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Association between tubular toxicity of cisplatin and expression of organic cation transporter rOCT2 (Slc22a2) in the rat

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Abstract

Cisplatin is an effective anticancer drug, but has its severe adverse effects, especially nephrotoxicity. The molecular mechanism of cisplatin-induced nephrotoxicity is still not clear. In the present study, we examined the role of rat (r)OCT2, an organic cation transporter predominantly expressed in the kidney, in the tubular toxicity of cisplatin. Using HEK293 cells stably expressing rOCT2 (HEK-rOCT2), we evaluated the cisplatin-induced release of lactate dehydrogenase and the uptake of cisplatin. The release of lactate dehydrogenase and the accumulation of platinum were greater in HEK-rOCT2 cells treated with cisplatin than in mock-transfected cells. Moreover, cimetidine and corticosterone, OCT2 inhibitors, inhibited the cytotoxicity and the transport of cisplatin in HEK-rOCT2 cells. Pharmacokinetics of cisplatin was investigated in male and female rats because the renal expression level of rOCT2 was higher in male than female rats. The renal uptake clearance of cisplatin was greater in male than female rats, while the hepatic uptake clearance was similar between the sexes. In addition, glomerular filtration rate and liver function were unchanged, but *N*-acetyl-β-D-glucosaminidase activity in the bladder urine and the urine volume were markedly increased 2 days after the administration of 2 mg/kg of cisplatin in male rats. Moreover, cisplatin did not induce the elevation of urinary *N*-acetyl-β-D-glucosaminidase activity in the castrated male rats whose renal rOCT2 level was lower than that of the sham-operated rats. In conclusion, the present results indicated that renal rOCT2 expression was the major determinant of cisplatin-induced tubular toxicity.

Keywords: Cisplatin; Organic cation transporter 2; Nephrotoxicity; Cytotoxicity; Renal tubular epithelial cells; Pharmacokinetics

1. Introduction

cis-Diamminedichloroplatinum II (CDDP, cisplatin) is widely used to treat solid tumors of prostate, bladder, colon, lung, testis and brain. Although cisplatin is an effective anticancer agent, severe nephrotoxicity limits its clinical application. It was reported that an increase in the serum creatinine concentration was observed in 41% of patients treated with high-dose cisplatin [1]. However, the major site of cisplatin-induced renal injury is the proximal tubule [2]. In addition, cisplatin induced tubular toxicity, followed by an increase in the serum creatinine level [3]. Moreover, the tubular toxicity caused a decrease in the glomerular filtration rate (GFR), resulting in acute renal failure [4]. Therefore, it was suggested that cisplatin was toxic primarily to renal tubular epithelial cells. But, the molecular mechanism of cisplatin-induced nephrotoxicity is still unknown.

Safirstein et al. [5] reported that cisplatin was concentrated in rat renal cortical slices five-fold above the concentration in medium. We previously demonstrated that cisplatin treatment from the basolateral side caused sever toxicity compared to the apical side in the porcine derived epithelial cell line LLC-PK_1 cells [6]. Recently, Ludwig et al. [7] reported that cisplatin-induced cytotoxicity was specifically observed from the basolateral side, and the toxicity was ameliorated in the presence of cimetidine in

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Madin-Darby canine kidney (MDCK) cells. These reports suggested that the uptake of cisplatin in tubular epithelial cells was mediated by basolateral drug transporter(s). Identification of the transporter(s) is essential to understand the mechanism of cisplatin-induced nephrotoxicity.

Human organic cation transporter 2 (hOCT2) is the most abundant organic cation transporter in the kidney among an organic cation transporter family which consists of hOCT1-3 (SLC22A1-3) and hOCTN1 and 2 (SLC22A4 and 5) [8,9]. Rat (r)OCT2 is expressed predominantly in the basolateral membranes of proximal tubules and mediated the accumulation of various cationic drugs into proximal tubular epithelial cells from the circulation [10–14]. Uptake of tetraethylammonium (TEA) by rOCT2 was suppressed by the replacement of Na^+ with K^+, suggesting that the transport activity of rOCT2 was membrane potential-dependent [9,15]. Based on such backgrounds and findings, we hypothesized that rOCT2 was the key molecule to clarifying the tubular accumulation and subsequent nephrotoxicity of cisplatin.

In the present study, we investigated whether rOCT2 affected the nephrotoxicity of cisplatin in rat proximal tubules. We examined the effect of rOCT2 expression on the cytotoxicity of cisplatin in HEK293 transfectants and on the pharmacokinetics of cisplatin in rats.

2. Materials and methods

2.1. Cell culture and transfection

HEK293 cells (American Type Culture Collection CRL-1573) were cultured in complete medium consisting of Dulbecco's modified Eagle's medium (Sigma Chemical Co., St. Louis, MO) with 10% fetal bovine serum (Whittaker Bioproducts Inc., St. Louis, MO) in an atmosphere of 5% CO_2–95% air at 37 °C.

