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Protective effect of concomitant administration of imatinib on cisplatin-induced nephrotoxicity focusing on renal organic cation transporter OCT2

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ABSTRACT

Although the organic cation transporter 2 (OCT2/SLC22A2) mediate renal tubular uptake of cisplatin from the circulation, neither apical multidrug and extrusion (MATE) 1 or MATE2-K mediate tubular secretion of the agent. Therefore, the highly concentrated tubular cisplatin potentiates nephrotoxicity, and these are considered to be a critical mechanism for cisplatin-induced nephrotoxicity. In the present study, we examined the protective effect of imatinib, a cationic anticancer agent, on that nephrotoxicity. Imatinib markedly reduced cisplatin-induced cytotoxicity and platinum accumulation in OCT2-expressing HEK293 cells, but almost no change was found in the cells expressing human MATE1, MATE2-K and rat MATE1. In rats, the renal accumulation of platinum and subsequent nephrotoxicity, based on the blood urea nitrogen, plasma creatinine and creatinine clearance, were significantly decreased with the oral administration of imatinib. The orally administered imatinib significantly increased the area under the plasma concentration–time curve of intravenously administered cisplatin for 3 min by an average of 120%. In additional, the concomitant administration of imatinib clearly avoided the severe renal impairment by the histological examination. In conclusion, the concomitant administration of imatinib with cisplatin prevents cisplatin-induced nephrotoxicity inhibiting the OCT2-mediated renal accumulation of cisplatin.

1. Introduction

Although Cis-diamminedichloroplatinum II (cisplatin, CDDP) is widely used against malignant solid tumors [1,2], severe nephrotoxicity limits its clinical application [3]. Recently, the renal organic cation transporter OCT2-mediated renal accumulation of cisplatin was found to be a key mechanism for its nephrotoxicity both in rats and humans [4,5].

Imatinib (STI-571), a potent inhibitor of ABL tyrosine kinase, has been used for the treatment of Philadelphia chromosome-positive (Ph^+) chronic myeloid leukemia (CML) and Ph^+ acute lymphoblastic leukemia (ALL) [6,7]. Previous studies have suggested that leukocytic hOCT1 mediated the uptake of imatinib and this expression was an important clinical determinant of the pharmacological response of imatinib in CML patients [8–11]. Based on these reports, imatinib might interact with other members of organic cation transporters, such as OCT2, MATE1 (multidrug and toxin extrusion) and MATE2-K [12,13], in addition to OCT1.

In the present study, we hypothesized and examined that the concomitant administration of imatinib prevents cisplatin-induced nephrotoxicity, mainly based on the inhibition of OCT2-mediated renal accumulation of cisplatin, using transporter-expressing cells and rat.

2. Materials and methods

2.1. Materials

Cisplatin was obtained from Sigma Chemical Co. (St. Louis, MO). Imatinib was a kindly gift from Novartis Pharma AG (Basel, Switzerland). NaCl, KCl, $CaCl_2$, $MgCl_2$, D-glucose, HEPES, NaOH, and dimethylsulfoxide were obtained from Nacalai Tesque (Kyoto, Japan). Ammonium chloride and Triton X-100 were obtained from Wako Pure Chemical Industries (Osaka, Japan).

2.2. Cell culture and transfection

Human embryonic kidney (HEK) 293 cells (American Type Culture Collection CRL-1573, Manassas, VA) were cultured in complete medium consisting of Dulbecco's modified Eagle's

Abbreviation: cisplatin, Cis-diamminedichloroplatinum II; hOCT, human organic cation transporter; rOCT, rat organic cation transporter; hMATE, human multidrug and toxin extrusion; rMATE, rat multidrug and toxin extrusion; HEK, human embryonic kidney; ICP-MS, inductively coupled plasma-mass spectrometry; LDH, lactate dehydrogenase.

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medium (Sigma) with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) in an atmosphere of 5% CO_2/95% air at 37 °C, and used as host cells. pCMV6-XL4 plasmid vector DNA (OriGene Technologies, Rockville, MD), pBK-CMV plasmid vector DNA (Stratagene, La Jolla, CA) or pcDNA3.1/Hygro(+) plasmid vector DNA (Invitrogen) containing hOCT1, hOCT2, hOCT2-A, rOCT1, rOCT2, hMATE1, hMATE2-K, and rMATE1 cDNAs were used to conduct the transient expression analysis. For transient expression systems, HEK293 cells were transfected with each cDNA or empty vector using LipofectAMINE 2000 Reagent® (Invitrogen) according to the manufacturer's instructions. At 48 h after transfection, the cells were used for uptake experiments.

2.3. Uptake experiments

Cellular uptake of [^{14}C]creatinine (2.035 GBq/mmol; American Radiolabeled Chemicals Inc., St. Louis, MO) was measured with cultures of HEK293 cells grown on poly-D-lysine-coated 24-well plates (Becton, Dickinson and Company, Tokyo, Japan). Typically, the cells were preincubated with 0.2 mL incubation medium (145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose, 5 mM HEPES, pH 7.4) for 10 min at 37 °C. The medium was then removed, and 0.2 mL incubation medium containing radiolabeled substrate was added. The medium was aspirated off at the end of incubation, and the monolayers were rapidly rinsed three times with 1 mL ice-cold incubation medium. The cells were solubilized in 0.5 mL of 0.5 N NaOH, and then the radioactivity in aliquots was determined by liquid scintillation counting. To manipulate the intracellular pH, intracellular acidification was performed by pretreatment in incubation medium with ammonium chloride (30 mM, 20 min at 37 °C, pH 7.4).

The accumulation of cisplatin was measured using monolayer cultures of HEK293 cells seeded on poly-D-lysine-coated 24-well plates. Both cisplatin and/or imatinib were dissolved with 0.5% dimethylsulfoxide to the final concentration in the treatment medium. After removal of the culture medium, 0.5 mL Dulbecco's modified Eagle's medium with 10% fetal bovine serum containing cisplatin with or without imatinib was added and the monolayers were incubated for 2 h in an atmosphere of 5% CO_2/95% air at 37 °C. After this incubation, the monolayers were rapidly washed twice with ice-cold incubation buffer containing 3% bovine serum albumin (Nacalai Tesque) 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 solubilized cells was determined using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA) with bovine γ-globulin as a standard.

