the NPX and SO rats that were treated with the 2.5-μmol/kg dose of HgCl$_2$ and the 10-mg/kg dose of DMPS excreted a significantly lesser amount of cellular enzymes during the 24-hr post-treatment period than did the corresponding NPX and SO rats treated with HgCl$_2$ alone. However, the NPX rats excreted significantly less LDH, AST, γ-GT and AP during the 24-hr period than did the SO rats. Only the rate of excretion of NAG was similar in both the NPX and SO rats given HgCl$_2$ and 10 mg/kg of DMPS.

The amount of cellular enzymes excreted by the NPX and SO rats treated with the 2.5-μmol/kg dose of HgCl$_2$ and the 100-mg/kg dose of DMPS during the 24-hr experimental period was, with little exception, not statistically different from that excreted by the same animals during the 24-hr period before the treatment with HgCl$_2$ and DMPS.

Urinary excretion of plasma solutes. During the 24-hr control period, the NPX and SO rats excreted similar amounts of glucose and α-amino nitrogen (fig. 2). However, the NPX rats excreted more protein and albumin than the SO rats.

The 24-hr urinary excretion of protein, albumin, glucose and α-amino nitrogen increased greatly above base-line levels in

### TABLE 1

Animal balance data 12 days after surgery

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal Wt.</th>
<th>Left Kidney Wt.</th>
<th>Total Renal Mass</th>
<th>V</th>
<th>U$_{Cr}$</th>
<th>U$_{Osm}$</th>
<th>U$_{Crea}$</th>
<th>U$_{α-N}$</th>
<th>P$_{Crea}$</th>
<th>P$_{Osm}$</th>
<th>P$_{α-N}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPX-Hg</td>
<td>246</td>
<td>1.54*</td>
<td>1.54</td>
<td>0.030</td>
<td>237</td>
<td>4.07</td>
<td>257</td>
<td>18.6</td>
<td>330</td>
<td>1558</td>
<td>57.0</td>
</tr>
<tr>
<td></td>
<td>±19</td>
<td>±0.13</td>
<td>±0.13</td>
<td>±0.006</td>
<td>±24</td>
<td>±128</td>
<td>±1013</td>
<td>±64</td>
<td>±20</td>
<td>±206</td>
<td>±5.3</td>
</tr>
<tr>
<td>NPX-HgDMPS</td>
<td>259</td>
<td>1.32*</td>
<td>1.32</td>
<td>0.017</td>
<td>619</td>
<td>860</td>
<td>1242</td>
<td>61</td>
<td>316</td>
<td>1903</td>
<td>45.5</td>
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<tr>
<td></td>
<td>±9</td>
<td>±0.13</td>
<td>±0.13</td>
<td>±0.004</td>
<td>±174</td>
<td>±295</td>
<td>±1293</td>
<td>±66</td>
<td>±4</td>
<td>±129</td>
<td>±7.0</td>
</tr>
<tr>
<td>NPX-HgDMPS</td>
<td>245</td>
<td>1.24</td>
<td>1.24</td>
<td>0.014</td>
<td>724</td>
<td>979</td>
<td>176</td>
<td>90</td>
<td>318</td>
<td>1851</td>
<td>45.3</td>
</tr>
<tr>
<td></td>
<td>±18</td>
<td>±0.13</td>
<td>±0.13</td>
<td>±0.003</td>
<td>±139</td>
<td>±370</td>
<td>±166</td>
<td>±30</td>
<td>±10</td>
<td>±341</td>
<td>±6.8</td>
</tr>
<tr>
<td>SO-Hg</td>
<td>249</td>
<td>1.14</td>
<td>2.22</td>
<td>0.025</td>
<td>413</td>
<td>711</td>
<td>735</td>
<td>449</td>
<td>333</td>
<td>1927</td>
<td>57.7</td>
</tr>
<tr>
<td></td>
<td>±4</td>
<td>±0.13</td>
<td>±0.24</td>
<td>±0.006</td>
<td>±73</td>
<td>±175</td>
<td>±3507</td>
<td>±114</td>
<td>±4.5</td>
<td>±154</td>
<td>±6.0</td>
</tr>
<tr>
<td>SO-HgDMPS</td>
<td>248</td>
<td>1.00</td>
<td>1.97</td>
<td>0.020</td>
<td>590</td>
<td>869</td>
<td>2673</td>
<td>241</td>
<td>316</td>
<td>2092</td>
<td>50.3</td>
</tr>
<tr>
<td></td>
<td>±11</td>
<td>±0.02</td>
<td>±0.09</td>
<td>±0.06</td>
<td>±321</td>
<td>±429</td>
<td>±2058</td>
<td>±103</td>
<td>±3.1</td>
<td>±22</td>
<td>±7.0</td>
</tr>
<tr>
<td>SO-HgDMPS</td>
<td>252</td>
<td>0.93</td>
<td>1.87</td>
<td>0.014</td>
<td>786</td>
<td>1168</td>
<td>86</td>
<td>117</td>
<td>332</td>
<td>2146</td>
<td>54.8</td>
</tr>
<tr>
<td></td>
<td>±9</td>
<td>±0.13</td>
<td>±0.27</td>
<td>±0.004</td>
<td>±182</td>
<td>±294</td>
<td>±55</td>
<td>±0.7</td>
<td>±13</td>
<td>±838</td>
<td>±9.4</td>
</tr>
</tbody>
</table>