The construction of HEK293 cells stably expressing rOCT2 (HEK-rOCT2) was performed as described [14]. The transfectants were used for the experiments at 48 h after seeding.

For a transient expression system, pBK-CMV plasmid vector DNA (Stratagene, La Jolla, CA), containing rOCT1 or rOCT2 cDNA, was purified using Wizard® Plus SV Minipreps DNA Purification System (Promega, San Luis Obispo, CA). The day before transfection, HEK293 cells were seeded onto poly-D-lysine-coated 24-well plates at a density of 2.0×10^5 cells per well. The cells were transfected with 50 ng of total plasmid DNA per well using 0.125 μl of LipofectAMINE 2000 (Invitrogen, Carlsbad, CA) per well according to the manufacturer's instructions. Forty-eight hours after the transfection, the cells were used for uptake experiments.

2.2. Uptake experiment

Cellular uptake of [^{14}C]TEA (88.8 MBq/mmol, Perkin-Elmer Inc., Wellesley, MA) was measured with monolayer cultures grown on poly-D-lysine-coated 24-well plates. The composition of the incubation buffer was as follows: 145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose and 5 mM HEPES (pH 7.4 adjusted with NaOH). Experimental procedures were performed as described previously [14].

For the measurement of cisplatin uptake, seeded cells were incubated with the medium containing cisplatin with or without cimetidine or corticosterone for 1 h. After this incubation, the monolayers were rapidly washed twice with ice-cold incubation buffer containing 1% bovine serum albumin (Nacalai Tesque, Kyoto, Japan) and then washed three times with ice-cold incubation buffer. The cells were solubilized in 0.5N NaOH, and the amount of platinum was determined using inductively coupled plasma-mass spectrometry (ICP-MS) by the Pharmacokinetics and Bioanalysis Center, Shin Nippon Biomedical Laboratories, Ltd. (Wakayama, Japan).

The protein content of the cell monolayers solubilized in 0.5N NaOH was determined by the method of Bradford with a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Richmond, CA).

2.3. Cytotoxicity experiment

The cytotoxicity of cisplatin was measured with monolayer cultures grown on poly-D-lysine-coated 12-well plates. Cells were incubated with the medium containing cisplatin with or without cimetidine or corticosterone for 2 h. After removal of the medium, drug-free medium was added. After incubation for 24 h, the medium was collected, and the lactate dehydrogenase (LDH) activity in the medium was measured using a LDH Cytotoxicity Detection Kit (Takara, Shiga, Japan), according to the manufacturer's instructions. Cytotoxicity was evaluated by measuring LDH activity in the medium. Total LDH activity was defined as LDH activity in the medium containing 1% TritonX-100. LDH release represents (LDH activity − LDH activity of control)/(total LDH activity − LDH activity of control).

2.4. Quantification of mRNA expression

Cellular total RNA was extracted using a MagNA Pure LC RNA isolation kit II (Roche Diagnostic GmbH, Mannheim, Germany) [8]. The total RNA was reverse-transcribed, and the single stranded DNA was used for the quantification of mRNA expression.

Real-time PCR was performed in a total volume of 20 μl containing 2 μl of reverse-transcribed cDNA, 1 μM forward and reverse primers, 0.2 μM TaqMan probe, and 10 μl of TaqMan Universal PCR Master Mix (Applied

Biosystems, Foster City, CA). The quantification of mRNA was performed as reported [8].

2.5. Pharmacokinetics of cisplatin

The pharmacokinetics experiment was performed using male or female Wistar/ST rats (8 weeks), as described previously with some modifications [16]. Cisplatin (0.5 mg/kg) was administered as a bolus via the catheterized right femoral vein. Blood samples were collected at 0.5, 1, 1.5, 2, 2.5 and 3 min from the left femoral artery. Three minutes after the injection, the kidney and liver were collected immediately after sacrificing the rats. The excised tissues were gently washed, weighed and homogenized in 3 volumes of 0.9% NaCl. The amounts of cisplatin were measured by ICP-MS. The animal experiments were performed in accordance with the ''Guidelines for Animal Experiments of Kyoto University''. All protocols were previously approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University.

2.6. Western blot analysis

The crude membrane fractions were prepared from rat kidneys as described previously [17]. The crude membrane fractions (25 μg) were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride membranes (Immobilon-P®, Millipore, Bedford, MA) by semi-dry electroblotting. The blots were blocked and incubated overnight at 4 °C with each primary antibody specific for rOCT2 [18], or the Na^+/K^+-ATPase α1 subunit (Upstate Biotechnology Inc., Lake Placid, NY). The bound antibody was detected on X-ray film using enhanced chemiluminescence (ECL) with horseradish peroxidase-conjugated secondary antibodies and cyclic diacylhydrazides (Amersham Pharmacia Biotech, Uppsala, Sweden).

