Effects of an oral adsorbent on cisplatin-induced nephropathy in rats

Tadashi SATO, Sumio MIYAZAKI, and Shirou MOHRI

Department of Pediatrics, and Center for Laboratory Animals, Saga Medical School, Nabeshima, Saga, Japan

A 26-week experiment was designed to determine the effect of an oral adsorbent, AST-120 (KremezinTM), in rats with cisplatin-induced interstitial nephropathy. We found that creatinine clearance during the 24th week of the experiment was significantly higher in the AST-120-treated rats (n=11) than in the control animals (n=11) (1.09±0.14, vs. 0.63±0.12 ml/min; p<0.05). Furthermore, the kidneys, which were examined morphometrically using a computerized image scan, revealed that the AST-120-treated rats had a significantly lower ratio of the tubular cross-sectional area over the cortex and outer medulla cross-sectional area than the control group (0.26±0.03 vs. 0.38±0.02; p<0.05). An additional experiment was conducted to see if the oral adsorbent affects absorption and excretion of intraperitoneally administered cisplatin. There was no difference between the control group (n=12) and the AST-120-treated group (n=12) in serum concentration or urinary excretion of cisplatin during a 72-hour period after the injection. The results of our experiments suggest that the oral absorbent, AST-120, blunts progressive deterioration of renal function and nephron architecture in cisplatin nephropathy.


Key words: cisplatin, nephropathy, oral adsorbent, AST-120, morphometry

Introduction

Cisplatin, one of the most potent anti-cancer drugs, is frequently used in treating a wide range of human tumors. However, it has adverse effects on the kidneys, primarily on the renal tubules. Many substances have been shown to be useful in reducing nephrotoxicity: these include mannitol [1], sodium thiosulfate [2], WR 2721 (Ethiofos) [3], glutathione [4], probenecid [5], sodium N-methyl D-glucamine dithiocarbamate (DDTC) [6], and several other agents [7]. Recently, Yoshida Y. et al. [8] reported that AST-120 (KremezinTM, Kureha Chemical Industry, Co., Ltd., Tokyo), a spherical carbonaceous oral adsorbent, was useful in delaying the progression of deteriorating renal function and preserving the glomerular architecture in the rat model of subtotal nephrectomy. They showed that the serum level of uremic toxins was lower in the AST-120-treated animals. However, it is not known whether or not the oral adsorbent plays a role in protection against renal failure due to cisplatin-induced interstitial nephropathy. Therefore, an experiment was designed to determine the effect of AST-120 on nephropathy.

Materials and Methods

Animal preparation

Cisplatin 0.5 mg/kg body weight was given intraperitoneally twice a week, for 14 weeks, to thirty 10-week-old Wistar rats (weight 260–290 g). During the 14th week, twenty-two rats were selected and divided into two groups to serve as an AST-120-treated group and a control group, so that the levels of blood urea nitrogen (BUN), serum creatinine and creatinine clearance were comparable between the two groups. Each animal of the control group was fed with the standard powdered food, and each rat of the AST-120-treated group was administered 1.2–1.4 g of AST-120 per day p.o. in food mixed with the standard powder. The dose of AST-120 was determined according to the literature [8, 14, 15]. A tapered dose of cisplatin (0.25 mg/kg twice a week) was given continuously for another 10 weeks until the

Accepted March 13, 1996
24th week. The amount of food consumed and the changes in body weight of each rat were closely monitored. All rats were given free access to water and housed in individual cages. During the 24th week of the experiment, blood and 24-hour urine were collected to determine the levels of BUN, serum creatinine, urinary creatinine, urinary N-acetyl-β-D-glucosaminidase (NAG), urinary osmolality, hematocrit, serum electrolytes (Na⁺, K⁺, Cl⁻), serum protein, and urinary protein. All blood samples were drawn from the caudal artery. The renal function was evaluated using serum creatinine, creatinine clearance, and BUN; and a comparison was made between the two groups of animals. At the end of the 26th week, all rats of both groups were killed by an overdose of sodium pentobarbital, and the kidneys were harvested for weight measurement and also for pathological analysis, then compared with age-matched normal kidneys obtained from twelve 36-week-old Wistar rats with a body weight in the range of 450–520 g.

Analyses
BUN was measured with a BUN analyzer (BUN Analyzer 2, Beckman Instrument, Inc., Fullerton, Calif., USA). Serum creatinine was assayed using a creatinine analyzer (Creatinine Analyzer, Beckman Instruments). Urinary NAG was determined by a spectrophotometric method using 6-methyl-2-pyridyl-N-acetyl-l-thio-β-D-glucosaminidase (MPT-NAG) as the substrate.

