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Title page

Altered pharmacokinetics of cationic drugs caused by down-regulation of renal rOCT2 (Slc22a2) and rMATE1 (Slc47a1) in ischemia/reperfusion-induced acute kidney injury

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Running Title Page

Running title: Down-regulation of rOCT2 and rMATE1 in ischemic kidney

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List of abbreviations:

AKI, acute kidney injury; AUC, area under the plasma concentration curve; BUN, blood urea nitrogen; CL_{tot}, total body clearance; CL_{ren}, renal clearance; HPLC, high performance liquid chromatography; I/R, ischemia/reperfusion; k_{el}, plasma elimination rate constant; Kp,

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tissue-to-plasma concentration ratio; MATE, multidrug and toxin extrusion; NHE, Na^+/H^+ exchanger; OAT, organic anion transporter; OCT, organic cation transporter; SCr, serum creatinine; $t_{1/2\alpha}$, α-phase half-life; $t_{1/2\beta}$, β-phase half-life; TEA, tetraethylammonium; V_1, central volume of distribution; V_{ss}, volume of distribution at steady state.

Abstract

In the proximal tubules of rat kidney, the polyspecific organic cation transporters, rOCT1 and rOCT2, mediate the basolateral uptake of various organic cations, including many drugs, toxins, and endogenous compounds, and the apical type of H^+/organic cation antiporters, rat multidrug and toxin extrusion 1, rMATE1, mediate the efflux of organic cations. Renal clearances of H_2-receptor antagonists, including famotidine, were reported to be decreased in patients kidney disease. Therefore, acute kidney injury (AKI) could influence renal excretion and disposition of organic cations accompanied by the regulation of organic cation transporters. The aim of this study was to investigate the pharmacokinetic alteration of cationic drugs and the expression of tubular organic cation transporters, rOCT1, rOCT2 and rMATE1, in ischemia/reperfusion (I/R)-induced AKI rats. I/R-induced AKI increased the plasma concentration of intravenously administrated famotidine, a substrate for rOCT1 and rOCT2, or tetraethylammonium (TEA), a substrate for rOCT1, rOCT2 and rMATE1. The area under the plasma concentration curve (AUC) for famotidine and TEA was 2-fold and 6-fold higher in I/R rats than in sham-operated rats, respectively. The accumulation of TEA into renal slices was significantly decreased, suggesting that organic cation transport activity at the basolateral membranes was reduced in I/R rat kidney. The protein expressions of basolateral rOCT2 and luminal rMATE1 were down-regulated in I/R rat kidneys. These data suggest that the urinary secretion of cationic drugs via epithelial organic cation transporters is decreased in AKI.

Introduction

The kidney mediates urinary excretion of a wide variety of xenobiotics, including drugs, toxins, and endogenous compounds. In renal proximal tubules, several directional organic solute transport systems facilitate active secretion of a wide range of exogenous and endogenous organic ions (Pritchard and Miller, 1996; Inui et al., 2000). Transport proteins for organic anions and cations localized specifically at the apical or basolateral membranes of the proximal tubular cells are responsible for urinary secretion of diverse drugs (Sweet and Pritchard, 1999; Inui et al., 2000; Sekine et al., 2000). The structures and functions of SLC22A gene family members of organic anion transporter (OAT) and organic cation transporter (OCT), which mediate transepithelial transport of various organic anions and cations, have been characterized (Sweet and Pritchard, 1999; Inui et al., 2000; Sekine et al., 2000). rOAT1 (Slc22a6) and rOAT3 (Slc22a8) appear to mediate organic anion/α-ketoglutarate exchange at the basolateral membrane of the proximal tubules, including various organic anions (Sekine et al., 1997; Sweet and Pritchard, 1999; Tojo et al., 1999; Cha et al., 2001). On the other hand, rOCT1 (Slc22a1) and rOCT2 (Slc22a2) were reported to be driven by inside-negative membrane potential (Busch et al., 1996; Okuda et al., 1996), mediating basolateral uptake of diverse organic cations such as tetraethylammonium (TEA) and the H_2-receptor antagonist cimetidine (Urakami et al., 2001). The H^+/organic cation antiporter in renal brush-border membranes mediates active extrusion of cationic drug or toxins out of renal tubular cells (Ullrich, 1997). The oppositely directed H^+-gradient was demonstrated to be a driving force for the transport of organic cations such as TEA, a prototype substrate (Takano et al., 1984). More recently, the apical type of H^+/organic cation antiporter, rat multidrug and toxin extrusion 1 (rMATE1/Slc47a1) have been identified and functionally characterized (Ohta et al., 2006; Terada et al., 2006). rMATE1 is mainly expressed in the kidney and placenta, and is considered to be responsible for the final step of

urinary excretion of cationic drugs (Terada et al., 2006). Therefore, the functional and molecular variations of these transporters under renal diseases have a great impact on renal clearance of their substrates.