2.7. Acute renal failure

Male Wistar/ST rats (8 weeks) were used or male rats (5 weeks) were surgically castrated 3 weeks before the experiment. Acute renal failure was induced by intraperitoneal administration of 2 mg/kg of cisplatin. Rats were maintained in metabolic cages for 24 h before the experiment to determine urine output and the urinary level of creatinine. Two days after the administration of cisplatin, plasma and bladder urine samples were collected and then the state of the kidneys was determined, as previously described [19]. The liver function data were determined using the assay kits from Wako Pure Chemical Industries (Osaka, Japan). The concentration of testosterone was measured using an ELISA kit (Cayman Chemical Co., Ann Arbor, MI).

2.8. Statistical analysis

Data are expressed as means ± S.E.M. Data were analyzed statistically using the unpaired Student *t* test. Multiple comparisons were performed with Dunnett's two-tailed test after a one-way ANOVA. Probability values of less than 0.05 were considered statistically significant.

3. Results

3.1. Uptake of TEA by HEK293 cells stably expressing rOCT2

We constructed HEK293 cells stably transfected with the pBK-CMV vector or rOCT2-containing vector (HEK-pBK or HEK-rOCT2). To check the function of these cells, the cellular uptake of [^{14}C]TEA by HEK-pBK cells or HEK-rOCT2 cells was measured. The amounts of [^{14}C]TEA in HEK-pBK cells and HEK-rOCT2 cells were 19.2 ± 4.0 and 317.4 ± 11.7 pmol/mg protein/2 min, respectively. Therefore, these cells were used in the subsequent experiments.

3.2. Cytotoxicity of cisplatin in HEK-pBK cells and HEK-rOCT2 cells

We compared the sensitivities of HEK-pBK cells and HEK-rOCT2 cells to cisplatin (Fig. 1). When HEK-pBK cells were treated with 30–300 μM cisplatin for 2 h and subsequently cultured in normal medium for 24 h, the release of LDH into the culture medium was not significantly enhanced. Treatment with 1000 μM cisplatin promoted the release of LDH in HEK-pBK cells. On the other hand, when HEK-rOCT2 cells were treated with 30–1000 μM cisplatin, the amount of LDH released increased in a dose-dependent manner. The release of LDH was greater in HEK-rOCT2 cells treated with 30–1000 μM cisplatin than in HEK-pBK cells (Fig. 1A). Further, we investigated the effects of OCT2 inhibitors, cimetidine and corticosterone, on the cisplatin-induced cytotoxicity. The cytotoxicity of cisplatin was completely inhibited in the presence of 1 mM cimetidine or 100 μM corticosterone in HEK-rOCT2 cells (Fig. 1B).

3.3. Transport of cisplatin by HEK-pBK cells and HEK-rOCT2 cells

To investigate whether rOCT2 recognizes cisplatin as its substrate, the effect of cisplatin on the uptake of [^{14}C]TEA and the accumulation of cisplatin by HEK-rOCT2 cells were examined (Fig. 2). Cisplatin, cimetidine and corticosterone had inhibitory effects on the uptake of [^{14}C]TEA by HEK-rOCT2 cells in a dose-dependent manner. The IC_{50} values of cisplatin, cimetidine and corticosterone were 2096.2 ± 59.5, 216.1 ± 4.5 and 2.50 ± 0.02 μM,

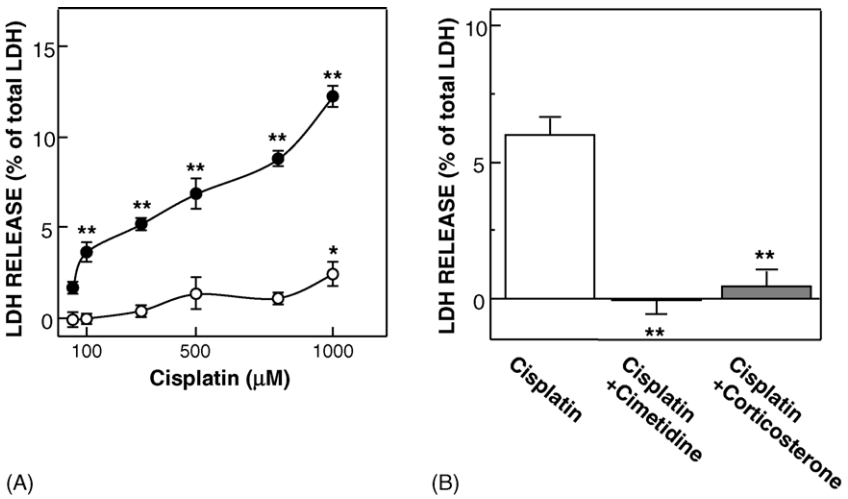

Fig. 1. Role of rOCT2 in the cytotoxicity of cisplatin. (A) Concentration-dependence of cytotoxicity in HEK-pBK cells (open circle) or HEK-rOCT2 cells (closed circle). Cells were exposed to cisplatin for 2 h, and then incubated in normal medium for 24 h. (B) Cimetidine (1 mM) or corticosterone (100 μM) was coadministrated with cisplatin (500 μM) for 2 h, and then the cells were incubated in normal medium for 24 h. Each point represents the mean ± S.E.M. of three wells. $^{*}P < 0.05$; $^{**}P < 0.01$, significantly different from control cells.