2.4. Measurement of cytotoxicity

The cytotoxicity of the platinum agents was measured with HEK293 cells seeded on poly-D-lysine-coated 24-well plates. Cells were incubated with medium containing cisplatin with or without imatinib or corticosterone (Sigma) for 2 h. After removal of the medium, a drug-free medium was added. After incubation for 24 h, the medium was collected, and the lactate dehydrogenase (LDH) activity and caspase-3 and -7 activities in the medium were measured using an LDH cytotoxicity detection kit (Takara Bio Inc., Shiga, Japan) and Caspase-Glo® 3/7 Assay (Promega, Madison, WI, USA), according to the manufacturer's instructions. The cytotoxicity was evaluated by measuring the LDH activity and caspase-3 and -7 activities in the medium. Total LDH activity was defined as LDH activity in the medium containing 1% Triton X-100. LDH release (percentage) represents (LDH activity − LDH activity of control)/(total LDH activity − LDH activity of control) × 100.

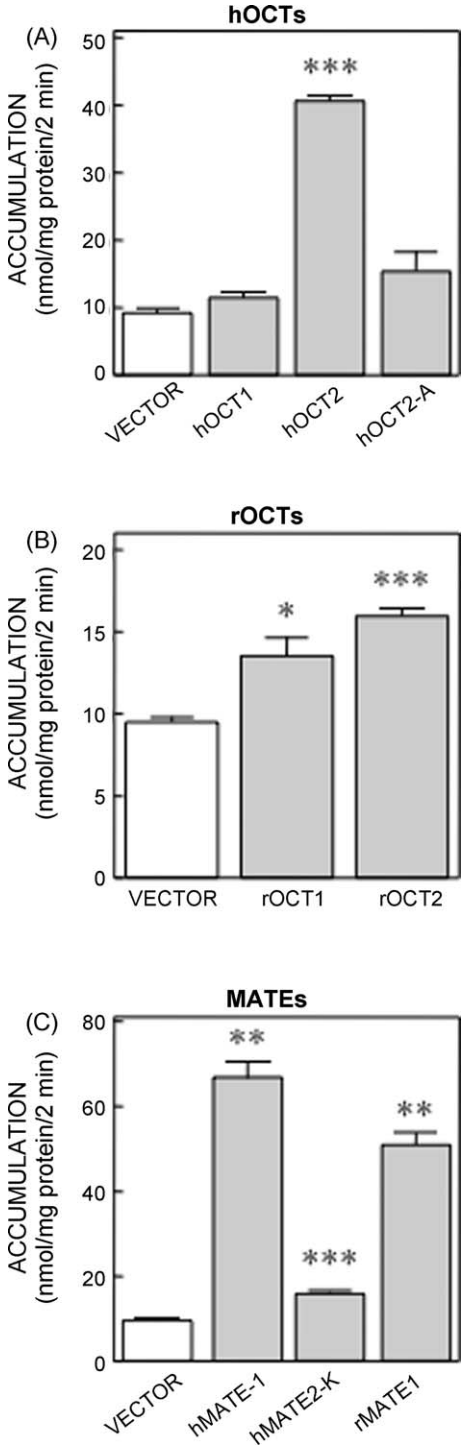

Fig. 1. Creatinine uptake by organic cation transporters. (A) HEK293 cells transfected with the empty vector, hOCT1, hOCT2, and hOCT2-A. (B) HEK293 cells transfected with the empty vector, rOCT1, and rOCT2. Cells transfected with hOCTs and rOCTs were incubated for 2 min at pH 7.4 at 37 °C with 10 μM [^{14}C]creatinine. (C) HEK293 cells transfected with the empty vector, hMATE1, hMATE2-K, and rMATE1. Cells transfected with MATEs were preincubated with incubation medium (pH 7.4) in the presence of 30 mM ammonium chloride for 20 min. The preincubation medium was removed, and the cells were incubated for 2 min at pH 7.4 at 37 °C with 10 μM [^{14}C]creatinine. Each column represents the mean ± S.E. of three monolayers from a typical experiment.

2.5. Animals

Male Wistar/ST rats (8 weeks) were purchased from SLC Animal Research Laboratories (Shizuoka, Japan). The rats were fed normal pellet food ad libitum, and given water freely. They were administered via gavage with 50 mg/kg imatinib three times and/or intraperitoneally with 5 mg/kg cisplatin (Randa®; Nippon Kayaku Co., Ltd., Tokyo, Japan) once after the second administration of imatinib. These drug solutions were prepared at concentrations of 30 and 0.5 mg/mL, respectively. Control rats were administered with the same volume of Ringer's solution. Three days after administration, the rats were maintained in metabolic cages for 24 h to determine the urinary levels of creatinine and urine output. Ninety-six hours after the administration of cisplatin, plasma, bladder urine, and kidneys were collected. The excised kidneys were gently washed, weighed, and homogenized in 9 volumes of saline. The amount of platinum was 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. Renal functional and histological studies

For the measurement of blood urea nitrogen (BUN), creatinine, and N-acetyl-β-D-glucosaminidase (NAG), we used commercial kits (BUN and creatinine; Wako Pure Chemical Industries; NAG; Shionogi & Co., Ltd., Osaka, Japan). Kidneys were fixed in ethyl Carnoy's solution and stained with periodic acid-Schiff (PAS) reagent by Sapporo General Pathology Laboratory Co., Ltd. (Hokkaido, Japan).