Histological Study
The kidneys were cut longitudinally, and the cross-sections were stained with periodic acid-Schiff. The histology of cisplatin-induced nephropathy is characterized by massive tubular dilatation and degeneration in the cortex and inner stripe of the outer medulla. We determined the degree of damage by measuring the ratio of tubular cross-sectional area over the cortex and outer medulla cross-sectional area (the cross-sectional area ratio of t/COM) in the kidneys of each animal using computerized image scan software called Personal Image Analysis (Pias Co. Tokyo, Japan). The computer-assisted morphometric studies of the kidneys were conducted previously in chronic cadmium [9] or amphotericin [10] nephropathy cases.

Effects of oral adsorbent on absorption and excretion of cisplatin
Twenty-four 12-week-old Wistar rats weighing from 285 g to 320 g were divided into two groups: one for a control group (n = 12), and the other for an AST-120-treated group (n = 12). All rats were housed in individual metabolic cages. The rats of the control group were given the standard powdered food while the rats of the AST-120-treated group were fed with 1.4 g of AST-120 mixed with standard powder for a week before 2.0 mg/kg of cisplatin was administered intraperitoneally to all rats. To determine the serum and urinary levels of cisplatin, blood was removed at 2, 4, 8, 24, and 72 hours, and urine was collected during the 72-hour period following administration of cisplatin. Platinum determinations were made using a Hitachi Z-8100 X-ray absorption spectrometer, and the concentrations of cisplatin were calculated. The values are expressed as µg and µg/ml, respectively.

Statistical analysis
Values are expressed as mean ± SEM. Comparisons among the normal group, the control group and the AST-120-treated group in the experiment were made using one-way analysis of variance (ANOVA) followed by Fisher's method of multiple comparison. The significance of differences between the means of two groups was tested by the unpaired t-test. The results were deemed to be statistically significant when the p value was less than 0.05.

Changes in body weight and renal function
Eleven animals in each group survived to the end of the experiment. The changes in body weight were similar in both groups of cisplatin-treated animals, but they gained significantly less weight than the normal rats (Fig. 1). The protein load on both the control and the AST-120-treated group was equivalent because the food

Fig. 1. Body weight changes in normal rats and rats with cisplatin-induced nephrotoxicity. The dotted line (—□—), broken line (—○—) and solid line (—●—) represent the body weight of the normal group, control group, and the AST-120-treated group, respectively. *p<0.05. **p<0.01 compared with the normal rats using one-way ANOVA.
consumed and changes in body weight were very similar. Although the BUN and the serum level of creatinine of the AST-120-treated rats were slightly lower than those of the control rats in the 24th week of the experiment, the difference was not significant. The creatinine clearance was significantly higher in the AST-120-treated group than in the control group (# p<0.05).

Histological analysis

When the animals were sacrificed at the end of the 26th week, the kidneys were removed for pathological evaluation. The tubules in the control group kidneys were markedly dilated and degenerated in comparison with those in the kidneys of the AST-120-treated rats (Fig. 3). The wet kidney weight, however, was similar: 2.96 ± 0.47 g in the control group, and 2.74 ± 0.14 g in the AST-120-treated group (the age-matched normal group: 1.45 ± 0.12 g). The morphometrical analysis conducted by a computerized image scan revealed that the cross-sectional area ratio of t/COM significantly lower in the AST-120-treated group than in the control group (0.26±0.03 vs. 0.38±0.02, p<0.05) (Normal 0.15 ± 0.00) (Fig. 4).

Absorption and excretion of cisplatin

An additional experiment was conducted to see if the oral adsorbent affects absorption and excretion of intraperitoneally administered cisplatin. The serum level of cisplatin at 2 hours after the administration was 0.62 ± 0.1 μg/ml in the control group (n=12), and 0.77 ± 0.1 μg/ml in an AST-120-treated group (n=12). It decreased thereafter in both groups. The urinary excretion of cisplatin during the first 8 hours was 77 ± 22.4 μg in the control rats, and 115 ± 20.6 μg in the AST-120-treated animals (Fig. 5). There was no difference between the control group and the AST-120-treated group in serum concentration or urinary excretion of cisplatin during the 72-hour period following the injection.

Discussion

Choie et al. [11] first investigated chronic cisplatin nephropathy in rats by injecting 1.0 mg/kg body weight of cisplatin intraperitoneally twice a week for 11 weeks. Using this method, the rats all died before the 15th