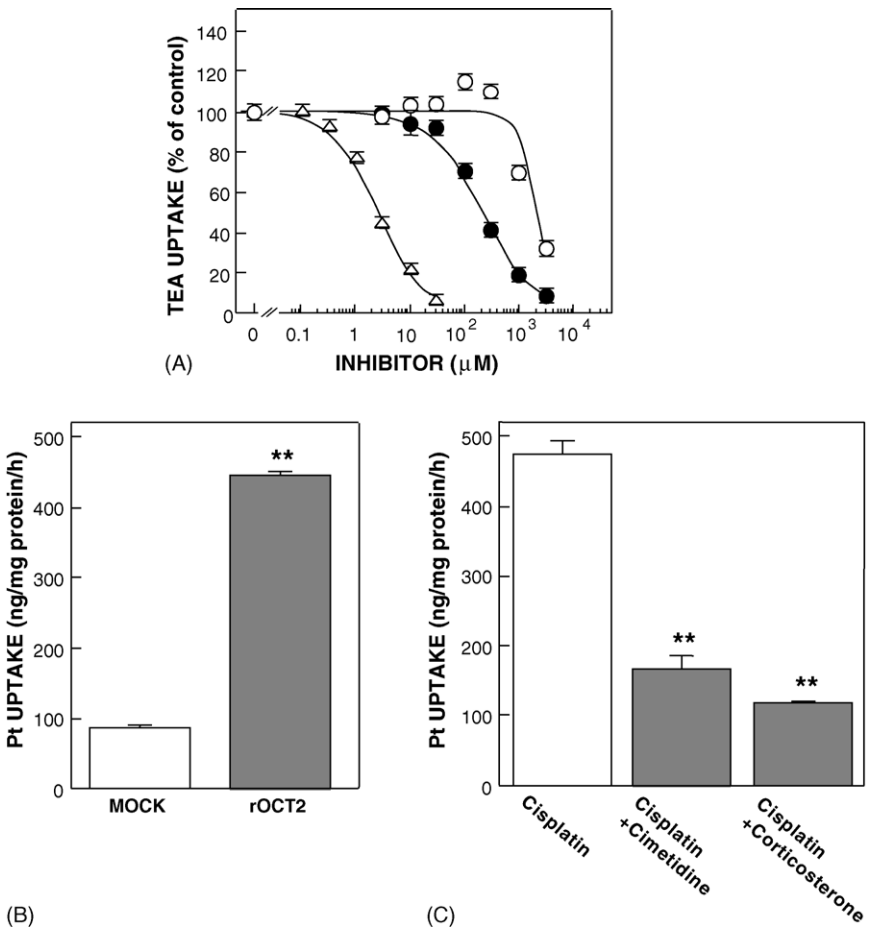

Fig. 2. Uptake of cisplatin by HEK-pBK cells or HEK-rOCT2 cells. (A) HEK-rOCT2 cells were incubated with 50 μM [^{14}C]TEA in the presence of cisplatin (open circle), cimetidine (closed circle) or corticosterone (open triangle) at various concentrations for 2 min. The amount of [^{14}C]TEA in HEK-rOCT2 cells was determined by measuring the radioactivity of solubilized cells. (B) HEK-pBK cells (MOCK) or HEK-rOCT2 cells (rOCT2) were incubated with 500 μM cisplatin for 1 h. (C) HEK-rOCT2 cells were incubated with 500 μM cisplatin in the presence or absence of 1 mM cimetidine or 100 μM corticosterone for 1 h. The amount of platinum in HEK-pBK cells or HEK-rOCT2 cells was determined by ICP-MS. Each point represents the mean ± S.E.M. of three or four wells. $^{**}P < 0.01$, significantly different from MOCK cells or control cells.