* Significant different (P < .05) from the mean for the corresponding SO rats treated the same way. † significantly different (P < .05) from the mean for the rats in the same surgical series given the 2.5-μmol/kg dose of HgCl$_2$. ** significantly different (P < .05) from the mean for the rats in the same surgical series given the 2.5-μmol/kg dose of HgCl$_2$ alone. ‡ significantly different (P < .05) from the mean for the rats with the 10-mg/kg dose of DMPS. § significantly different (P < .05) from the mean for the rats in the same surgical series given the 2.5-μmol/kg dose of HgCl$_2$ and the 10-mg/kg dose of DMPS.
Table 2
Mean rank scores of cellular and tubular neurosis in pars recta segments of proximal tubules in the cortex and outer stripe of the outer medulla

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Surgical Group</th>
<th>Mean Rank Scores for Severity of Cellular and Tubular Neurosis</th>
<th>Statistical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0  1  1.5  2  2.5  3  3.5  4</td>
<td></td>
</tr>
<tr>
<td>2.5 μmol/kg of HgCl2</td>
<td></td>
<td>1  1  4  3.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SO (n = 6)</td>
<td>2  4  3.92</td>
<td></td>
</tr>
<tr>
<td>2.5 μmol/kg of HgCl2 + 10 mg/kg of DMPS</td>
<td></td>
<td>1  1  1  1  1  1  2.67</td>
<td>—*</td>
</tr>
<tr>
<td></td>
<td>NPX (n = 6)</td>
<td>1  1  3  3.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SO (n = 6)</td>
<td>6  6  —**</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different (P < .05) from the values for the SO rats given the same treatment; † significantly different (P < .05) from the values for the NPX rats given only the 2.5-μmol/kg dose of HgCl2; ‡ significantly different (P < .05) from the values for the animals in the same surgical series given the 2.5-μmol/kg dose of HgCl2 alone and the 2.5-μmol/kg dose of HgCl2 plus the 10-mg/kg dose of DMPS.

Both the NPX and SO rats immediately after the 2.5-μmol/kg dose of HgCl2. With the exception of glucose, the NPX and SO rats excreted similar amounts of plasma solutes during the 24-hr experimental period. The NPX rats excreted glucose at a lower rate than did the SO rats.

When the 10-mg/kg dose of DMPS was given to NPX and SO rats pretreated with the 2.5-μmol/kg dose of HgCl2, they excreted a significantly smaller amount of all the plasma solutes than the corresponding NPX and SO rats given the 2.5-μmol/kg dose of HgCl2 alone.

