Regulation of Renal Organic Ion Transporters in Cisplatin-Induced Acute Kidney Injury and Uremia in Rats

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ABSTRACT

Purpose. The purpose of this study was to examine the regulation of renal organic ion transporters in cisplatin-induced acute kidney injury (AKI) and its relation with indoxyl sulfate (IS), a uremic toxin.

Methods. The IS concentrations in the serum and kidney were monitored by high-performance liquid chromatography. Uptake of *p*-aminohippuric acid, estrone-3-sulfate and tetraethylammonium were examined using renal slices. Real-time PCR and immunoblotting were performed to examine the mRNA and protein expression of rOATs, rOCTs and rMATE1 in the kidney, respectively.

Results. The serum and renal IS levels were markedly elevated in cisplatin-treated rats. However, this effect was largely reversed by administration of AST-120, an oral charcoal adsorbent. The functions of renal basolateral organic anion and cation transporters were reduced in cisplatin-treated rats. The levels of mRNA and protein corresponding to rOAT1, rOAT3, rOCT2 and rMATE1, but not rOCT1, were depressed in the kidney of cisplatin-treated rats. Administration of AST-120 to cisplatin-treated rats partially restored the function and expression level of these transporters.

Conclusions. Cisplatin-induced AKI causes down-regulation of renal organic ion transporters accompanied by accumulation of serum and renal IS. IS could be involved in the mechanism of down-regulation of rOAT1, rOAT3 and rMATE1 under cisplatin-induced AKI.

KEY WORDS: acute kidney injury; cisplatin; indoxyl sulfate; organic anion transporter; organic cation transporter

INTRODUCTION

Cisplatin is a widely used chemotherapeutic agent for the treatment of several solid tumors, including ovarian, head and neck, and testicular germ cell tumors (1). A well-known complication of cisplatin administration is acute kidney injury (AKI) (2). The nephrotoxic effect of cisplatin is cumulative and dose-dependent, and often requires its dose reduction or withdrawal. It is known that cisplatin treatment damages the proximal tubules of the kidney by several different mechanisms, including oxidative stress, inflammation, DNA damage and apoptosis (3). Additionally, we previously reported that indoxyl sulfate (IS) , a representative uremic toxin, could be involved in the progression of cisplatin-induced AKI. Understanding of the pathogenesis of cisplatin-induced AKI is a clinical issue for the development of adjunctive renoprotective strategies.

IS is an anionic uremic toxin that accumulates in the serum of patients with uremia. IS is generated enzymatically in the liver from indole, which is produced from tryptophan by the intestinal flora including *Escherichia coli* (5). Normally, IS is excreted into the urine primarily by proximal tubular secretion (6, 7). Oral administration of IS in uremic rats stimulates glomerular sclerosis, which is accompanied by a reduction in renal function (5). Administration of AST-120, an oral adsorbent for intestinal indole, decreases the intensity of IS staining in the proximal tubules as well as the serum and urinary concentrations of IS, thereby preventing progression of renal dysfunction in rats (8, 9). Therefore, we reasoned that IS is likely to play a pathophysiological role in the development and progression of cisplatin-induced AKI.

The kidney plays an important role in the elimination of numerous organic anions and cations, including uremic toxins, from blood into the urine. In the renal proximal tubules, transporter-mediated secretory pathways for organic anions and cations exist that facilitate active

secretion of a wide range of exogenous and endogenous organic ions (10, 11). Renal basolateral isoforms of multispecific organic anion and cation transporters, OAT1 (Slc22a6), OAT3 (Slc22a8), OCT1 (Slc22a1) and OCT2 (Slc22a2), have been cloned and characterized (12-15). These transporters appeared to mediate the basolateral uptake of various organic anions and cations into the renal tubular epithelial cells. In contrast, rMATE1 (Slc47a1) is a renal apical membrane-localized H^+/organic cation antiporter, which has recently been functionally identified (17-19). rMATE1 is thought to mediate the final step of renal tubular secretion of cationic compounds (19). Therefore, the functional and molecular variations of these transporters could result in impaired renal excretion of substrate drugs, thereby causing the pharmacokinetic alterations and/or unexpected adverse side effects of administered ionic drugs. Previously, it was reported that the renal clearance of endogenous hippurate decreased in 5/6 nephrectomized rats, accompanied with the reduced levels of protein expression for rOAT1 and rOAT3 (16). Similarly, in 5/6 nephrectomized rats, a correlation between the renal clearance of unbound cimetidine and the expression of rOCT2 was also reported (20). In addition, we recently reported that ischemia/reperfusion-induced AKI caused a down-regulation of rOAT1, rOAT3 and rOCT2 in rat kidney, and pharmacokinetic behavior of famotidine observed (21, 22). Alternatively, renal uptake of IS is reported to be mediated by rOAT1 and rOAT3 (6, 7). Thus, the elevated IS level might be caused by the down-regulation of these two transporter proteins (4). However, there is a paucity of information concerning the functional and molecular variation of renal organic ion transporters, especially in relation to cisplatin-induced AKI and uremia. In the present study, we examined the effect of cisplatin-induced AKI on the expression and function of organic ion transporters in rat kidney, and its relation to accumulation of IS in the serum and kidney.