Acute kidney injury (AKI) caused by ischemia/reperfusion (I/R) is a critical syndrome associated with high mortality in humans (Thadhani et al., 1996; Star, 1998; Schrier et al., 2004). I/R-induced AKI is evoked by a complicated interaction among renal hemodynamics, inflammatory cytokines, and tubular cell damages (Bonventre and Weinberg, 2003). AKI is principally characterized by tubular dysfunction with impaired sodium and water reabsorption which are associated with the shedding and excretion of renal brush-border membranes and epithelial tubule cells into the urine (Thadhani et al., 1996). Following I/R, morphological changes occur in the proximal tubules, including loss of polarity, loss of the brush border, and redistribution of integrins and Na^+/K^+-ATPase to the apical membrane (Molitoris et al., 1992; Thadhani et al., 1996; Schrier et al., 2004). Therefore, renal tubular secretion of xenobiotics and endogenous toxins could be also affected by AKI, since this important secretory process is performed by several transporting systems localized in the renal tubular cells. In patients with renal diseases, it was reported that the plasma elimination and renal clearance of the H_2-receptor antagonist famotidine were decreased compared with healthy volunteers (Manlucu et al., 2005). Famotidine is mainly eliminated by the kidney as the intact form by tubular secretion in addition to glomerular filtration (Lin, 1991). Famotidine was reported to be transported by the rat and human OAT members rOAT3 and hOAT3, and the OCT members rOCT1, rOCT2, and hOCT2 (Tahara et al., 2005). Taking these findings into consideration, we hypothesized that decreased renal excretion of famotidine in patients with renal diseases could be caused by the decreased expression and function of OAT and/or OCT members in the kidney. We reported that renal organic anion transport activity at the basolateral membranes was suppressed in

rats with I/R-induced AKI, which was accompanied by the down-regulation of both rOAT1 and rOAT3 (Matsuzaki et al., 2007). In contrast, there is little information concerning the regulation of renal OCT family members in AKI. Previously, it was reported that the transport activity of organic cations in renal brush-border membranes was decreased in I/R rats (Maeda et al., 1993). However, there is no information regarding the expression of luminal rMATE1 in association with AKI. In the present study, we examined the pharmacokinetics of cationic drugs and the expression levels of tubular organic cation transporters in I/R-induced AKI rats.

Methods

Materials

[1-^{14}C]Tetraethylammonium bromide (118.4 MBq/mmol) and D-[1-^{3}H(N)]mannitol (525.4 GBq/mmol) were obtained from PerkinElmer Life and Analytical Sciences (Boston, MA). Radiochemical purity of these products was greater than 97% guaranteed by the company. Famotidine was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals used were of the highest purity available.

Experimental animals

Male Sprague-Dawley rats, initially weighing 200 to 210 g (Clea Japan, Inc., Tokyo, Japan), were housed in a standard animal maintenance facility at constant temperature (21°C - 23°C), humidity (50% - 70%) and a 12h:12h light/dark cycle 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 anesthetized using sodium pentobarbital (50 mg/kg intraperitoneally), and placed on a heating plate (39°C) to maintain a constant temperature. The kidneys were exposed via midline abdominal incisions. Renal ischemia was induced using vascular clamps (AS ONE, Osaka, Japan) over both pedicles for 30 min. After the clamps were released, the incision was closed in two layers with 3-0 sutures. Sham animals underwent anesthesia, laparotomy and renal pedicle dissection only. All animals received warm saline solution instilled in the peritoneal cavity during the surgical procedure, and were then allowed to recover with ad libitum access to food and water. All experiments were performed under surgical anesthesia at 48 hr after I/R. Twenty two of 27 rats survived 48 hrs after surgery. Blood samples were collected for measurement of blood urea nitrogen

(BUN) and serum creatinine (SCr). BUN and SCr in serum were measured at the SRL laboratory (Tokyo, Japan).