The NPX and SO rats given the 2.5-μmol/kg dose of HgCl2 and the 100-mg/kg dose of DMPS excreted similar amounts of plasma solutes during the control and experimental 24-hr collecting periods. Thus, there was no increase in the excretion of plasma solutes above the base-line levels in these animals.

Plasma creatinine and creatinine clearance. Plasma creatinine was elevated in the NPX and SO rats given the 2.5-μmol/kg dose of HgCl2 24 hr after the dose of HgCl2 was injected (table 1; fig. 3). The levels of plasma creatinine in the NPX and SO rats were similar. When the 10-mg/kg dose of DMPS was given to NPX and SO rats pretreated with HgCl2, plasma creatinine was significantly less than that in the corresponding NPX and SO rats given the HgCl2 alone. Again, there were no differences between the NPX and SO rats with respect to the levels of plasma creatinine. The same was true when the 2.5-μmol/kg dose of HgCl2DMNS and the 100-mg/kg dose of DMPS was given to NPX and SO rats.

Because plasma creatinine was elevated in the NPX and SO rats given the dose of HgCl2DMNS alone, it was not surprising to find that creatinine clearance was very low in these animals (fig. 9). Creatinine clearance was significantly greater in the NPX and SO rats treated with the 2.5-μmol/kg dose of HgCl2 and the 10-mg/kg dose of DMPS than in the corresponding NPX and SO rats given the dose of HgCl2 alone. In the SO rats treated with the HgCl2 and the 100-mg/kg dose of DMPS creatinine clearance was significantly greater than in the SO rats given the 10 mg/kg dose of DMPS.

Fractional excretion data. The fractional excretion of glucose, osmotic particles, α-amino nitrogen and water was elevated in the NPX and SO rats given the 2.5-μmol/kg dose of HgCl2 during the 24 hr immediately after the administration of the HgCl2 (fig. 4). No statistical difference in the fractional excretion of glucose, α-amino nitrogen and osmotic particles was detected between the NPX and SO rats. However, the fractional excretion of water was significantly greater in the NPX rats than in the SO rats.

In the NPX and SO rats given both the 2.5-μmol/kg dose of HgCl2 and the 10-mg/kg dose of DMPS, the fractional excretion of the four compounds measured in this study was significantly less than that in the corresponding NPX and SO rats given just the HgCl2.

In the NPX and SO rats that received the 100-mg/kg dose of DMPS after pretreatment with the 2.5-μmol/kg dose of HgCl2, the fractional excretion of all the compounds measured in this study was also significantly less than that in the corresponding NPX and SO rats given the HgCl2 alone. In addition, the fractional excretion of glucose, α-amino nitrogen and osmotic particles in the SO rats was significantly less than that in the SO rats given the 10-mg/kg dose of DMPS. The level for the fractional excretion of all four compounds measured in this study was similar in the NPX rats given the 10 mg/kg dose of DMPS and in the NPX rats given the 100 mg/kg dose of DMPS. No statistical differences in the fractional excretion of any of the compounds was detected between the NPX and SO rats given the 100 mg/kg dose of DMPS.

Mercury in urine and renal tissue. There was no difference in the amount of mercury that was excreted in urine in 24 hr between the NPX and SO rats that received the 2.5-μmol/kg dose of HgCl2 (fig. 5). The NPX and SO rats given the 10 mg/kg dose of DMPS after treatment with HgCl2 excreted significantly more mercury in the urine than did the corresponding NPX and SO rats that were treated with HgCl2 alone. Moreover, the NPX rats excreted significantly more mercury than did the SO rats. The NPX rats that received the 100 mg/kg dose of DMPS excreted a similar amount of mercury that the NPX rats given the 10 mg/kg dose of DMPS excreted. By contrast, the SO rats that received the 100 mg/kg dose of DMPS excreted a significantly greater amount of mercury than did the SO rats that received the 10 mg/kg dose of DMPS.