MATERIALS AND METHODS

Materials. Cisplatin was kindly donated by Nipponkayaku Co. (Tokyo, Japan). AST-120 was kindly supplied by Daiichi Sankyo Co., LTD., (Tokyo, Japan). IS was obtained from Sigma-Aldrich (St Louis, MO, USA). Famotidine was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). [6,7-^{3}H(N)]estrone-3-sulfate (ES) ammonium salt (2,120 GBq/mmol), D-[1-^{3}H(N)]mannitol (525.4 GBq/mmol), *p*-[Glycyl-^{14}C]aminohippuric acid (PAH) (1.95 GBq/mmol), [1-^{14}C]tetraethylammonium (TEA) bromide (118.4 MBq/mmol) and D-[1-^{14}C]mannitol (1.89 GBq/mmol) were obtained from PerkinElmer Life and Analytical Sciences (Boston, MA, USA). All other chemicals used were of the highest purity available.

Experimental animals. Male Sprague-Dawley rats, initially weighing 200 to 220 g (Clea Japan, Inc., Tokyo, Japan), were housed in a standard animal maintenance facility at constant temperature (21-23°C) and humidity (50-70%) for at least 1 week before the day of the experiment. Our protocol of animal experiments was approved by the committee of the Kumamoto University Institute of Resource Development and Analysis (18-024, 19-004). Rats were divided into three different groups as follows: saline-treated control rats, cisplatin (10 mg/kg)-treated rats, and cisplatin-treated rats with AST-120. Saline or cisplatin was administered intraperitoneally to rats. AST-120 suspended in water was administered orally on a daily basis (0.5 g/day) for 4 consecutive days since 24 h before administration of cisplatin. Animals were sacrificed under surgical anesthesia using sodium pentobarbital (50 mg/kg i.p.) 72 h after cisplatin administration. Blood samples were collected for measurement of blood urea nitrogen (BUN) and serum creatinine (SCr) values. BUN and SCr values in serum were measured at the SRL laboratory (Tokyo, Japan).

High-performance liquid chromatography determination of IS. IS concentration of serum and kidney was measured as described previously (4, 22). Briefly, the high-performance liquid chromatography system consisted of a Shimadzu LC-10ADVP pump and a Shimadzu RF-10AXL fluorescence spectrophotometer. A column of LiChrosorb RP-18 (Cica Merck, Tokyo, Japan) was used as the stationary phase and the mobile phase was acetate buffer (0.2 M, pH 4.5). The flow rate was 1.0 mL/min. IS was detected by means of a fluorescence monitor (excitation 280 nm, emission 375 nm).

Uptake by rat renal slices. Uptake studies using isolated rat renal slices were carried out as described in a previous report (22). Briefly, slices of whole kidney from control, cisplatin-treated, or cisplatin-treated rats with AST-120 were stored in ice-cold oxygenated incubation buffer composed of 120 mM NaCl, 16.2 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgSO_4$ and 10 mM NaH_2PO_4/Na_2HPO_4, pH 7.5. Renal slices were randomly selected and placed for incubation in flasks containing 6 mL of the incubation buffer with [^{14}C]PAH (5 µM, 0.37 kBq/mL), [^{3}H]ES (5 µM, 1.85 kBq/mL) or [^{14}C]TEA (5 µM, 0.56 kBq/mL). The uptake of these compounds was carried out at 37°C under an atmosphere of 100% oxygen. [^{3}H]Mannitol (5 µM, 1.85 kBq/mL) was used to calculate the extracellular trapping and non-specific uptake of [^{14}C]PAH and [^{14}C]TEA as well as to evaluate the viability of slices. Similarly, [^{14}C]mannitol (5 µM, 0.37 kBq/mL) was used to determine the non-specific uptake of [^{3}H]ES. After incubation for 60 min, the incubation buffer containing radiolabeled compounds was quickly removed from the flask and the renal slices were then washed three times with 5 mL of ice-cold phosphate-buffered saline, blotted on filter paper, weighed, and solubilized in 0.5 mL of NCSII. The amount of radioactivity was then determined in a liquid scintillation counter after adding

5 mL of OCS. Data were expressed as the slice-to-medium concentration ratio (i.e., slice concentration is expressed as milligrams PAH per gram of wet tissue, and medium concentration is in milligram PAH per milliliter of medium).

Real-time PCR analysis. The isolation of mRNA from kidney and reverse transcription were performed as described previously (22). We carried out TaqMan quantitative real-time RT-PCR using an ABI PRISM 7900 sequence detection system (Applied Biosystems Inc., Foster City, CA) to determine the mRNA expression level of rOAT1, rOAT3, rOCT1, rOCT2, rMATE1 and eukaryotic 18S ribosomal RNA (18S rRNA). The following TaqMan 18S rRNA control reagents, primer sets, products of TaqMan Gene Expression Assays were purchased from Applied Biosystems Inc.: rOAT1, Rn00568143_m1; rOAT3, Rn00580082_m1; rOCT1, Rn00562250_m1; rOCT2, Rn00580893_m1; rMATE1, Rn01497159_m1; and 18S rRNA, 4319413E.