2.7. Phamacokinetics of cisplatin

The pharmacokinetics experiment was performed using male Wistar/ST rats (8 weeks), as described previously with some modifications [4]. They were administered 50 mg/kg imatinib via gavage 3 h and just before cisplatin administration. Cisplatin (0.5 mg/kg) was administered as a bolus via the catheterized right femoral vein under pentobarbital anesthesia. 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 was collected immediately after sacrificing the rats by blood loss. 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. At the same time, the concentration of imatinib was also determined using the high performance liquid chromatography (HPLC) method described below.

2.8. HPLC analysis for imatinib

The concentration of imatinib was determined using HPLC (model LC-10A; Shimadzu, Kyoto, Japan). HPLC analysis was performed as

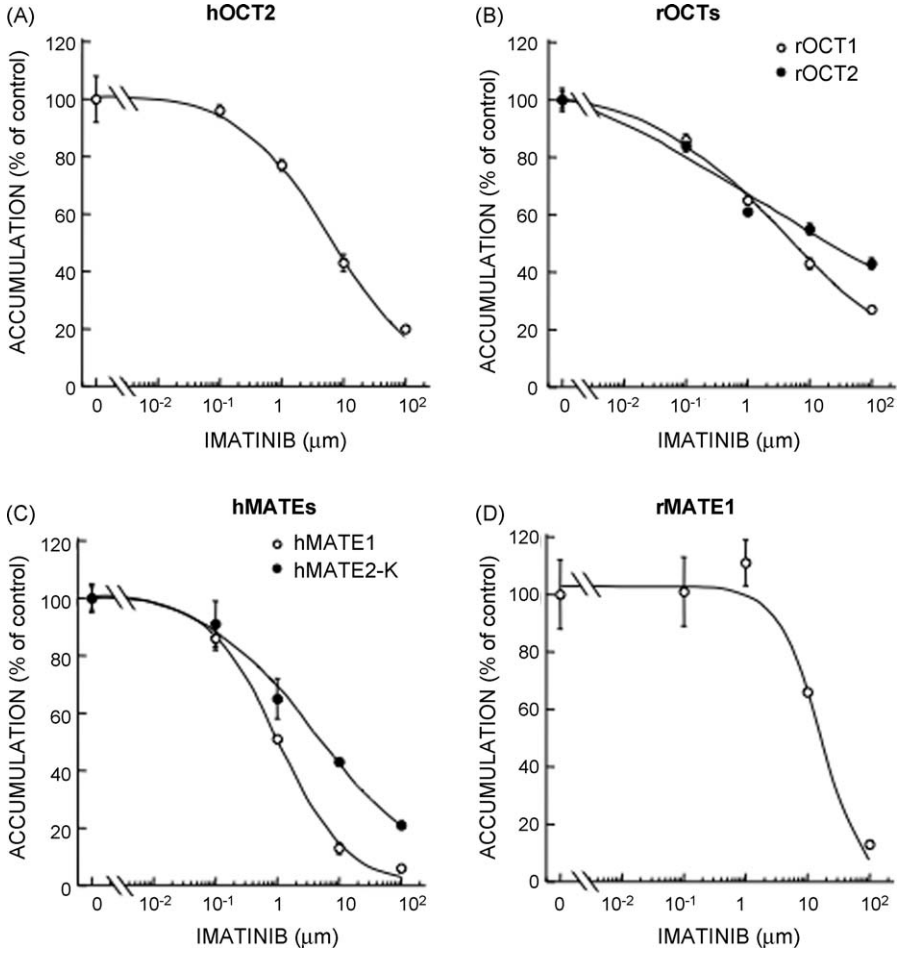

Fig. 2. Effect of imatinib on [^{14}C]creatinine uptake by HEK293 cells transiently expressing organic cation transport systems. (A) HEK293 cells transfected with hOCT2. (B) HEK293 cells transfected with rOCT1 (open circle) and rOCT2 (closed circle). (C) HEK293 cells transfected with hMATE1 (open circle) and hMATE2-K (closed circle). (D) HEK293 cells transfected with rMATE1. Cells were incubated with 20 μM [^{14}C]creatinine for 2 min at pH 7.4 at 37 °C in the presence of various concentrations of imatinib. For kinetic analyses, ammonium chloride (30 mM, pH 7.4, 20 min) was used to achieve intracellular acidification (C and D). Each point represents the mean ± S.E. of three monolayers from a typical experiment in several separate experiments.

described previously with some modification [14]. Separation was performed using a reversed-phase column (TSK gel ODS-80TM, 5-μm particle size, 150 mm × 4.6 mm i.d.; TOSOH, Tokyo, Japan) at 40 °C. Imatinib was detected by UV absorption at 267 nm.

2.9. Statistical analysis

Data are expressed as the mean ± S.E. Data were analyzed statistically using an unpaired *t* test or Bonferroni's multiple comparison test after one-way ANOVA. Significance was set at $P < 0.05$. In all figures except Fig. 6, where error bars are not shown, they are smaller than the symbols.

3. Results

3.1. Effect of imatinib on [^{14}C]creatinine uptake by HEK293 cells transiently expressing hOCT1, hOCT2, hOCT2-A, rOCT1, rOCT2, hMATE1, hMATE2-K and rMATE1

Based on our previous reports [4,5], the affinities of organic cation transporters against cisplatin were markedly low compared to tetraethyl ammonium, a typical substrate for organic cation transporters. Therefore, we chose low affinity substrate creatinine as a probe substrate for mimicking the interaction between cisplatin and imatinib via renal rOCT2 and hOCT2 [15].

First, we examined creatinine transport by organic cation transport systems. Consistent with previous reports, creatinine transport was stimulated by the expressions of hOCT2, rOCT1, rOCT2, hMATE1, hMATE2-K and rMATE1 (Fig. 1). Next, the inhibitory effect of imatinib on creatinine transport by these transporters was examined. The apparent IC_{50} values of imatinib on creatinine transport via hOCT2, rOCT1, rOCT2, hMATE1, hMATE2-K and rMATE1 were (in μM) 6.7, 4.1, 1.3, 1.0, 4.3, 16.6, respectively (Fig. 2). Therefore, we chose 10 μM imatinib as a potent inhibitor in the following experiments.