Measurement of plasma concentration of famotidine

At 48 h after I/R, famotidine was intravenously administered to rats at 20 mg/kg via the left jugular vein for 1 min. Blood samples (0.4 mL) were collected from the right jugular vein at 5, 15, 30, 60, 120, and 240 min after the injection, and plasma samples were obtained by centrifugation. Urine was also collected for 240 min after injection for determining urinary recovery. The concentration of famotidine in plasma and urine was measured by high-performance liquid chromatography (HPLC). 100 μL of plasma or urine sample was deproteinized by adding 0.2 mL of methanol and 0.1 mL of the mobile phase, and centrifuged at 6000 *g* for 10 min. 10 μL of the supernatant was injected into HPLC. The HPLC system consisted of a Shimadzu LC-10ADVP pump (Shimadzu, Kyoto, Japan) and a Shimadzu SPD-10AVP ultraviolet spectrophotometric detector (Shimadzu), and a column of TSK-gel ODS 80TM (4.6 mm inside diameter, 150 mm length) (Tosoh, Tokyo, Japan). The mobile phase consisted of a mixture of 30 mM phosphate buffer (pH 7.0) and acetonitrile (95:5, v/v), and the flow rate was 1.0 mL/min at a column temperature of 40°C. Ultraviolet absorbance was determined at a wavelength of 280 nm. Standard curves for famotidine were prepared over a range of 0.25-100 μg/mL and shown to be linear. The coefficients of variation for the desired concentration (2.5, 25 and 100 μg/mL) ranged from 1.4% to 3.9%. The limit of quantification was 0.25 μg/mL. Blank plasma and urine samples showed no interference with the peak corresponding to famotidine.

Measurement of plasma and kidney concentrations of TEA

At 48 h after I/R, [^{14}C]TEA was intravenously administered to rats at 1.0 mg/kg via the left jugular vein for 1 min. Blood samples (0.4 mL) were collected from the right jugular vein at 5, 15, 30, 60, 120, and 240 min after the injection, and plasma samples were obtained by centrifugation. Urine was also collected for 240 min after injection for determining urinary recovery. At 240 min post-injection, kidneys were collected immediately after sacrificing rats. The excised kidneys were gently washed and weighed. 100 μL of plasma, urine or kidney homogenates were solubilized in 0.5 mL of NCSII (GE Healthcare Bio-sciences Corp., Piscataway, NJ), and the radioactivity was determined in a liquid scintillation counter after adding 5 mL of OCS (GE Healthcare Bio-sciences Corp.).

Pharmacokinetic analysis

A conventional two-compartment model was used to analyze the plasma concentration-time profiles of famotidine and TEA after intravenous administration in rats. The area under plasma concentration-time curve (AUC) for famotidine and TEA were determined by the trapezoidal rule with extrapolation to infinity. Pharmacokinetic parameters calculated using standard formulae were central volume of distribution (V_1), volume of distribution at steady state (V_{ss}), plasma elimination rate constant (k_{el}), α-phase half-life ($t_{1/2\alpha}$), β-phase half-life ($t_{1/2\beta}$), total body clearance (CL_{tot}), and renal clearance (CL_{ren}).

Uptake by rat renal slices

Uptake studies in isolated rat renal slices were carried out as described in a previous report (Matsuzaki et al., 2007). Briefly, 12-15 slices prepared from whole kidney of sham and ischemic rats (n=3) 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 were randomly selected and incubated in flasks containing 6 mL of the incubation buffer with [^{14}C]TEA (5 μM, 0.56 kBq/mL). The uptake of these compounds was measured 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]TEA as well as to evaluate the viability of slices. After incubation for a specified period, the incubation buffer containing radiolabeled compounds was rapidly removed from the flask, and the renal slices were washed twice 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.

Western blot analysis

Kidneys (n=3) 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 (40 μg) was mixed in loading buffer (2% SDS, 125 mM Tris-HCl, 20% glycerol, 5% 2-mercaptoethanol) and heated at 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 1 h at room temperature with primary antibody specific for rOCT1 (Ji et al., 2002), rOCT2 (Ji et al., 2002), rOAT1 (Matsuzaki et al., 2007),

rOAT3 (Matsuzaki et al., 2007), rMATE1 (Nishihara et al., 2007) or β-actin (Sigma Chemical Co., St Louis, MO). 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 sham-operated rats were used as the reference and accorded an arbitrary value of 100.