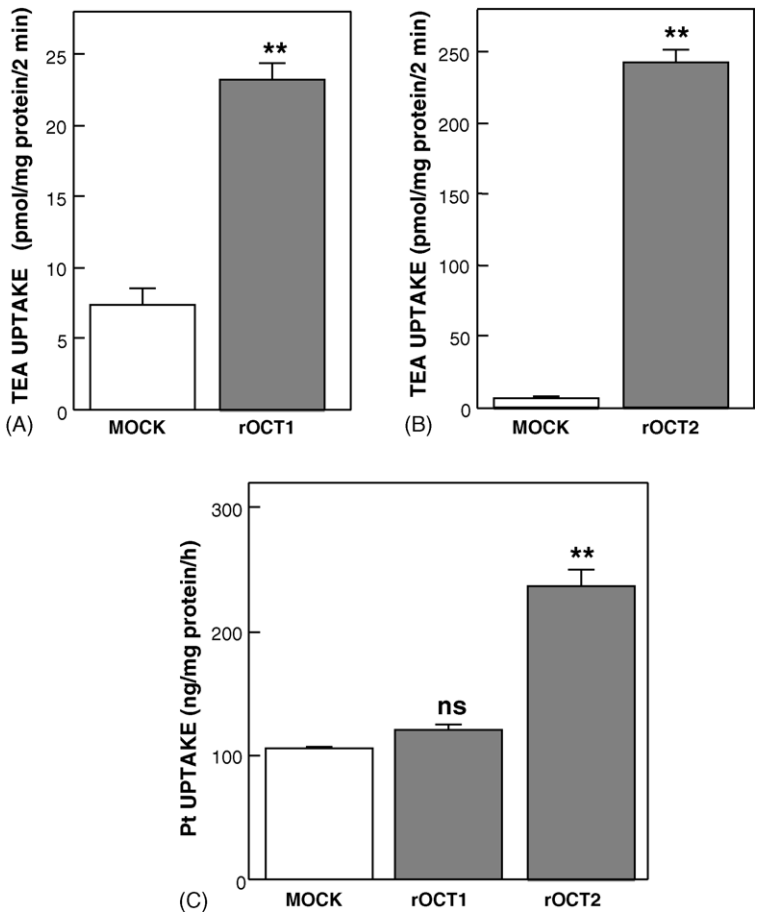

Fig. 3. rOCT1 or rOCT2-mediated uptake of cisplatin. HEK293 cells transiently expressing pBK-CMV (MOCK), rOCT1 or rOCT2 were incubated with 50 μM [^{14}C]TEA for 2 min (A and B) or 500 μM cisplatin for 1 h (C). The amount of [^{14}C]TEA or platinum was determined. Each point represents the mean ± S.E.M. of three or four wells. ns, not significantly; $^{**}P < 0.01$, significantly different from MOCK cells.

respectively (Fig. 2A). After the incubation with 500 μM cisplatin for 1 h, the amounts of platinum in HEK-pBK cells and HEK-rOCT2 cells were 90.0 ± 2.8 and 447.7 ± 5.3 ng/mg protein/h, respectively (Fig. 2B). Moreover, the accumulation of platinum by HEK-rOCT2 cells was inhibited in the presence of 1 mM cimetidine or 100 μM corticosterone (Fig. 2C).

3.4. Transport of cisplatin by HEK293 cells transiently expressing rOCT1 and rOCT2

Following the transfection of rOCT1 or rOCT2 cDNA, the mRNA expression levels of these transporters were 8037 ± 701 and 5834 ± 306 amol/mg protein (mean ± S.E.M. of four monolayers), respectively. Uptake of [^{14}C]TEA was observed in HEK293 cells transiently expressing rOCT1 or rOCT2 (Fig. 3A and B). However, the amount of platinum accumulated in the HEK293 cells transiently transfected with pBK-CMV, rOCT1 and rOCT2 was 107.8 ± 1.1, 122.4 ± 4.0 (ns versus pBK-CMV) and 237.6 ± 13.8 ($P < 0.01$ versus pBK-CMV) ng/mg protein/h, respectively (Fig. 3C).

3.5. Pharmacokinetics of cisplatin in male and female rats

We compared the pharmacokinetics of cisplatin between male and female rats, because it was found that the expression level of renal rOCT2, but not rOCT1, was much higher in male rats than female rats [18]. The plasma concentrations of platinum up to 3 min after the administration of cisplatin as a bolus were determined (Fig. 4A). The total clearance (CL_{total}) of cisplatin, which was calculated from the dose and area under the concentration-time curve (AUC) of cisplatin for 3 min, was 35.1 ± 3.5 ml/min and 16.8 ± 1.1 ml/min in male and female rats, respectively (Fig. 4B). The tissue uptake clearance (CL_{tissue}), which was calculated from the AUC of cisplatin for 3 min and the amount of cisplatin in tissue at 3 min, was also examined. The renal CL_{tissue} was significantly greater in male rats (13.2 ± 1.3 ml/min) than female rats (5.9 ± 0.4 ml/min). However, hepatic CL_{tissue} in male rats (3.7 ± 0.3 ml/min) was not significantly different from that in female rats (2.6 ± 0.2 ml/min) (Fig. 4C). In addition, the male-dominant expression of