3.2. Effects of imatinib on cisplatin-induced cytotoxicity in HEK293 cells transiently expressing basolateral organic cation transporters, hOCT1, hOCT2, rOCT1, and rOCT2

To assess the cytotoxicity of cisplatin, LDH assay and caspase 3/7 assay were employed. Results from the LDH assay as well as the

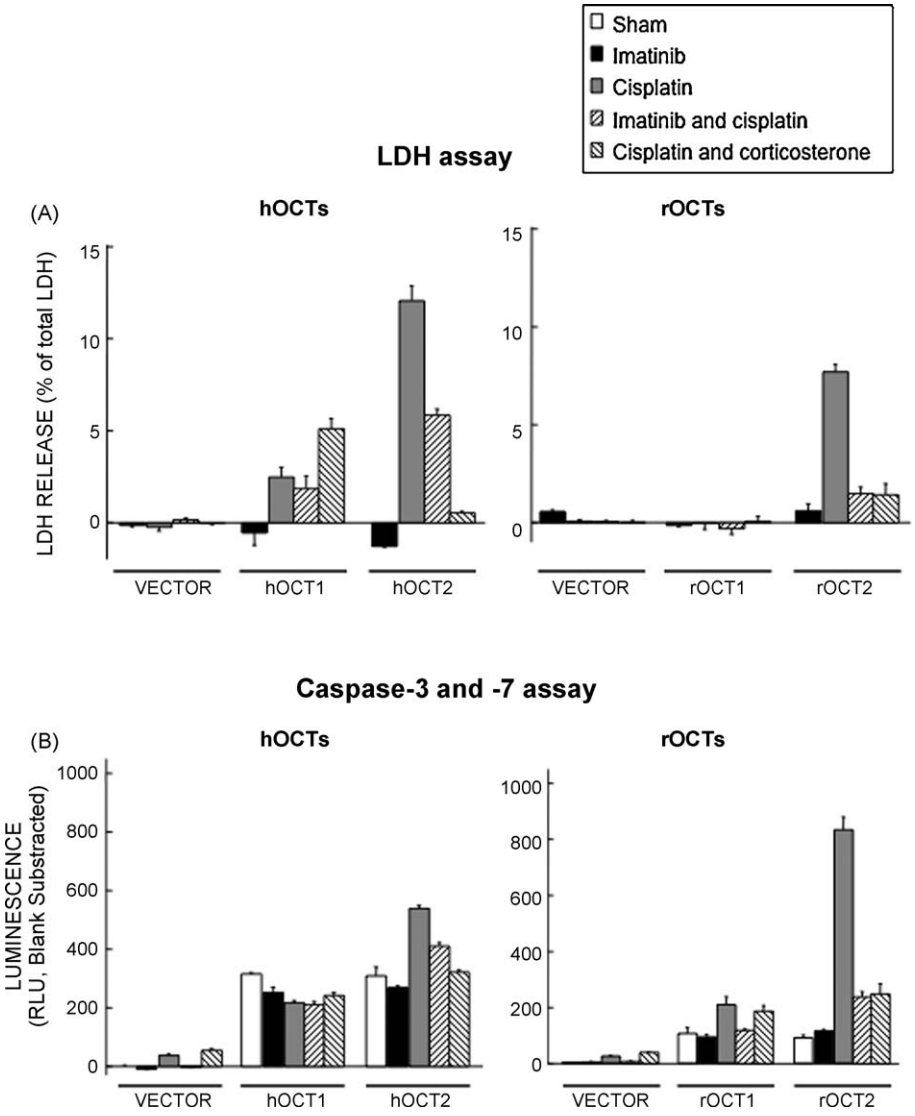

Fig. 3. Effect of imatinib on cisplatin-induced cytotoxicity in HEK293 cells transiently expressing basolateral organic cation transporters. HEK293 cells were transfected with empty vector, hOCT1, hOCT2, rOCT1, or rOCT2. Cells were treated with medium containing imatinib (10 μM; black column) or cisplatin (50 μM) with or without imatinib (positive sloped column and gray column, respectively) or corticosterone (100 μM; negative sloped column) for 2 h and then incubated in normal medium for 24 h. LDH (A) or caspase-3 and -7 (B) released into the medium was measured. Each column represents the mean ± S.E. of three monolayers from a typical experiment in several separate experiments.

caspase 3/7 assay showed that cisplatin-induced cytotoxicity via both human and rat OCT2 was markedly reduced by concurrent treatment with imatinib (Fig. 3) ($P < 0.001$ except hOCT2 for caspase 3/7 assay; $P < 0.01$). Corticosterone, a potent antagonist of OCT2, also attenuated cisplatin-induced cytotoxicity (Fig. 3). However, both human and rat OCT1-mediated cytotoxicities by cisplatin were much lower than OCT2-mediated cytotoxicities and were not prevented in the presence of imatinib and corticosterone.

3.3. Effects of imatinib on cisplatin-induced cytotoxicity in HEK293 cells transiently expressing apical organic cation transporters, hMATE1, hMATE2-K, and rMATE1

The expressions of hMATE1, hMATE2-K and rMATE1 did not affect cisplatin-induced cytotoxicity in LDH assay (Fig. 4A). However, a slight significant increase of caspase-3 and -7 activities was observed by cisplatin treatment in HEK293 cells expressing hMATE1 and rMATE1 compared to sham treatment ($P < 0.01$ and $P < 0.001$, respectively) (Fig. 4B). In addition, these increase caused by cisplatin treatment significantly decreased by the presence of imatinib ($P < 0.001$, Fig. 4B).