The percentage of the administered dose of mercury that was in each gram of renal tissue from the NPX and SO rats 24 hr after the 2.5-μmol/kg dose of HgCl2 was administered was similar (fig. 5). The same levels of mercury per gram of renal tissue were also found in the NPX and SO rats given the 10 mg/kg dose of DMPS. In the renal tissue from the NPX and SO rats given the 100 mg/kg dose of DMPS, the concentration...
Discussion

The findings from the present study show that DMPS can protect or prevent (depending on the dose) NPX and SO rats from developing the acute nephropathy induced by a toxic dose of HgCl₂. This is the first study to demonstrate the effects of DMPS on the acute nephropathy induced by HgCl₂ using sensitive indicators of renal injury, such as the urinary excretion of cellular enzymes and plasma solutes and histopathological scoring of cellular necrosis. The excretion of various cellular enzymes, such as γ-GT, NAG, LDH, AST and AP, has been used recently to demonstrate injury to renal tubular epithelial

of mercury was significantly lower than that in the renal tissue from the corresponding NPX and SO rats given the 10-mg/kg dose of DMPS or the 2.5-μmol/kg dose of HgCl₂ alone.

The left kidney of the NPX rats accumulated more of the administered dose of mercury than did the left kidney from the SO rats (fig. 5). This difference appeared to related to the fact that the left remnant kidney of the NPX rats had undergone compensatory renal growth. Because the remnant left kidney of the NPX rats did not equal the combined mass of the two kidneys in the SO rats, the amount of mercury in the total renal mass was greater in the SO rats than in the NPX rats.
cells induced by HgCl₂ (Cal et al., 1989; Zalups and Diamond, 1987), lead (Khalil-Manesh et al., 1989) and uranyl fluoride (Zalups et al., 1988; Diamond et al., 1989). It has been established that enzynuria is a very sensitive indicator of injury to renal tubular epithelial cells (Price, 1982). Other sensitive markers of renal injury were also used in the present study. The profiles for the urinary excretion of cellular enzymes and plasma solutes and other functional markers may provide some insights in evaluating the effects of DMPS in the clinical setting.

Protection against the acute nephrotoxic effects of inorganic mercury by the administration of DMPS has been suggested in two previous preliminary studies (Jones et al., 1980; Wannag and Aaseth, 1980). The data supporting this suggestion are

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**Fig. 2.** Urinary excretion of total protein, albumin, glucose and α-amino nitrogen in SO rats (S) and NPX rats (N) 24 hr before and 24 hr after treatment with HgCl₂. Each value represents the mean ± S.D. for six animals. M, treated with a 2.5-μmol/kg dose of HgCl₂. MD, treated with a 2.5-μmol/kg dose of HgCl₂ followed by a 10-μmol/kg dose of DMPS 1 hr later. **Significantly different (P < .05) from the mean for the control data from the animals in the same group of animals and significantly different (P < .05) from the means for the experimental data from the animals in the same surgical series (S or N) that received the 2.5-μmol/kg dose of HgCl₂ plus the 10-μmol/kg dose of DMPS and the 2.5-μmol/kg dose of HgCl₂ plus the 100-μmol/kg dose of DMPS. * Significantly different (P < .05) from the means for the control data from the same group of animals and significantly different (P < .05) from the mean for the experimental data from the animals in the same surgical series (S or N) that received the 2.5-μmol/kg dose of HgCl₂ plus the 10-μmol/kg dose of DMPS. × Significantly different (P < .05) from the mean for the experimental data from the corresponding S rats treated identically.

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**Fig. 3.** Plasma creatinine and creatinine clearance in NPX rats and SO rats (SHAM) during the 24 hr after treatment with a 2.5-μmol dose of HgCl₂ or a 2.5-μmol dose of HgCl₂ plus a 10-μmol/kg dose of DMPS or 100-μmol/kg dose of DMPS (DMPS2). **Significantly different (P < .05) from the means for the animals in the same surgical series (SHAM or NPX) treated with the 2.5-μmol/kg dose of HgCl₂ plus the 10-μmol/kg dose of DMPS and the 2.5-μmol/kg dose of HgCl₂ plus the 100-μmol/kg dose of DMPS. * Significantly different (P < .05) from the means for the animals in the same surgical series (SHAM or NPX) treated with the 2.5-μmol/kg dose of HgCl₂ plus the 100-μmol/kg dose of DMPS.
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quite preliminary in nature and have hitherto left much room for speculation as to how much protection is afforded by DMPS.