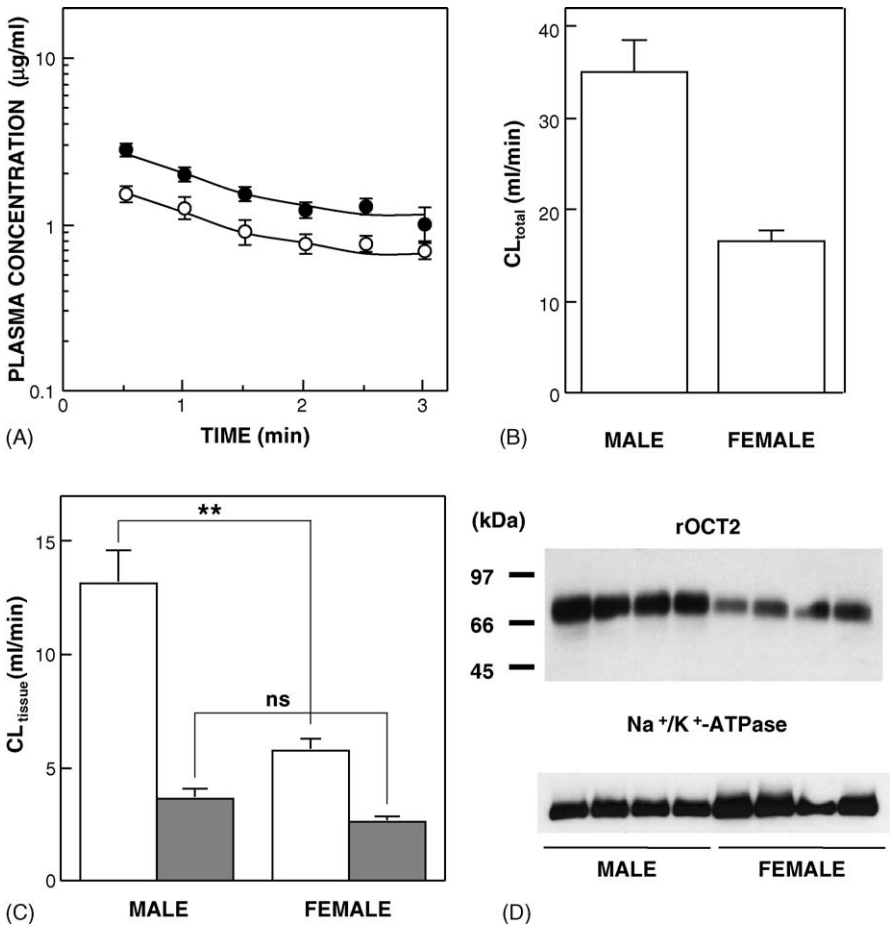

Fig. 4. Pharmacokinetics of cisplatin in male and female rats. (A) Plasma concentrations of platinum at various points were determined in male (open circle) and female (closed circle) rats. Total clearance (CL_{total}; B) and tissue uptake clearance (CL_{tissue}; C: renal CL_{tissue} (open column) and hepatic CL_{tissue} (closed column)) were calculated by dividing the administered dose or the amount in tissue at 3 min by the area under the curve (AUC) from 0 to 3 min, respectively. Each column represents the mean ± S.E.M. of five rats. ns, not significantly different; $^{**}P < 0.01$, significantly different. (D) Protein expression of rOCT2 and Na^+/K^+-ATPase in male and female rats. Representative photographs of Western blotting are shown.

the renal rOCT2 was confirmed by Western blotting (Fig. 4D).

3.6. Renal functional data of male rats treated with cisplatin

The male rats showed renal tubular toxicity 2 days after the administration of 2 mg/kg of cisplatin. The body weight, plasma creatinine level, creatinine clearance, blood urea nitrogen (BUN), urinary albumin level, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin level were unaffected. However, *N*-acetyl-β-D-glucosaminidase (NAG) activity and urine volume were significantly increased in the male rats treated with cisplatin compared to the control rats (Table 1).

To confirm the contribution of renal rOCT2 expression on cisplatin-induced tubulotoxicity, we compared the NAG

Table 1
Biochemical parameters in male rats treated with cisplatin (2 mg/kg)

	Sham	Cisplatin
Body weight (g)	281 ± 4	274 ± 3
Urine volume (ml/24 h)	10.9 ± 0.9	22.4 ± 2.9**
NAG (U/day)	129 ± 42	317 ± 47**
Pcre (mg/dl)	0.42 ± 0.02	0.50 ± 0.03
Ccr (ml/min/kg)	5.81 ± 0.08	5.96 ± 0.06
BUN (mg/dl)	14.9 ± 0.3	14.2 ± 0.6
Urinary albumin (mg/day)	0.24 ± 0.06	0.44 ± 0.08
AST (IU/l)	61.8 ± 4.0	76.1 ± 6.2
ALT (IU/l)	15.0 ± 1.3	18.8 ± 1.7
T-Bil (mg/dl)	0.05 ± 0.01	0.11 ± 0.04

Values represent means ± S.E.M. of nine rats. NAG, *N*-acetyl-β-D-glucosaminidase; Pcre, plasma creatinine; Ccr, creatinine clearance; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-Bil, total bilirubin.