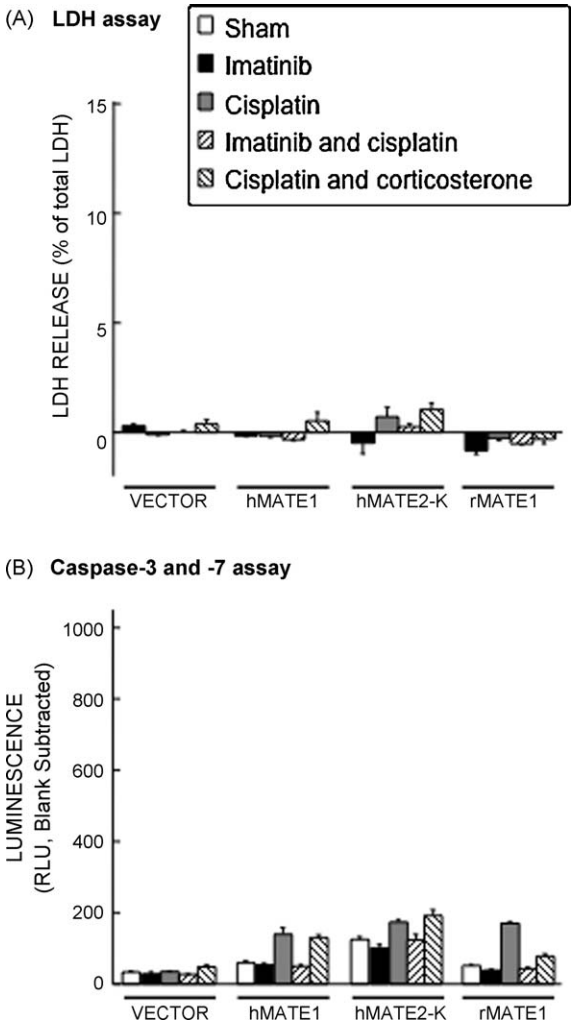

Fig. 4. Effect of imatinib on cisplatin-induced cytotoxicity in HEK293 cells transiently expressing apical organic cation transporters. HEK293 cells were transfected with empty vector, hMATE1, hMATE2-K, or rMATE1. Cells were treated with medium containing imatinib (10 μM; black column) or cisplatin (50 μM) with or without imatinib (positive sloped column and gray column, respectively) or corticosterone (100 μM; negative sloped column) for 2 h and then incubated in normal medium for 24 h. LDH (A) or caspase-3 and -7 (B) released into the medium was measured. Each column represents the mean ± S.E. of three monolayers from a typical experiment in several separate experiments.

3.4. Effects of imatinib on platinum accumulation in HEK293 cells transiently expressing basolateral organic cation transporters, hOCT1, hOCT2, rOCT1, and rOCT2

To obtain more information about the association between cisplatin-induced cytotoxicity and intracellular accumulation of

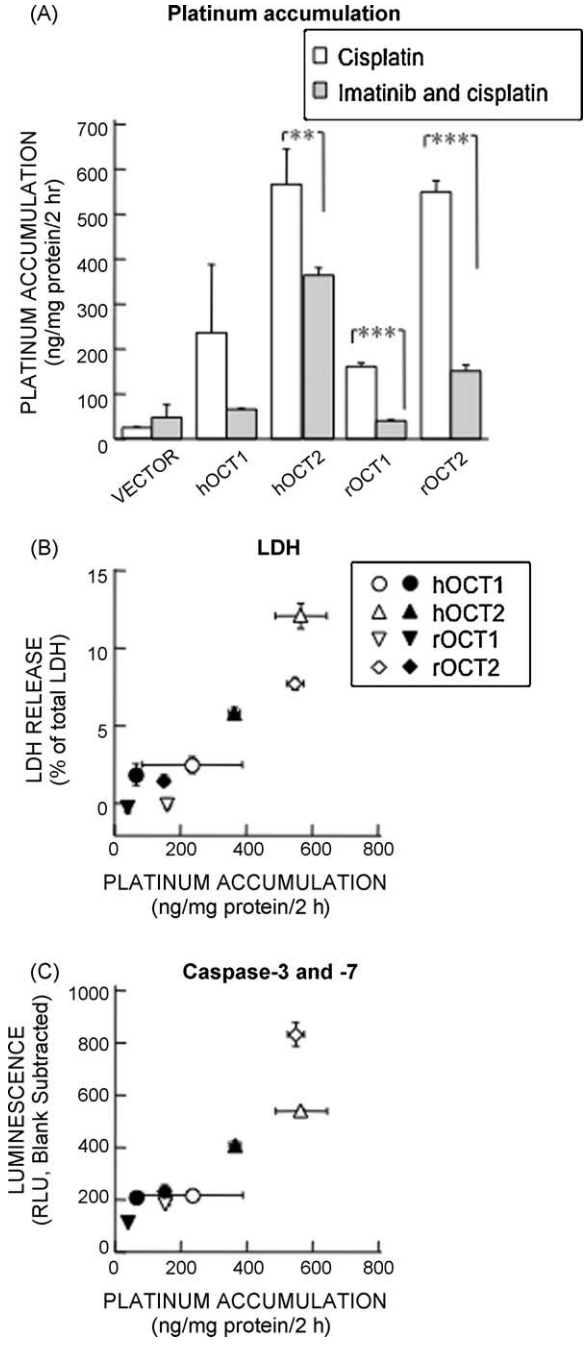

Fig. 5. Effect of imatinib on platinum accumulation in HEK293 cells transiently expressing basolateral organic cation transporters (A), and relation between platinum accumulation and cytotoxicity (B and C). (A) HEK293 cells were transfected with empty vector, hOCT1, hOCT2, rOCT1, or rOCT2. Cells were treated with medium containing cisplatin (50 μM) with or without imatinib (10 μM; closed column and open column, respectively) for 2 h. After washing, the cells were solubilized in 0.5 N NaOH, and the amount of platinum was determined by ICP-MS. Each column represents the mean ± S.E. of three monolayers. $^{**}P < 0.01$; $^{***}P < 0.001$, significantly different. (B and C) The data on LDH and caspase-3 and -7 are from Fig. 3. Platinum accumulation versus LDH release (B) or caspase-3 and -7 activities (C) on treatment with 50 μM cisplatin with or without imatinib (10 μM; closed symbol and open symbol, respectively).