Real-time PCR analysis

The isolation of mRNA from kidney and reverse transcription were performed as described previously (Matsuzaki et al., 2007). We performed a 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 rOCT1, rOCT2, rOAT1, rOAT3, 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.: rOCT1, Rn00562250_m1; rOCT2, Rn00580893_m1; rOAT1, Rn00568143_m1; rOAT3, Rn00580082_m1; rMATE1, Rn01497159_m1; and 18S rRNA, 4319413E.

Statistical Analysis

Statistically significance was determined by Student's t-test. A *P*-value of less than 0.05 was considered statistically significant.

Results

Renal functional data of I/R-induced AKI rats

Renal function was first examined in rats with renal I/R. As summarized in Table 1, the body weights were slightly yet significantly decreased in I/R rats. The levels of both BUN and SCr were significantly elevated in I/R rats compared with sham-operated rats, indicating AKI was evoked by I/R treatment.

Effects of I/R-induced AKI on famotidine pharmacokinetics

To examine whether the renal disposition of famotidine is decreased in I/R rats in comparison with that in sham-operated rats, we assessed the pharmacokinetics of famotidine. The plasma concentration-time profile of famotidine up to 240 min after intravenous administration is shown Fig. 1. The plasma concentration of famotidine in I/R rats was higher than in sham-operated rats. Table 2 summarizes the pharmacokinetic parameters of famotidine in the sham-operated and I/R rats. The AUC for famotidine in I/R rats was 2-fold higher than in sham-operated rats. The CL_{tot} and CL_{ren} values for famotidine in I/R rats were significantly decreased to 49% and 14%, respectively, of the corresponding values in sham-operated rats. The $t_{1/2\beta}$ of famotidine was significantly prolonged in I/R rats compared with sham-operated rats, whereas there was no significant differences in the $t_{1/2\alpha}$ of famotidine between the sham-operated and I/R rats.

Effects of I/R-induced AKI on TEA pharmacokinetics

We next examined the pharmacokinetics of TEA, a typical cationic substrate for rOCT1 and rOCT2. The plasma concentration-time profile of TEA up to 240 min after intravenous administration is depicted in Fig. 2A, and the pharmacokinetic parameters of TEA are summarized

in Table 3. I/R caused significant changes in the pharmacokinetic behavior of TEA. The AUC for TEA was markedly elevated in I/R rats as compared with sham-operated rats, while the CL_{tot} for TEA was significantly decreased in I/R rats. The AUC for TEA was almost 7-fold higher in I/R rats than in sham-operated rats. The CL_{tot} and CL_{ren} values for TEA in I/R rats were significantly decreased to 20% and 14%, respectively, of the values in sham-operated rats. The $t_{1/2\beta}$ of TEA in I/R rats was almost 4-fold higher than in sham-operated rats, whereas the $t_{1/2\alpha}$ of TEA was not affected.

Figures 2B and 2C show the kidney concentrations and tissue-to-plasma concentration ratio (apparent Kp) for TEA in sham-operated and I/R rats at 240 min after intravenous administration. The concentration of TEA in the kidney was significantly elevated in I/R rats as compared with sham-operated rats. The Kp value in I/R rat kidneys was significantly decreased to 20% of that in sham-operated rats.

Uptake of TEA by renal slices

To evaluate organic cation transport activity in the renal basolateral membrane, we measured the accumulation of TEA in renal slices prepared from sham-operated and I/R rat kidneys. As illustrated in Fig. 3, the accumulation of TEA was significantly lower in the I/R rats at each time point. The accumulation of TEA into renal slices at 60 min was significantly decreased to 36% of those in sham-operated rats.

Protein and mRNA expression of rOCTs in I/R-induced AKI rats

To get precise information about the decreases in accumulation of TEA in renal slices of I/R rat kidney, we measured renal rOCT1 and rOCT2 expression using Western blot analyses. As is evident in Fig. 4, rOCT2 protein expression was markedly suppressed in I/R rat kidney compared to

that in sham-operated rat kidney, whereas there was no significant difference in the expression of rOCT1. The expressions of rOAT1 and rOAT3 protein was significantly depressed in I/R rat kidney, which is consistent with our previous report (Matsuzaki et al., 2007).