Western blot analysis. Kidneys were homogenized in homogenization buffer consisting of 230 mM sucrose, 5 mM Tris-HCl (pH 7.5), 2 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride, 1 mg/mL leupeptin, and 1 mg/mL pepstatin A. After measurement of the protein content using bicinchoninic acid (BCA) protein assay reagent (Pierce, Rockford, IL), each sample (30 μg) was mixed in loading buffer (2% SDS, 125 mM Tris-HCl, 20% glycerol, 5% 2-mercaptoetanol) and heated to 100°C for 2 min. The samples were separated by 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Immobilon-P; Millipore, Bedford, MA) by semi-dry electroblotting. The blots were blocked overnight at 4°C with 2% ECL Advance Blocking Agent (GE Healthcare Bio-sciences Corp.) in

Tris-buffered saline (TBS) containing 0.3% Tween 20 (TBS-T) and incubated for 1 h at room temperature with primary antibody specific for rOAT1 (22), rOAT3 (22), rOCT1 (20), rOCT2 (20), rMATE1 (21, 23) or β-actin (Sigma-Aldrich). The blots were washed with TBS-T and incubated with the secondary antibody (horseradish peroxidase-linked anti-rabbit immunoglobulin F $(ab)_2$ or horseradish peroxidase-linked anti-mouse immunoglobulin F $(ab)_2$, GE Healthcare Bio-sciences Corp.) for 1 h at room temperature. Immunoblots were visualized with an ECL system (ECL Advance Western Blotting Detection Kit, GE Healthcare Bio-sciences Corp.). The relative amount of each band was determined densitometrically using Densitograph Imaging Software (ATTO Corporation, Tokyo, Japan). Densitometric ratios relative to control rats were used as the reference and accorded an arbitrary unit of 100.

Statistical analysis. Data were analyzed statistically by analysis of variance (ANOVA) followed by Scheffe's multiple comparison test. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Renal functional data of cisplatin-treated rats with or without AST-120. The renal function of rats treated with cisplatin was evaluated. Cisplatin-treated rats displayed a marked decrease in body weight compared with the untreated control group (Table 1). Furthermore, the levels of both BUN and SCr values were significantly elevated in cisplatin-treated rats compared with control rats, indicating AKI was evoked by cisplatin administration. However, the changes in body weight, BUN and SCr levels in cisplatin-treated rats were significantly reduced by administration of AST-120. These results suggested that cisplatin-induced AKI is attenuated by AST-120 administration, and that uremic toxins could be involved in the AKI evoked by cisplatin.

Endogenous IS levels of cisplatin-treated rats with or without AST-120. As anticipated during cisplatin-induced AKI, endogenous IS levels in serum and kidney were markedly elevated (Figs. 1A and 1B). By contrast, the cisplatin-associated increase in the IS level in both serum and kidney was significantly suppressed upon administration of AST-120. Fig. 1C shows the tissue-to-plasma concentration ratio (apparent K_p value) for IS. The K_p value of the cisplatin-treated rats was significantly lower than that of the control rats. A similar decrease in K_p value was also observed in cisplatin-treated rats administered with AST-120.

Uptake of PAH and ES by renal slices We reasoned that the decrease in K_p value for IS may be due to down-regulation of the organic anion transporters rOAT1 and rOAT3, which are specifically localized in the renal basolateral membrane. The activities of the two organic anion transporters were measured using renal slices by analyzing the uptake of PAH and ES, which are high-affinity transport

substrates of rOAT1 and rOAT3, respectively. As shown in Figs. 2A and 2B, accumulation of both PAH and ES in the cisplatin-treated rat kidney was significantly reduced compared with control rat kidney. However, the uptake of PAH showed a tendency to be recovered, whereas that of ES was significantly restored by AST-120 administration.

mRNA and protein expression of rat organic anion transporters in cisplatin-treated rats with or without administration of AST-120. We next examined whether the renal expression of rOAT1 and rOAT3 mRNA was altered after treatment with cisplatin and AST-120. The relative mRNA expression levels of rOAT1 and rOAT3 in the kidney derived from the control and cisplatin-treated rats with or without AST-120 are shown in Figs. 3A and 3B, respectively. The mRNA levels of rOAT1 and rOAT3 were markedly suppressed in cisplatin-treated rats. Intriguingly, however, cisplatin-treated rats administered with AST-120 displayed significantly higher levels of rOAT1 and rOAT3 mRNA compared with cisplatin-treated rats. The level of rOAT1 and rOAT3 at the protein level was examined by Western blot analysis (Fig. 3C). As observed for the corresponding mRNA expression, the protein levels of rOAT1 and rOAT3 were greatly depressed in cisplatin-treated rat kidney. Furthermore, administration of AST-120 partially restored the cisplatin-induced down-regulation of rOAT1 and rOAT3 at the protein level.

Uptake of TEA by renal slices. The basolateral membrane transport activity of Slc22a organic cation transporters was further examined. We measured the accumulation of TEA, a typical substrate for rOCT1 and rOCT2, in renal slices prepared from rat kidney. As shown in Fig. 4, the accumulation of TEA in cisplatin-treated rat kidney was significantly depressed compared with control rat kidney.