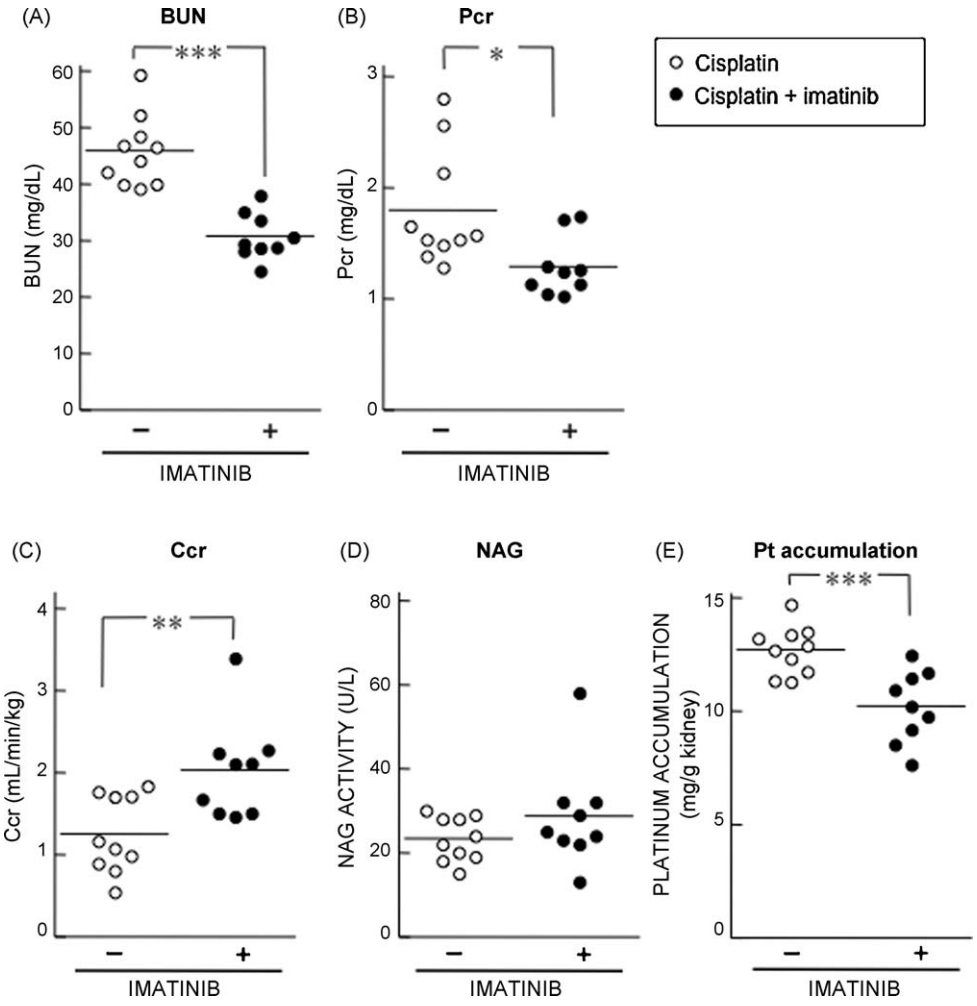

Fig. 6. Effect of coadministered imatinib on renal function and platinum accumulation of rats treated with cisplatin (5 mg/kg). Biochemical parameters (A: BUN; B: P_{cr}; C: C_{cr}; D: NAG) and platinum accumulation (E) in the kidney were examined on the fifth day after treatment with 5 mg/kg cisplatin with or without coadministration of 50 mg/kg imatinib. The kidney was homogenized in 9 volumes of buffer. Renal accumulation of platinum was then evaluated by ICP-MS. BUN, blood urea nitrogen; P_{cr}, plasma creatinine; C_{cr}, creatinine clearance; NAG, N-acetyl-β-D-glucosaminidase. Each bar represents the mean of nine or 10 rats from two independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, significantly different from cisplatin-treated rats.

cisplatin, we examined the effect of imatinib on platinum accumulation in HEK293 cells transiently expressing hOCT1, hOCT2, rOCT1 and rOCT2. Consistent with the results of cytotoxicity, platinum accumulation significantly decreased by the presence of imatinib in hOCT2- or rOCT2-expressing cells. In addition, imatinib decreased platinum accumulation in rOCT1-expressing cells, but accumulations via rOCT1 were less than a quarter of accumulations via rOCT2 (Fig. 5A). In combination of the data of cytotoxicity (Fig. 3) and cellular accumulation of cisplatin (Fig. 5A), the cellular cisplatin closely related with the magnitude of cytotoxicity with or without imatinib (Fig. 5B and C).

3.5. Effect of coadministrated imatinib on renal function and platinum accumulation of rats treated with cisplatin

Biochemical parameters on the 4 days after treatment in imatinib-treated rats were 13.0 ± 0.9 mg/dL (BUN), 0.42 ± 0.03 mg/dL (P_{cr}), 2.34 ± 0.20 mL/min (C_{cr}), and 26.4 ± 9.0 U/L (urinary NAG) (mean ± S.E. of five rats). These data were comparable with sham-operated rats (BUN, 13.1 ± 0.8 mg/dL; P_{cr}, 0.48 ± 0.08 mg/dL; C_{cr}, 1.77 ± 0.37 mL/min; urinary NAG, 23.7 ± 9.4 U/L, statistically not significant). Compared to the cisplatin-treated group, concurrent treatment with cisplatin and imatinib significantly reduced nephrotoxicity in BUN, P_{cr}, and C_{cr} (Fig. 6A–C). However, coadministered imatinib had no effect on the urinary NAG activity (Fig. 6D).

The renal accumulation of platinum was also measured 4 days after the intraperitoneal administration of 5 mg/kg cisplatin with or without gavage administration of 50 mg/kg imatinib. When rats were coadministered cisplatin with imatinib, the renal platinum accumulation decreased significantly compared to cisplatin alone (Fig. 6E).