Next, we examined mRNA expression levels of organic cation transporters, rOCT1 and rOCT2, in the kidney (Fig. 5). When compared with sham-operated rat kidneys, the levels of rOCT1 and rOCT2 mRNA in I/R rat kidneys were significantly depressed to 48% and 4%, respectively.

Protein and mRNA expression of rMATE1 in I/R-induced AKI rats

The effect of I/R-induced AKI on mRNA and protein expression of rMATE1 was examined. The rMATE1 protein level was markedly depressed by I/R rat kidneys (Fig. 6). As observed for the corresponding protein expression, the relative mRNA expression level of rMATE1 was significantly decreased in the I/R rats (Fig. 7).

Discussion

Functional changes in renal organic ion transporters may be of clinical relevance, particularly to the use of drugs with high toxicity or narrow therapeutic range. Serious kidney diseases, such as AKI, influence renal disposition of diverse organic ions in association with the decreased glomerular filtration and function of transport systems. Our previous study demonstrated that the mRNA and protein expression levels of Slc22A organic anion transporters, rOAT1 and rOAT3, were markedly suppressed under I/R-induced AKI, which was accompanied with significant elevation of the serum level of indoxyl sulfate, a uremic toxin that is a substrate of both rOAT1 and rOAT3 (Matsuzaki et al., 2007). We investigated the change in renal organic cation transporters, rOCT1, rOCT2 and rMATE1, in this study.

Three isoforms of Slc22A organic cation transporter family members, OCT1, 2 and 3, were identified, and their physiological and pharmacokinetic roles have been evaluated (Inui et al., 2000; Jonker and Schinkel, 2004). rOCT1 is expressed abundantly in the liver and kidney (Grundemann et al., 1994), whereas rOCT2 is expressed predominantly in the kidney but not in the liver (Okuda et al., 1996). These transporters are localized to the basolateral membranes of renal proximal tubules. rOCT3 is expressed predominantly in the placenta but also has been detected in the intestine, heart, brain, lung, and very weakly in the kidney (Kekuda et al., 1998). In the renal proximal tubules of rats, rOCT1 and rOCT2 are considered to mediate the basolateral uptake of various cationic compounds. Previous reports suggested that the pharmacokinetics of famotidine are related to renal function (Manlucu et al., 2005). We found that the renal excretion of famotidine was significantly decreased in I/R rats (Fig. 1 and Table 2). Transport study demonstrated that famotidine was a substrate for rOCT1, rOCT2 and rOAT3 (Tahara et al., 2005). Basolateral OCTs are known to be driven by the K^+-gradient associated with the inside-negative electrical potential difference, generated by Na^+/K^+-ATPase

(Wright and Dantzler, 2004). We reported that Na^+/K^+-ATPase expression was markedly depressed in the I/R rat kidney (Matsuzaki et al., 2007), thereby the driving force for OCTs at basolateral membrane could be decreased in I/R rats. As shown in Figs. 2C and 3, organic cation transport activity at the basolateral membranes was reduced in I/R rat kidney, as the Kp value of TEA after intravenous administration and the accumulation of TEA into renal slices were significantly decreased to 20% and 36% of those in sham-operated rats, respectively. We previously reported that the transport activity of rOAT3 in I/R rats was significantly reduced to 52% of that in sham-operated rats, since the accumulation of estrone sulfate, a substrate of rOAT3, was decreased in renal slices from I/R rat kidney (Matsuzaki et al., 2007). It was reported that the Michaelis-Menten constant (K_m) values of famotidine for rOCT1, rOCT2 and rOAT3 were 87, 61 and 345 μM, respectively (Tahara et al., 2005). In this study, the estimated maximum plasma concentrations of famotidine in sham-operated and I/R rats were 154 and 148 μM, respectively. Considering the transporter affinity, decreased expression levels, and plasma concentration of famotidine in AKI rats, the decreased renal excretion of famotidine in I/R may be mainly evoked by the decreased organic cation transport activity of the basolateral membrane in renal proximal tubules. Alternatively, the serum level of indoxyl sulfate, one of the high-affinity substrates for rOAT1 and rOAT3, was markedly elevated in I/R rats (Matsuzaki et al., 2007). Therefore, elevation of serum IS could inhibit rOAT3 in a competitive manner, thereby decreasing renal accumulation of famotidine mediated by rOAT3.