Renal slices derived from cisplatin-treated rats with AST-120 displayed an intermediate level of TEA uptake.

mRNA and protein expression of rat organic cation transporters in cisplatin-treated rats with or without administration of AST-120. The relative mRNA expression levels of rOCT1 and rOCT2 in the kidney of control and cisplatin-treated rats with or without AST-120 are shown in Figs. 5A and 5B, respectively. The kidney from cisplatin-treated rats displayed a slightly, but not significant, lower rOCT1 mRNA level compared with control kidney. However, the mRNA level of rOCT2 in the kidney from cisplatin-treated rats was markedly lower than in the control rats. The decreased rOCT2 mRNA level was unaffected by administration of AST-120 to cisplatin-treated rats, although the rOAT1 and rOAT3 mRNA was restored. The level of rOCT1 and rOCT2 at the protein level was also examined by Western blot analysis (Fig. 5D). The kidney from cisplatin-treated rats contained lower levels of rOCT2 protein compared with control kidney, but the level of rOCT1 protein level was the same. AST-120 administration partially, but not significantly, restored the decrease in rOCT2 protein expression observed in cisplatin-treated rats.

Additionally, we examined the mRNA and protein expression of rMATE1. In cisplatin-treated rats, rMATE1 mRNA expression was significantly lower than that of control rats (Fig. 5C). However, administration of AST-120 to cisplatin-treated rats resulted in a slight recovery in the level of rMATE1 mRNA. In terms of protein, the level of rMATE1 was slightly lower in the kidney of cisplatin-treated rats compared to control rats (Fig. 5D). However, administration of AST-120 to cisplatin-treated rats resulted in an almost complete recovery of the rMATE1 protein level.

DISCUSSION

In the present study, AST-120 administration decreased IS levels in the serum and kidney, and ameliorated kidney injury (Fig. 1; Table 1). This finding was the corresponding observation of our previous report (4). In addition, it was reported that AST-120 did not affect the pharmacokinetics of cisplatin in rats (24). Thus, we suggest that IS could accelerate cisplatin-induced AKI as a mediator and that AST-120 treatment may be useful in preventing the progression of renal dysfunction by decreasing the accumulation of IS in the serum and kidney.

As shown in Figs. 2 and 4, the uptake of PAH, ES and TEA by renal slices from cisplatin-treated rat kidney was significantly lower than that of the control. *In vivo* studies have shown that Na^+/K^+-ATPase activity is inhibited by cisplatin treatment (25). Both rOAT1 and rOAT3 were suggested to be driven by an inward gradient of dicarboxylic acids, such as α-ketoglutarate, which could be accumulated in the renal cells *via* the Na^+/dicarboxylate cotransporter 3 (26). The Na^+ gradient is generated by Na^+/K^+-ATPase. Furthermore, rOCT1 and rOCT2 are known to be driven by the K^+-gradient associated with the inside-negative electrical potential difference, generated by Na^+/K^+-ATPase (10, 11, 27). Taken together, these results suggest that the impaired transport activities of rOAT and rOCT during cisplatin-induced AKI is the cause of the lower uptake of PAH, ES and TEA. Furthermore, the mRNA and protein expression levels of rOAT1, rOAT3 and rOCT2, but not rOCT1, were depressed as a result of cisplatin-induced AKI (Figs. 3 and 5). Thus, down-regulation of these transporters probably involves disturbed transcriptional regulation.

The down-regulation of the organic anion transporters results in the impaired renal secretion of IS, thereby enhancing serum accumulation of IS. This could lead further accumulation of IS in the kidney. The fact that AST-120 prevented serum and renal accumulations of IS in association with

significant amelioration of renal functions suggests that IS should be involved, at least in part, in cisplatin-induced AKI. In other words, the down-regulation of rOAT1 and rOAT3 caused by cisplatin could accelerate IS accumulation in both serum and kidney, thereby deteriorating renal tubular damages which may further down-regulate these transporters. The decrease in renal IS levels was correlated to the expression levels of rOAT1 and rOAT3, but not rOCT1 and rOCT2, demonstrating a possibility that IS may affect the transcriptional regulation of these organic anion transporters, in addition to renal toxic responses. In contrast to rOCT1, rOAT1 and rOAT3 would be highly sensitive or adaptive to toxic cellular events, thereby preventing further cell injury due to accumulation of toxic substances including IS and/or other uremic toxins. Basically, because serum and renal levels of IS appear to be very low in control rats without kidney injury, AST-120 may not have a significant impact on renal handling and accumulation of IS. Thus, we consider that rOAT expressions would not be affected, even if AST-120 slightly decreases renal IS level in control rats.

Recently, it was reported that prostaglandin E_2 down-regulated the expression of rOAT1 and rOAT3 in a time- and dose-dependent manner in a cell line from rat proximal tubule (28). The expression of cyclooxygenase-2, which plays an important role in inflammation processes by producing prostaglandin E_2, was increased in ischemia/reperfusion rat kidney (29). Because an inflammation reaction is involved in the progression of cisplatin-induced AKI, prostaglandin E_2 may be elevated in cisplatin-treated rats. Meanwhile, it was also reported that the expression of rOCT2 is up-regulated by testosterone and down-regulated by estradiol in rats (30). Furthermore, the level of serum testosterone is lower during cisplatin-induced AKI (31), suggesting that decreased rOCT2 expression might be caused by a decline in plasma testosterone level.