3.6. Pharmacokinetics of cisplatin in rats coadministrated with imatinib

The pharmacokinetics of cisplatin in rats with or without the coadministration of imatinib was examined. Plasma concentrations of platinum up to 3 min after the bolus administration of cisplatin via the catheterized right femoral vein were determined (Fig. 7A). Imatinib (50 mg/kg) or Ringer's solution was administered via gavage 3 h before and in the final minute before the administration of cisplatin. The total clearance (CL_{total}) of cisplatin was significantly decreased in combined-modality rats (24.0 ± 1.1 mL/min) compared to cisplatin-treated rats (30.3 ± 1.3 mL/min) ($P < 0.01$) (Fig. 7B). The tissue uptake clearance (CL_{tissue}) of the kidney was also significantly decreased in combined-modality rats (1.7 ± 0.1 mL/min) compared to cisplatin-treated rats (2.2 ± 0.2 mL/min) ($P < 0.05$) (Fig. 7C). In

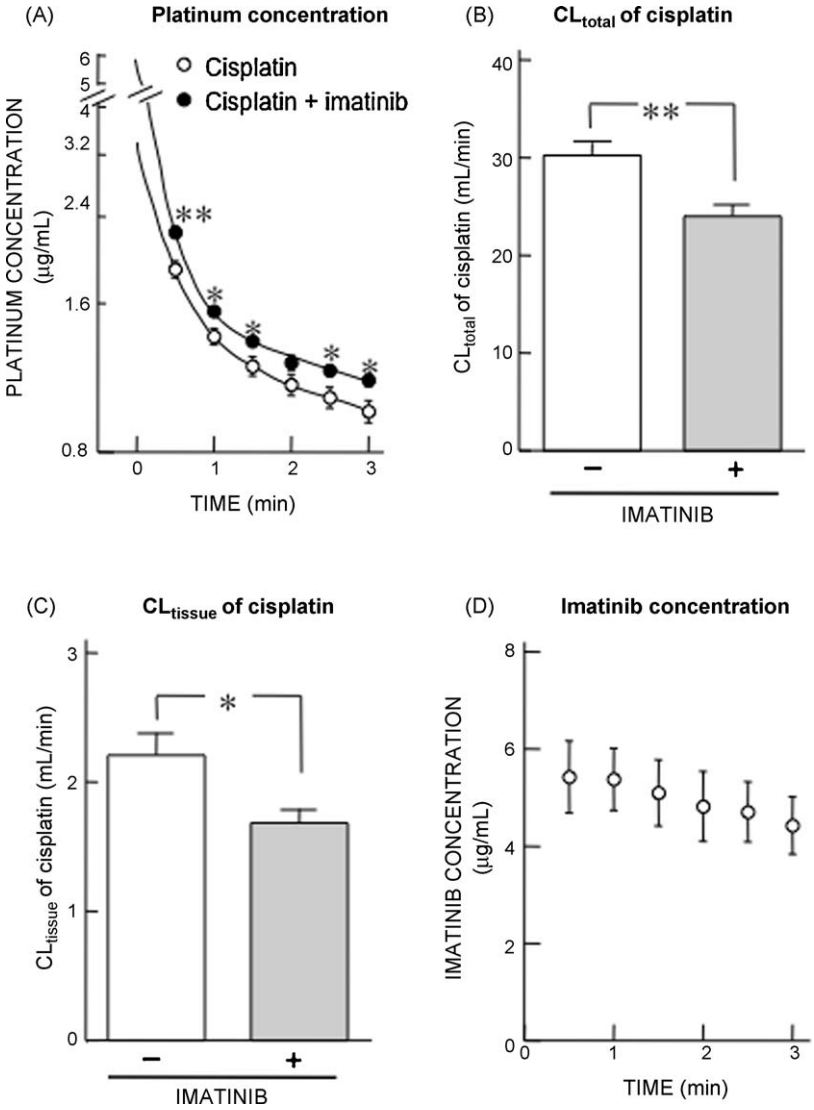

Fig. 7. Pharmacokinetics of cisplatin in rats coadministered with imatinib. (A) Plasma concentrations of platinum at various points were determined in male rats treated with cisplatin with or without coadministration of imatinib (closed circle and open circle, respectively). Total clearance (CL_{total}; B) and tissue uptake clearance (renal CL_{tissue}; C) 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. (D) Plasma concentrations of imatinib at various points were determined in male rats treated with cisplatin with coadministration of imatinib (open circle). Each column represents the mean ± S.E. of 10 rats from two independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$, significantly different from cisplatin-treated rats.

addition, the plasma concentration of imatinib during 3 min was stable around 5 μg/mL (8 μM) (Fig. 7D), and the renal concentration of imatinib was 182 ± 22 μg/mL (309 ± 37 μM, mean ± S.E. of 10 rats).

3.7. Histology of rat kidney coadministered cisplatin with imatinib

The pathology of the rat kidney treated with cisplatin with or without coadministration of imatinib was examined (Fig. 8). The degeneration of tubular cells, including tubular dilatation, tubular cell vacuolation, tubular cell detachment from the basement membrane and brush-border detachment, was prominent in rats treated with cisplatin (Fig. 8A and B). These changes were seen to a lesser degree in the rat kidney after the coadministration of cisplatin and imatinib (Fig. 8C and D).

4. Discussion

For more than four decades, there has been no successful treatment to avoid cisplatin-induced nephrotoxicity while maintaining its strong anticancer efficacy. Only diuretics in combination with hydration of more than 4000 mL/day are used in the present clinical practice to prevent its severe nephrotoxicity. However, the continuous urge to urinate reduces patients' quality-of-life. Previously, the renal toxicity of cisplatin was suggested to be a result of rOCT2-mediated extensive renal accumulation from the circulation, and weak tubular secretion into the urine by rMATE1 in rats [16]. The preventative effect of imatinib against cisplatin-induced nephrotoxicity may be the first effective treatment to maintain its anticancer effect without hydration. In addition, combination therapy with imatinib may bring additional anticancer effects in some ABL or c-kit-positive tumors.