Table 1. Changes in urinary NAG (U/g creatinine) and urinary osmolality (mOsm/kg) in the normal (N=11), the cisplatin-treated (N=11), and the cisplatin plus AST-120-treated rats (N=11) before the experiment, during the 14th week, and during the 24th week of the experiment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>Control</th>
<th>AST-120</th>
</tr>
</thead>
<tbody>
<tr>
<td>0w</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uNAG</td>
<td>28.1 ± 1.6</td>
<td>25.1 ± 1.8</td>
<td>19.6 ± 2.1</td>
</tr>
<tr>
<td>Control</td>
<td>28.5 ± 1.5</td>
<td>14.7 ± 0.7**</td>
<td>15.7 ± 0.7**</td>
</tr>
<tr>
<td>AST-120</td>
<td>28.1 ± 1.5</td>
<td>15.1 ± 0.7**</td>
<td>14.2 ± 1.8**</td>
</tr>
<tr>
<td>uOsmolality</td>
<td>Normal</td>
<td>745 ± 89</td>
<td>833 ± 98</td>
</tr>
<tr>
<td>Control</td>
<td>831 ± 105</td>
<td>385 ± 41**</td>
<td>407 ± 62**</td>
</tr>
<tr>
<td>AST-120</td>
<td>786 ± 66</td>
<td>356 ± 47**</td>
<td>405 ± 52**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. * p<0.05, ** p<0.01 compared with the normal rats during the 14th week using one-way ANOVA followed by Fisher's multiple comparison method.
Oral adsorbent in cisplatin-induced nephropathy

Fig. 3. PAS-stained kidneys of an age-matched normal (A), cisplatin-treated (B), and cisplatin and AST-120-treated (C) rats examined in the 26th week of the experiment. The interstitial lesions of dilatation and degeneration were more striking in the control group than in the AST-120-treated group.

Fig. 4. Wet kidney weight and the cross-sectional area ratio of t/COM in the age-matched normal group, the control group, and the AST-120-treated group. The area ratio was determined by computer image analyzer and compared using one-way ANOVA. The ratio was significantly lower in the AST-120-treated group than in the control group. (* p<0.05, ** p<0.01, # p<0.05)

Fig. 5. Absorption and excretion during the 72 hours after the intraperitoneally administered cisplatin in the control group rats (the open bars) and the AST-120-treated rats (the closed bars).
Tadashi Sato et al

played a significant role in protecting the kidneys against nephropathy. The age-matched normal control animals did not exhibit the pathological lesions seen in the animals receiving cisplatin. The creatinine clearance and urinary osmolality in the cisplatin-treated rats were significantly reduced compared to the normal rats of the same age as demonstrated in the Table 1 and Fig. 2. Taken together, the decreased renal function seen in cisplatin-treated animals in this experiment was not aging-related nephropathy, but cisplatin nephropathy.

Our experiment demonstrated that the oral adsorbent played a significant role in protecting the kidneys against cisplatin nephrotoxicity, but the mechanism by which the oral adsorbent blunted the progress of interstitial nephropathy is not clear. The oral adsorbent failed to ameliorate cisplatin-induced nephropathy in a 12-week or 16-week protocol (unpublished data). Therefore we concluded that AST-120 had no significant protective action during acute injury induced by cisplatin. The oral adsorbent seemed to work well in long-standing and advanced renal failure that was unrelated to the etiology underlying the renal failure due to either glomerular or interstitial injury.

We measured the serum and urinary concentration of cisplatin during the 72-hour period after intraperitoneal administration of cisplatin, and we found that the oral adsorbent did not adsorb parenterally administered cisplatin. This is consistent with the findings of others that after parenteral administration of cisplatin, biliary or intestinal excretion of cisplatin was minimal, and the drug was poorly absorbed in the intestines [12, 13]. This indicates that AST-120 did not act as a cisplatin adsorbent.

According to the literature, AST-120 played a significant role in ameliorating the progression of chronic renal failure in subtotally nephrectomized rats by eradicating nephrotoxic substances, such as indoxyl sulfate produced by bacteria in the intestines [8, 14, 15]. Creatol and methylguanidine were shown to accumulate in the body accelerating the progress of chronic renal failure [16]. The blood levels of these nephrotoxic substances were not assayed in our experiment; however, the glomerular filtration rate (GFR) decreased as the nephropathy progressed, suggesting that the nephrotoxic substances may have intensified the nephropathy induced by cisplatin.

Recently, Sugihara et al. [17] suggested that cisplatin affected renal tissues by generating free radicals which may interact with membrane lipids to cause the production of lipid peroxidation. The oral adsorbent AST-120 does not seem to be a direct oxygen free radical scavenger of the kidneys because it does not play a role in protecting cisplatin-induced acute nephropathy. The oral adsorbent may have adsorbed some substance that facilitates free radical toxicity. However, no study has been conducted to investigate this hypothesis.

The concentration of urinary NAG is known to increase with the progress of acute tubulopathy. However, in long-term nephrotoxicity of cisplatin neither β2-microglobulin nor NAG in the urine were shown to be predictive parameters [18], and the result of our experiment was consistent with this finding. NAG may be depleted in an advanced chronic tubulopathy. The urinary osmolality decreased constantly with the progress of cisplatin-induced nephropathy.

In conclusion, we found that the AST-120 was useful to ameliorate progression of long-standing and advanced renal failure in rats with cisplatin-induced nephrotoxicity as seen in this 24-week model.

Address to: Dr. Tadashi Sato, Department of Pediatrics, Saga Medical School, Nabeshima, Saga, Japan

References

Oral adsorbent in cisplatin-induced nephropathy