IS appears to be a high-affinity substrate for rOAT1 and rOAT3 (6, 7). Therefore, an

elevation of IS concentration and a decrease in K_p value for IS in cisplatin-treated rats appear to be related to the down-regulation of these transporters. We reasoned that IS may play a pathological role, at least in part, in the altered function and expression of organic ion transporters as a mediator. This proposal is supported by our finding that AST-120 can partially restore the decrease in function and expression of organic ion transporters during cisplatin-induced AKI. AST-120 administration attenuated the mRNA and protein expression of both rOAT1 and rOAT3 (Fig. 3). The transport activity of rOAT3 was also significantly restored, although that of rOAT1 showed only a slight positive response after administration of AST-120 (Fig. 2). Hence, the observed recovery of transport activity might be attributed to the restored expression of these transporters. However, AST-120 had no effect on the K_p value of IS. It may be possible that other uremic toxins being substrates for rOATs act as competitive inhibitors of IS. Our results show that the transport activity of rOCT2 was partially restored by administration of AST-120 to cisplatin-treated rats (Figs. 4 and 5), whereas the mRNA and protein expression of rOCT2 were unaffected. It might be possible that partial prevention of cisplatin-induced renal injury by AST-120 is associated with a recovery of driving force for rOCTs, i.e., inside-negative membrane potential. In other words, cisplatin-caused decrease in TEA uptake by renal slices may be due to both down-regulation of rOCT2 expression and decline of its driving force. AST-120 would protect partially the activity of Na^+/K^+-ATPase accompanied by prevention of renal cell injury. It was previously reported that the protein expression of rMATE1 was depressed in 5/6 nephrectomized rats (23). We found that the expression of rMATE1 in the kidney of cisplatin-treated rats was reduced at both the mRNA and protein level. However, this reduction in the expression level of rMATE1 could be largely restored by administration of AST-120 (Fig. 5). Therefore, it was suggested that the expression of rMATE1 is regulated under cisplatin-induced AKI, and that IS might

be involved in the regulation.

In conclusion, the present studies suggest that the pathophysiological variations of the renal organic ion transporters, rOAT1, rOAT3, rOCT2 and rMATE1, under cisplatin-induced AKI could influence the pharmacokinetic behavior of various ionic drugs. Furthermore, IS was suggested to be involved in cisplatin-induced AKI being associated with the down-regulation of these renal transporters. These findings could be useful in understanding the mechanisms of cisplatin-induced AKI and the pharmacokinetic and physiological roles of IS and the renal organic ion transporters.

ABBREVIATIONS

AKI, acute kidney injury; BUN, blood urea nitrogen; SCr, serum creatinine; ES, estrone-3-sulfate; IS, indoxyl sulfate; MATE, multidrug and toxin extrusion; OAT, organic anion transporter; OCT, organic cation transporter; PAH, *p*-aminohippuric acid; TEA, tetraethylammonium.

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LEGENDS FOR FIGURES

Fig. 1. Concentration of IS in control and cisplatin-treated rats with or without administration of AST-120; (A) serum, (B) kidney. IS concentrations in serum and kidney were measured 72 h after treatment with saline or cisplatin by high-performance liquid chromatography. (C) The K_p values were estimated by dividing the kidney IS concentration by the serum IS concentration. Each column represents the mean ± S.E. for 5 rats. $^{**}p < 0.01$, significantly different from control rats. $^{\#\#}p < 0.01$, significantly different from cisplatin-treated rats.

Fig. 2. Uptake of (A) PAH and (B) ES in renal slices derived from control and cisplatin-treated rats with or without AST-120. Renal slices from control, cisplatin-treated and cisplatin-treated rats with AST-120 were incubated at 37°C in incubation buffer containing 5 μM [^{14}C]PAH or 5 μM [^{3}H]ES, for 60 min. D-[^{3}H]Mannitol or D-[^{14}C]mannitol was used to estimate the extracellular trapping and non-specific uptake of [^{14}C]PAH or [^{3}H]ES, respectively. Each column represents the mean ± S.E. for 10 slices. $^{**}p < 0.01$, significantly different from control rats. $^{\#\#}p < 0.01$, significantly different from cisplatin-treated rats.

Fig. 3. mRNA and protein expression of rat organic anion transporters in the kidney of control and cisplatin-treated rats with or without AST-120. mRNA expression levels of (A) rOAT1 and (B) rOAT3 in control and cisplatin-treated rats with or without AST-120 were determined by real-time PCR analysis. The relative amount of rOAT1 and rOAT3 mRNA was normalized to that of 18S ribosomal RNA. The expression level in the control was arbitrarily set at 1.0. Each column represents the mean ± S.E. for 5 rats. (C) Immunoblots of kidney from control and cisplatin-treated rats with or

without AST-120. Antisera specific for rOAT1 (77 kDa), rOAT3 (72 kDa) and β-actin were used as primary antibodies. Densitometric ratio of (D) rOAT1 and (E) rOAT3 to β-actin. The values for control rats were arbitrarily defined as 100%. Each column represents the mean ± S.E. from 3 rats. *p < 0.05, and **p < 0.01, significantly different from control rats. $^{\#}p$ < 0.05, and $^{\#\#}p$ < 0.01, significantly different from cisplatin-treated rats.

Fig. 4. Uptake of TEA in renal slices of control and cisplatin-treated rats with or without AST-120. Renal slices from control, cisplatin-treated and cisplatin-treated rats with AST-120 were incubated at 37°C in incubation buffer containing [^{14}C]TEA for 60 min. D-[^{3}H]Mannitol was used to estimate the extracellular trapping and non-specific uptake of [^{14}C]TEA. Each column represents the mean ± S.E. for 10 slices. **p < 0.01, significantly different from control rats. $^{\#}p$ < 0.05, significantly different from cisplatin-treated rats.