** $P < 0.01$, significantly different from sham.

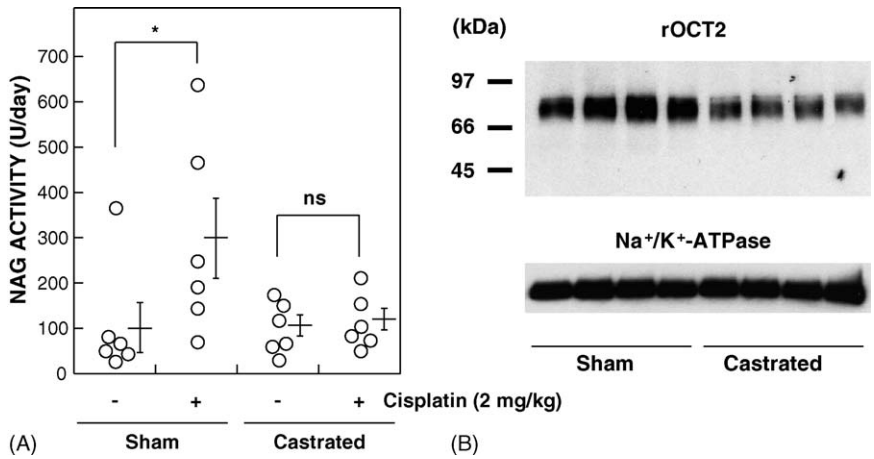

Fig. 5. Effect of the castration on cisplatin-induced tubular toxicity and renal rOCT2 expression. Male rats (5 weeks) were surgically castrated, and fed and given water freely for 3 weeks. (A) The NAG activity in bladder urine in the sham-operated or castrated rats 2 days after the administration of 2 mg/mg of cisplatin ($n = 6$). Each bar represents the mean ± S.E.M. of six rats. ns, not significantly different; $^{*}P < 0.05$, significantly different. (B) Protein expression of rOCT2 and Na^+/K^+-ATPase in the sham-operated or castrated rats. Representative photographs of Western blotting are shown.

activity in bladder urine between the sham-operated and castrated male rats. The serum concentrations of testosterone were 1.56 ± 0.11 and 0.25 ± 0.04 ng/ml (mean ± S.E.M. of 12 rats; $P < 0.01$) in the sham-operated and castrated rats, respectively. Two days after the administration of 2 mg/kg of cisplatin, NAG activity was significantly increased in the sham-operated rats. On the other hand, 2 mg/kg of cisplatin was not enough to increase urinary NAG activity in the castrated rats (Fig. 5A). The renal rOCT2 expression was decreased in the castrated rats compared to the sham-operated rats (Fig. 5B).

4. Discussion

Using cultured renal epithelial cells, cisplatin was found to be accumulated from the basolateral membranes via a specific carrier system [6,7,20]. In OK cells, the basolateral uptake of cisplatin was decreased in the presence of TEA [20]. Ludwig et al. [7] reported that cisplatin-induced cytotoxicity was ameliorated in the presence of cimetidine in MDCK-C7 cells. Although a polyclonal antibody against rat OCT2 (OCT2-A®, Alpha-diagnostic, San Antonio, TX) showed two signals, 65 and 50 kDa [7], the amino acid sequence of the antigen peptide was conserved 76% in mouse, 66% in porcine and 57% in human (http://www.4adi.com/data/oat/oct21.html). Therefore, the 65-kDa signal was speculated to be a canine OCT2 [7], but this should be confirmed using an actual antibody against an appropriate antigen. In the present study, we have demonstrated that rOCT2 mediated the uptake of cisplatin into the stable transfectant HEK-rOCT2 cells, and stimulated cisplatin-sensitivity in comparison with the control cells (Figs. 1 and 2). Hitherto it has not been clear which transporter protein mediated the uptake of cisplatin in renal tubular cells. The present study in vitro indicated that rOCT2 was a cisplatin transporter in the kidney.