Western blot analysis revealed that the expression of rOCT2, but not rOCT1, was significantly suppressed in I/R rat kidneys (Fig. 4). In addition, the mRNA expression of not only rOCT2 but also rOCT1 was significantly depressed (Fig. 5). The decrease in rOCT2 mRNA was remarkable compared to that in rOCT1 mRNA, suggesting that rOCT2 was more sensitive to I/R-induced AKI. Recently, it was reported that the expression of rOCT2 was decreased in rats with

chronic renal failure (Ji et al., 2002), hyperuricemia (Habu et al., 2003), and diabetes mellitus (Thomas et al., 2004). Urakami et al. reported that the expression of rOCT2 was up-regulated by testosterone and down-regulated by estradiol in rats (Urakami et al., 2000). It was also suggested that the lowered plasma level of testosterone was responsible for the decreased rOCT2 expression (Ji et al., 2002). Testosterone induces the expression of rOCT2, but not rOCT1 and rOCT3, via the androgen receptor-mediated transcriptional pathway (Asaka et al., 2006). However, it has been reported that there were no significant changes in plasma testosterone and estradiol after renal I/R-induced AKI (Park et al., 2004), although the serum testosterone levels was decreased in bilateral ureteral ligation, uranyl nitrate or cisplatin-induced AKI (Ivic et al., 1988; Masubuchi et al., 2006). Therefore, further study on the factor(s) and mechanisms of the decreased expression of rOCT2 is required to understand its regulation in AKI states.

In vivo renal clearances of famotidine and TEA were significantly decreased in I/R rats. Renal clearance of famotidine and TEA may be affected by organic cation transport activity not only at the basolateral membranes but also at the brush-border membranes, since renal secretion is performed by two transport steps in both membranes. In the rat renal tubular brush-border membranes, rMATE1 can mediate the organic cation transport energized by an inward-directed H^+-gradient, which is mainly generated by Na^+/H^+ exchanger (NHE) 3 (Moe, 1999). In the 5/6 nephrectomized rats, the down-regulated expression of luminal rMATE1 was correlated well with the tubular secretion of cimetidine, and the expression of NHE3 was markedly depressed (Nishihara et al., 2007). TEA and cimetidine are substrates for rMATE1 (Ohta et al., 2006; Terada et al., 2006), although the ability of rMATE1 to recognize famotidine as a substrate is unknown yet. We found that the protein and mRNA expressions of rMATE1 were markedly depressed in I/R rats (Figs. 6 and 7). Previously, it was reported that NHE3 expression was markedly depressed in I/R rats (Wang et al.,

1997; Kwon et al., 2000), and the transport activity of organic cations in renal brush-border membranes was decreased in I/R rats (Maeda et al., 1993). Therefore, the down-regulation of rMATE1 could be involved in the decreased renal clearance of TEA in I/R rats at the luminal membranes.

We have reported that I/R-induced AKI caused the down-regulation of basolateral rOCT2, accompanied by decreased organic cation transport activities at basolateral membrane. Furthermore, the luminal rMATE1 expression was markedly depressed in I/R rats, suggesting decreased organic cation transport activities at brush-border membranes. The present results suggest that the renal expression of rOCT2 and rMATE1 are down-regulated and the urinary secretion of cationic drugs is decreased in AKI. Our findings would provide information for understanding the mechanisms involved in pharmacokinetic alteration of drugs excreted mainly into urine under AKI, and the pathophysiological roles of basolateral rOCTs and luminal rMATE1.

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Footnotes

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Legends for figures

Fig. 1. The plasma concentration versus time profiles for famotidine in sham-operated (open circle) and I/R rats (closed circle) after intravenous administration of famotidine (20 mg/kg). Each point represents the mean±SD from 6 rats. **p<0.01, and ***p<0.001, vs. sham-operated rats.

Fig. 2. (A) The plasma concentration versus time profiles for TEA in sham-operated (open circle) and I/R rats (closed circle) after intravenous administration of [^{14}C]TEA (1.0 mg/kg). (B) Kidney concentration and (C) tissue-to-plasma concentration ratio (Kp) value of TEA in sham-operated (open column) and I/R rats (closed column) at 240 min after intravenous administration of [^{14}C]TEA (1.0 mg/kg). Each point or column represents the mean±SD from 4 rats. *p<0.05, and **p<0.01, vs. sham-operated rats.