Fig. 5. mRNA and protein expression of rat organic cation transporters in the kidney of control and cisplatin-treated rats with or without AST-120. mRNA expression level of (A) rOCT1, (B) rOCT2 and (C) rMATE1 in control and cisplatin-treated rats with or without AST-120 were determined by real-time PCR analysis. The relative amount of rOCT1, rOCT2 and rMATE1 mRNA was normalized to that of 18S ribosomal RNA. The expression level in the control was arbitrarily set at 1.0. Each column represents the mean ± S.E. for 5 rats. (D) Immunoblots of kidney from control and cisplatin-treated rats with or without AST-120. Antisera specific for rOCT1 (66 kDa), rOCT2 (65 kDa), rMATE1 (70 kDa) and β-actin were used as primary antibodies. Densitometric ratio of (E) rOCT1, (F) rOCT2 and (G) rMATE1 to β-actin. The values for control rats were arbitrarily defined

as 100%. Each column represents the mean ± S.E. from 3 rats. *$p < 0.05$, significantly different from control rats. **$p < 0.01$, significantly different from control rats.

Table 1. Renal functional data after cisplatin administration

	control	cisplatin	cisplatin + AST-120
Body weight (g)	238.4 ± 2.6	178.0 ± 4.3 **	193.6 ± 4.4 ** #
BUN (mg/dL)	21.9 ± 1.0	136.0 ± 10.1**	74.7 ± 9.8** ##
SCr (mg/dL)	0.20 ± 0.01	1.48 ± 0.15**	0.95 ± 0.11** ##

Each value represents the mean ± S.E. from 11-17 rats.
**$p < 0.01$, significantly different from control rats. #$p < 0.05$, significantly different from cisplatin-treated rats, and ##$p < 0.01$, significantly different from cisplatin-treated rats.

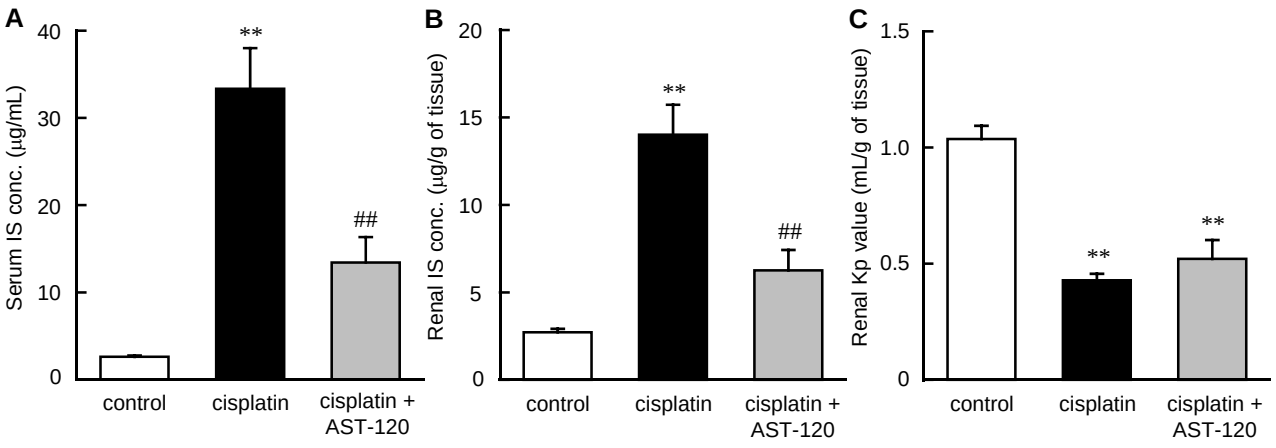
A
Serum IS conc. (μg/mL)
40
30
20
10
0
**
##
control
cisplatin
cisplatin +
AST-120
B
Renal IS conc. (μg/g of tissue)
20
15
10
5
0
**
##
control
cisplatin
cisplatin +
AST-120
C
Renal Kp value (mL/g of tissue)
1.5
1.0
0.5
0
**
**
control
cisplatin
cisplatin +
AST-120

Fig. 1.

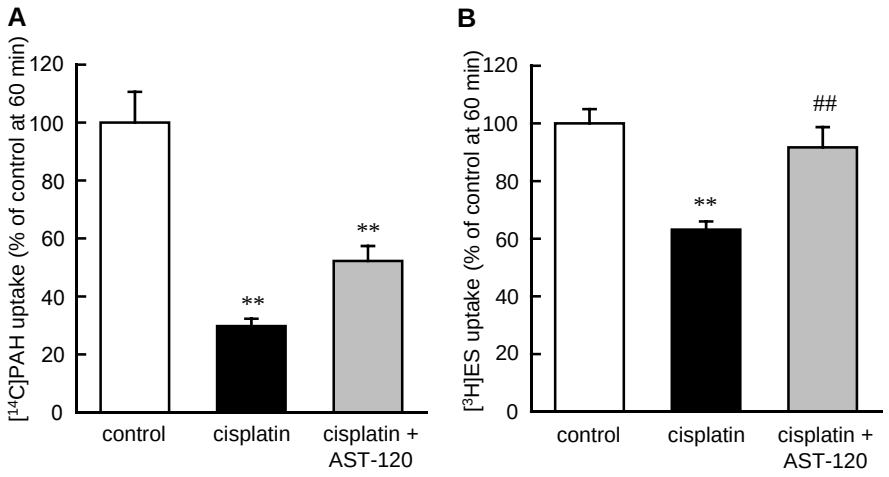
A
[14C]PAH uptake (% of control at 60 min)
120
100
80
60
40
20
0
**
**
control
cisplatin
cisplatin +
AST-120
B
[3H]ES uptake (% of control at 60 min)
120
100
80
60
40
20
0
**
##
control
cisplatin
cisplatin +
AST-120

Fig. 2.