In one study observations were made that DMPS increased the survival ratio for mice treated with HgCl₂ (Jones et al., 1980). In the other study it was observed that DMPS caused less oliguria and less macroscopic renal changes in rats treated with twice the dose of HgCl₂ used in the present study (Wannag and Asseth, 1980). Although there were indications that DMPS provided some protection against the nephrotoxic effects of HgCl₂, the question as to whether renal damage occurred in the animals treated with DMPS was never addressed by the investigators in the previous studies. By contrast, the present study is the first to demonstrate detailed structural and functional relationships associated with the effects of DMPS on the acute nephropathy induced by HgCl₂. The present findings show clearly that in both NPX and SO rats a 100-mg/kg dose of DMPS given shortly after treatment with HgCl₂ completely prevents the acute nephropathy induced by HgCl₂ from occurring.

NPX rats were examined in the present study because the accumulation of, and the nephropathy induced by, inorganic mercury is altered in rats that have undergone unilateral nephrectomy (Ramos-Frendo et al., 1979; Houser and Berndt, 1986; Zalups et al., 1987; Zalups and Diamond, 1987). These changes are somehow related to the numerous morphological, physiological and biochemical changes that occur in the remnant kidney of NPX rats during compensatory growth (Bricker and Fine, 1981; Zalups et al., 1985; Zalups, 1989). Because the renal handling of mercury is altered after unilateral nephrectomy and compensatory renal growth, it was hypothesized that the rescue effects of DMPS are also altered.

In the present study several sets of data indicate that NPX rats treated with a 10-mg/kg dose of DMPS are protected from the nephrotoxic effects of a 2.5-μmol/kg dose of HgCl₂ to a significantly greater extent than SO rats. The mechanism for this increased protection is not known. However, it appears that the increased protection may be related to the increased urinary excretion of mercury. The NPX rats excreted nearly 20% more mercury during the initial 24 hr after the dose of HgCl₂ was given than did the SO rats. There are a couple of possible explanations for the increased urinary excretion of mercury in the NPX rats. Inasmuch as solute transport and renal blood flow have been demonstrated to increase in the remnant kidney (Bricker and Fine, 1981), it is possible that the renal tubular secretion of DMPS (Stewart and Diamond, 1987; Klotzbach and Diamond, 1988) by the proximal tubular epithelial cells may also increase. Increased transport of DMPS would increase the probability of DMPS removing more mercury from the cytosol of the epithelial cells in the proximal tubules. The increased removal of mercury from the cytosol would probably serve a protective role as well. The increased renal blood flow may also increase the availability of mercury-DMPS complexes
formed in the blood and other tissues to be excreted in the urine by the remnant kidney.

Although a 10-mg/kg dose of DMPS causes significant changes in the urinary excretion of mercury, it does not cause a significant change in the concentration of mercury in the renal tissue of either NPX or SO rats given a 2.5-μmol/kg dose of HgCl₂. In the present study, DMPS caused a decrease in the amount of mercury in the total renal mass by only about 12% in the SO rats and by only about 17% in the NPX rats within 24 hr. However, as stated above, this dose of DMPS did cause urinary mercury excretion to increase in the NPX and SO rats. Urinary excretion of mercury increased about 2.4 times in NPX rats and by about 1.9 times in SO rats. In the final analysis, only about 10% of the mercury excreted in the urine of the NPX rats can be presumed to be of renal origin. Only about 13% of the mercury excreted in the urine of the SO rats can be assumed to be of renal origin. As a result of these calculations, it appears the majority of mercury excreted in the urine of both the NPX and SO rats treated with the 10-mg/kg dose of DMPS does not come from renal tissue. The above findings might lead one to suggest that the protection afforded by the 10-mg/kg dose of DMPS, especially to the NPX rats, may not be completely dependent on altering the renal burden of mercury.