Intracellularly, the two chlorides of cisplatin are rapidly replaced by hydroxyl groups to produce a toxic agent [21]. This agent can induce nuclear damage [22], cause mitochondrial damage [23] or trigger several other mechanisms [24], resulting in cell death. Therefore, it is considered that the entering into the cells is the most important step in the cytotoxicity of cisplatin. It was reported that the deletion of copper transporter 1 (Ctr1) resulted in resistance to cisplatin in yeast and mammal cells, and therefore, Ctr1 was suggested to be a candidate transporter mediating cisplatin uptake. [25]. On the other hand, copper-transporting P-type adenosine triphosphate (ATP7B) [26–28] and ATP-binding cassette, subfamily C2 (ABCC2, known as MRP2 or cMOAT) [29,30] promoted cisplatin efflux and had a role in cisplatin resistance. However, these three transporters were suggested to have a minor role in cisplatin-induced tubular toxicity, because the tissue distribution of these transporter proteins was not limited to the kidney, but was in the liver, intestine and brain [31–33].

Urakami et al. [18] reported that the renal expression of rOCT2, but not rOCT1, was markedly higher in male than female rats. In addition, there was a gender difference in the uptake of TEA but not *p*-aminohippurate, a typical substrate of organic anion transporter 1, in rat renal slices [18]. Because there is no selective and non-toxic rOCT2 antagonist, we performed the cisplatin pharmacokinetic experiments using the gender difference in the renal rOCT2 expression. It was expected that the renal distribution of cisplatin would be much greater in male than female rats. As expected, the renal CL_{tissue} of cisplatin was significantly greater in male rats than female rats, while hepatic CL_{tissue} did not differ between males and females. Moreover, the renal CL_{tissue} was 3.5 times higher than the hepatic CL_{tissue} in male rats (Fig. 4C). In addition, the transport of cisplatin was mediated by rOCT2 predominantly expressed in kidney [10], but not rOCT1 expressed

in liver, kidney and intestine [34] (Fig. 3C). These results suggested that rOCT2 played a major role in the distribution of cisplatin. As previously reported, the renal clearance of cisplatin was inhibited by cationic compounds having the potential to interact with rOCT2 such as quinidine, cimetidine and ranitidine in dogs [35]. Based on these findings, it was suggested that rOCT2 mainly mediated the tubular accumulation of cisplatin.

There are numerous reports suggesting that cisplatin causes nephrotoxicity, glomerular and tubular injury [3,19,36–38]. In these studies, cisplatin was used at dose of more than 5 mg/kg in rats, which was severe compared to clinical use. The peak concentration of cisplatin in humans undergoing chemotherapy was reported to be 3.4 μg/ml [39]. In the present study, the blood concentration of cisplatin in rats after the administration of cisplatin (0.5 mg/kg) as a bolus was between 1 and 3 μg/ml (Fig. 4A). When rats were administered 2 mg/kg of cisplatin, the activity of NAG in bladder urine and the urine volume were increased, while plasma creatinine level, creatinine clearance, BUN, urinary albumin level, AST, ALT and total bilirubin level were unchanged (Table 1). These results indicated that kidney was more sensitive to cisplatin than liver. It corresponded to the pharmacokinetics of cisplatin (Fig. 4). Kishore et al. [38] demonstrated that the polyuria after the cisplatin treatment was accompanied by decreased expression levels of aquaporin (AQP) 1 in the proximal tubules as well as AQP2 and AQP3 in the collecting ducts in rats. Using the specific antibody, rOCT2 protein was expressed in proximal tubular cells at the basolateral membrane [12] and mRNA of rOCT2 was detected abundantly in proximal tubules and weakly in distal convoluted tubules and collecting ducts [11]. Based on these findings, the proximal tubules were the most sensitive to cisplatin, because they consisted of rOCT2-rich cells.

We previously reported that testosterone increased the expression level of renal rOCT2 and stimulated TEA accumulation by kidney slices [40] and that testosterone recovered the renal rOCT2 expression and the clearance of cimetidine in chronic renal failure rats [41]. In the present study, to investigate the role of rOCT2 in cisplatin-induced tubular toxicity, we used the castrated rats as a model for the depression of rOCT2. As a result, cisplatin induced the increase of urinary NAG activity in the sham-operated rats, but not in the castrated rats whose renal rOCT2 expression was lower than the sham-operated rats (Fig. 5). In addition, cellular uptake and renal distribution of cisplatin depended on rOCT2 expression (Figs. 2B and 4). Therefore, it was suggested that rOCT2 was the determinant of the tissue distribution of cisplatin and cisplatin-induced tubular toxicity in vivo.

The tissue distribution and renal distribution of hOCT2 in humans are similar with those of rats [8,12,13]. The functional characteristics including substrate specificity of hOCT2 was consistent with rOCT2 [9]. Therefore, the present data of rOCT2 on cisplatin-induced nephrotoxicity may be reflected in humans. The renal toxicity of cisplatin in humans should be confirmed in future focusing on hOCT2.

We propose OCT2 as the transporter responsible for cisplatin-induced renal tubular toxicity. Some OCT2-specific antagonists may prevent the nephrotoxicity of cisplatin.

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