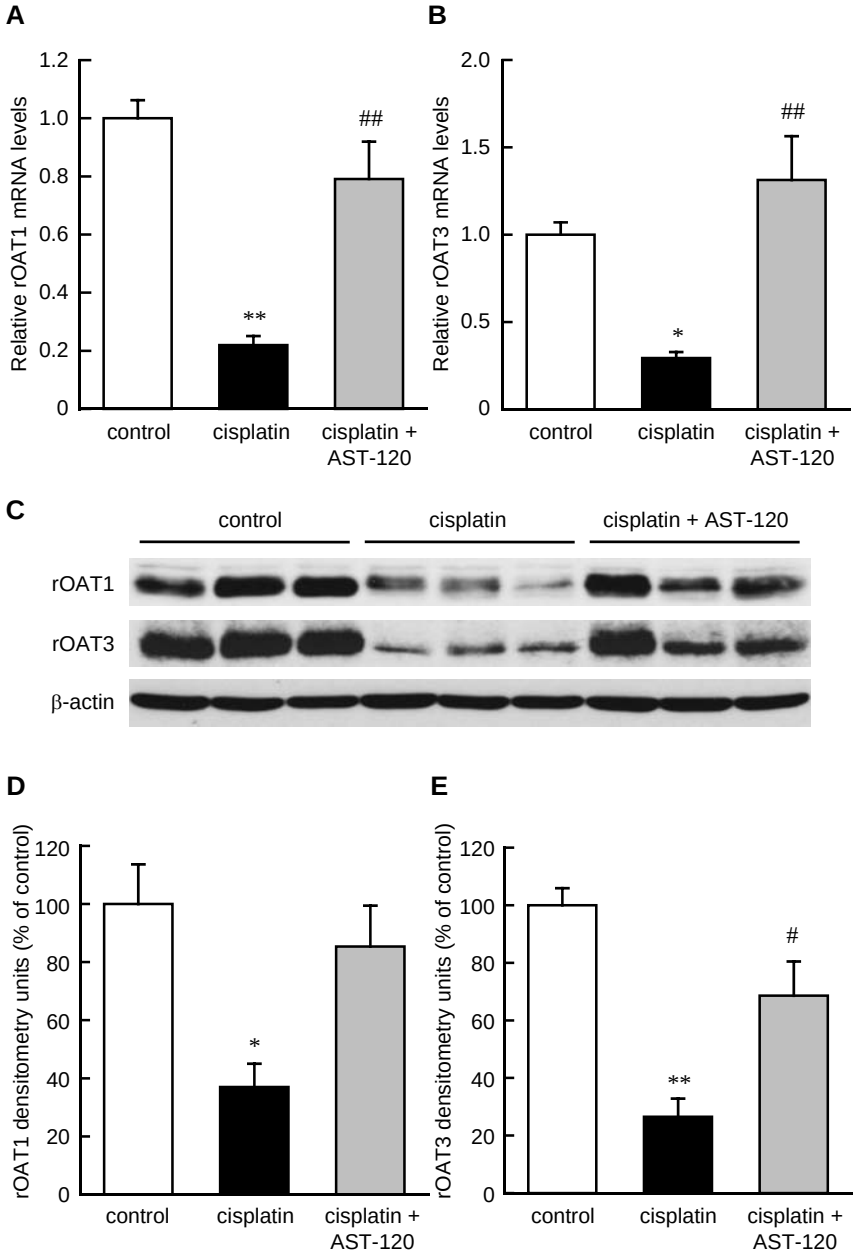
A
Relative rOAT1 mRNA levels
1.2
1.0
0.8
0.6
0.4
0.2
0
**
##
control
cisplatin
cisplatin + AST-120
B
Relative rOAT3 mRNA levels
2.0
1.5
1.0
0.5
0
*
##
control
cisplatin
cisplatin + AST-120
C
control
cisplatin
cisplatin + AST-120
rOAT1
rOAT3
β-actin
D
rOAT1 densitometry units (% of control)
120
100
80
60
40
20
0
*
control
cisplatin
cisplatin + AST-120
E
rOAT3 densitometry units (% of control)
120
100
80
60
40
20
0
**
#
control
cisplatin
cisplatin + AST-120

Fig. 3.