Peng et al. [17] reported that the mean peak plasma concentration of imatinib ranged from 72 ng/mL (0.12 μM; at a dose of 25 mg) to 3016 ng/mL (5.11 μM; at a dose of 750 mg) after once-daily administration and from 2315 ng/mL (3.93 μM; 800 mg/day) to 3880 ng/mL (5.73 μM; 1000 mg/day) after twice-daily administration. Considering the IC_{50} values of imatinib against renal organic cation transporters (<10 μM), the present concentration of imatinib is reasonable compared to its clinical use. In the pharmacokinetic study (Fig. 7D), the plasma concen-

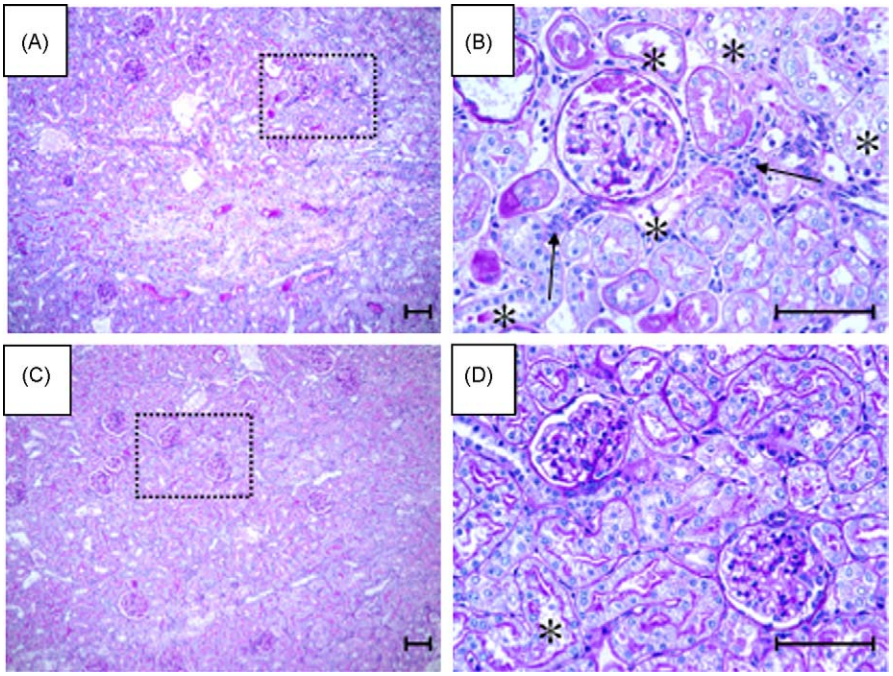

Fig. 8. Histology of rat kidney coadministered cisplatin with imatinib. Kidney was obtained on the fifth day after treatment with 5 mg/kg cisplatin with (C and D) or without (A and B) coadministration of 50 mg/kg imatinib. (A and C) PAS 100×. (B and D) PAS 400×. Scale bar: 100 μm. Kidney treated with cisplatin showed more severe tubular degeneration (B, asterisks) than the kidney coadministered cisplatin with imatinib (D). Arrow indicates cellular infiltration (B).

tration profile of imatinib (average, 5.0 ± 0.3 μg/mL (8.4 ± 0.5 μM)) was comparable to clinical use and enough to prevent the cisplatin-induced cytotoxicity and nephropathy (Figs. 3, 7 and 8) even in much lower free imatinib concentration than IC_{50} values because of high plasma protein binding rate of imatinib (about 95%; [18]) (Figs. 3, 7 and 8). Although clinical trials will be needed in the future, the range of the clinical dosage of imatinib may have a potential inhibitory effect on the renal accumulation of cisplatin and subsequent cisplatin-induced nephropathy, and reduce the total volume of hydration in cisplatin-based chemotherapy.

Consistent with previous results, the expressions of hMATE1, hMATE2-K and rMATE1 did not affect LDH release into culture medium (Fig. 4A) [5,16]. However, it was observed a slight but significant increase of caspase-3 and -7 activities caused by cisplatin treatment compared to sham treatment and a significant decrease by the presence of imatinib in HEK293 cells expressing hMATE1 and rMATE1 (Fig. 4B). It was quite a lower magnitude compared to the results of cells expressing OCT2 (Figs. 3B and 4B). We have previously reported that LDH release was markedly stimulated and γ-glutamyl transferase activity and amount of protein in the cell homogenate were markedly decreased by basolateral, not by apical treatment with cisplatin in LLC-PK_1 cell [19]. These results and reports strongly suggested that cisplatin-induced nephrotoxicity was caused by basolateral OCT2 and imatinib reduced cisplatin-induced toxicity via basolateral OCT2.

Recently, several in vitro studies demonstrated that co-treatment with cisplatin and imatinib resulted in synergistic cell killing and/or antiproliferative effects in non-small-cell lung cancer [20], CML [21], head and neck cancer [22,23], Ewing sarcoma, and breast cancer [24]. Moreover, preclinical and clinical studies indicated that combination therapy with imatinib and cisplatin was effective in a xenograft model in nasopharyngeal carcinoma [25], and in patients with small-cell lung carcinoma with irinotecan [26] and non-small-cell lung cancer [27]. However, there is no description relating renal impairment after chemotherapy in these clinical studies, and further studies are needed to clarify whether imatinib reduces cisplatin-induced nephrotoxicity.

In conclusion, the drug interaction between cisplatin and imatinib via renal OCT2 was revealed to be a useful strategy preventing cisplatin-induced nephrotoxicity in vivo due to the inhibition of renal accumulation of cisplatin. In addition, concomitant administration of cisplatin and imatinib may show synergistic anticancer effects because of alleviation of dose-limitation of cisplatin and different pharmacological target of cisplatin and imatinib. Further human studies focusing on renal function will evaluate the effect of the concomitant administration of imatinib on cisplatin-based chemotherapy, reducing its severe nephrotoxicity.

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