In contrast to what occurs with the 10-mg/kg dose of DMPS, no differences in the urinary excretion of mercury or the rescue effects of DMPS occur between NPX and SO rats given a 100-mg/kg dose of DMPS 1.0 hr after being given a 2.5-μmol/kg dose of HgCl₂. At least during the first 24 hr, there is no evidence of any renal injury in either group of animals. It appears that the 100-mg/kg dose of DMPS saturates the protective mechanism in both the NPX and SO rats and, thus, complete protection against the nephrotoxic effects of a 2.5-μmol/kg dose of HgCl₂ is afforded to both groups of animals.

A significant reduction in the amount of mercury that accumulates in the total renal mass occurs in both NPX and SO rats given the 100-mg/kg dose of DMPS. About 40% less mercury was found to accumulate in the total renal mass of both the NPX and SO rats within 24 hr. Compared with the level of mercury excretion in the NPX rats given the 10-mg/kg dose of DMPS, the urinary excretion of mercury in the NPX rats given the 100-mg/kg dose was not found to be significantly different. However, the urinary excretion of mercury in the SO rats given the 100-mg/kg dose of DMPS was 42% greater than the urinary excretion of mercury in the SO rats given the 10-mg/kg dose of DMPS. Approximately 26% of the amount of mercury-excreted in the urine from the NPX rats and 31% of the amount of mercury excreted in the urine from the SO rats can be presumed to be of renal origin. Thus, at the 100-mg/kg dose of DMPS, a significant amount of mercury is removed from the kidney. With this larger dose of DMPS, protection
against the nephrotoxic effects of HgCl$_2$ may involve both a decrease in the renal mercury burden and an increase in the urinary excretion of mercury.

In a previous study (Wannag and Asath, 1980) it was shown that about a 50-mg/kg dose of DMPS actually caused a slight increase in the concentration of mercury in the kidney when given shortly after a 5.0-μmol/kg dose of HgCl$_2$. In two other reports (Cherian et al., 1988; Diamond et al., 1988a) it was shown in rats given nontoxic doses of HgCl$_2$ that the urinary excretion of mercury induced by DMPS was almost equal to the renal loss of mercury. Based on the previous findings and those of the present study, it seems that the effects of DMPS on the renal mercury burden are dependent on the dose of inorganic mercury and the dose DMPS used.

Part of the problem in evaluating the effects of DMPS on the renal mercury burden in animals given nephrotoxic doses of mercury is that the urinary excretion of mercury increases as the dose of mercury is increased (Magos, 1972; Zalups and Diamond, 1987). This increased urinary excretion of mercury is presumed to be due to the release of mercury from necrotic renal proximal tubular epithelial cells (Zalups et al., 1987). Furthermore, the urinary excretion of mercury on a per gram of kidney basis is increased in NPX rats more so than in SO rats, particularly at low toxic doses of inorganic mercury (Zalups and Diamond, 1987). These issues need to be addressed more fully in the future in order to better understand the relationship between renal mercury content and the urinary excretion of mercury in NPX and SO rats treated with DMPS.

Because DMPS is relatively nontoxic in humans (Aposhian, 1983), DMPS may be useful in the clinical setting as a rescue agent against the acute nephrotoxic effects of inorganic mercury in situations of acute overdose or overexposure. The 100-mg/kg dose of DMPS used in this study is a large dose, and in humans some necrosis and ulcerations have been observed at the site of injection of this dose of DMPS (Glukharev, 1965). However, this would seem to be a small side effect when compared to the benefit gained by the injection of DMPS. It is also possible that a dose below 100 mg/kg would be effective as an antidote for inorganic mercury intoxication.

References


**Gabad, B.:** The excretion and distribution of inorganic mercury in the rat as influenced by several chelating agents. Arch. Toxicol. 35: 15–24, 1976a.


Send reprint requests to: Dr. Rudolfs K. Zalups, Division of Basic Medical Sciences, Mercer University School of Medicine, 1550 College St., Macon, GA 31207.