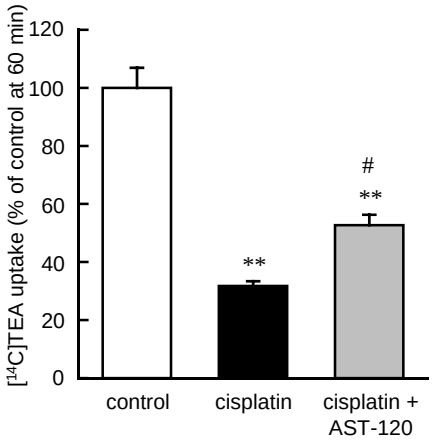
[14C]TEA uptake (% of control at 60 min)
120
100
80
60
40
20
0
**
#
**
control
cisplatin
cisplatin +
AST-120

Fig. 4.

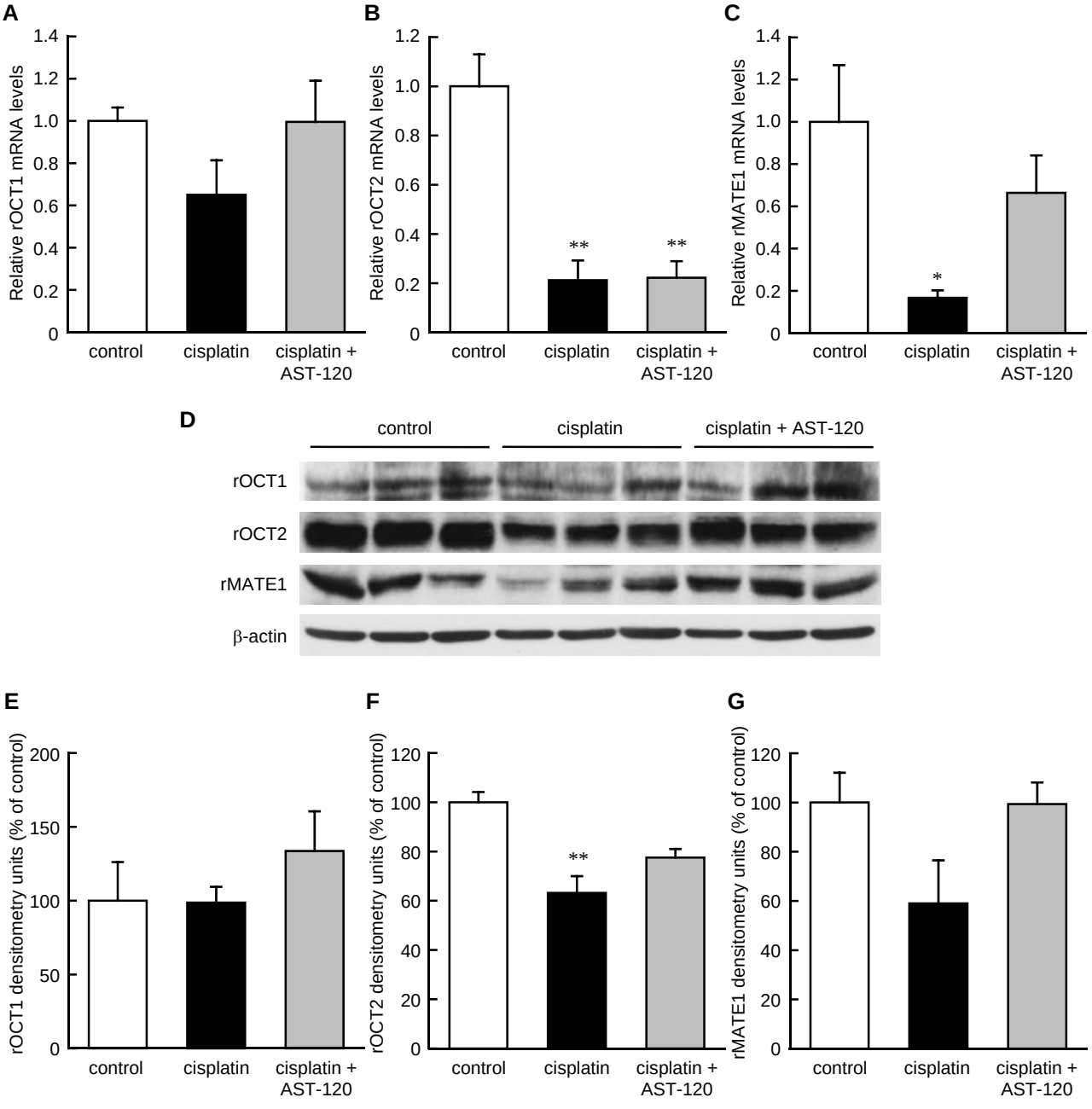
A
Relative rOCT1 mRNA levels
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
control
cisplatin
cisplatin + AST-120
B
Relative rOCT2 mRNA levels
1.2
1.0
0.8
0.6
0.4
0.2
0
**
**
control
cisplatin
cisplatin + AST-120
C
Relative rMATE1 mRNA levels
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
*
control
cisplatin
cisplatin + AST-120
D
control
cisplatin
cisplatin + AST-120
rOCT1
rOCT2
rMATE1
β-actin
E
rOCT1 densitometry units (% of control)
200
150
100
50
0
control
cisplatin
cisplatin + AST-120
F
rOCT2 densitometry units (% of control)
120
100
80
60
40
20
0
**
control
cisplatin
cisplatin + AST-120
G
rMATE1 densitometry units (% of control)
120
100
80
60
40
20
0
control
cisplatin
cisplatin + AST-120

Fig. 5.