Fig. 3. Uptake of TEA in renal slices of sham-operated and I/R rats. Renal slices from sham-operated (open circle) and I/R rats (closed circle) were incubated at 37°C in incubation buffer containing 5 μM [^{14}C]TEA, for the period indicated. D-[^{3}H]Mannitol was used to estimate the extracellular trapping and non-specific uptake of [^{14}C]TEA. Each point represents the mean±SD for 4-5 slices from different rats. *p<0.05, and ***p<0.001, vs. sham-operated rats.

Fig. 4. Protein and mRNA expressions of basolateral organic ion transporters in the kidney of sham-operated and I/R rats. (A) Antisera specific for rOAT1, rOAT3, rOCT1, rOCT2 or β-actin were used as primary antibodies. (B) The ratio of rOAT1, rOAT3, rOCT1 and rOCT2 density to β-actin density in sham-operated (open column) and I/R rats (closed column). The values for sham-operated rats were arbitrarily defined as 100%. Each column represents the mean±SD from 3

rats. (C) mRNA expressions of basolateral organic ion transporters in the kidney of sham-operated and I/R rats. rOAT1, rOAT3, rOCT1 and rOCT2 mRNA expression levels in sham-operated (open column) and I/R rats (closed column) were determined by real-time PCR analysis. The relative amount of rOAT1, rOAT3, rOCT1 and rOCT2 mRNA were normalized to that of 18S ribosomal RNA. Each column represents the mean±SD from 7-8 rats. ***p<0.001, vs. sham-operated rats.

Fig. 5. Protein and mRNA expression of luminal rMATE1 in the kidney of sham-operated and I/R rats. (A) Antisera specific for rMATE1 or β-actin were used as primary antibodies. (B) The ratio of rMATE1 density to β-actin density in sham-operated and I/R rats. The values for sham-operated rats were arbitrarily defined as 100%. Each column represents the mean±SD from 3 rats. (C) mRNA expression of luminal rMATE1 in the kidney of sham-operated and I/R rats. rMATE1 mRNA expression levels was determined by real-time PCR analysis. The relative amount of rMATE1 mRNA was normalized to that of 18S ribosomal RNA. Each column represents the mean±SD from 7-8 rats. **p<0.01, and ***p<0.001, vs. sham-operated rats.

Table 1. Renal functional data at 48 hr after I/R

	Sham	I/R
Body weight (g)	210.6±8.3	190.3±9.2***
BUN (mg/dL)	18.7±4.9	188.9±47.1***
SCr (mg/dL)	0.20±0.03	2.83±1.30***

Each value represents the mean±SD from 17-18 rats. ***P<0.001, vs. sham-operated rats.

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Table 2. Pharmacokinetic parameters of famotidine in sham-operated and I/R rats

	Sham	I/R
Percent dose excreted in urine (%)	67.3±7.5	18.1±14.6***
AUC (μg·min/mL)	911±94	1925±406***
V_1 (mL/kg)	428±157	424±109
V_{ss} (mL/kg)	801±168	810±223
CL_{tot} (mL/min/kg)	22.1±2.3	10.7±2.0***
CL_{ren} (mL/min/kg)	14.9±2.4	2.1±1.8***
k_{el} (min^{-1})	0.058±0.021	0.026±0.007***
$t_{1/2\alpha}$ (min)	6.7±3.2	7.0±4.2
$t_{1/2\beta}$ (min)	38.8±13.2	64.0±19.7*

Each value represents the mean±SD from 6 rats. *P<0.05, and ***P<0.001, vs. sham-operated rats.

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Table 3. Pharmacokinetic parameters of TEA in sham-operated and I/R rats

	Sham	I/R
Percent dose excreted in urine (%)	78.0±16.2	48.2±21.6
AUC (μg·min/mL)	29.3±5.8	198.0±134.9*
V_1 (mL/kg)	977±133	714±295
V_{ss} (mL/kg)	2313±374	1813±547
CL_{tot} (mL/min/kg)	35.3±8.1	7.1±4.5**
CL_{ren} (mL/min/kg)	27.6±9.3	3.8±3.8**
k_{el} (min^{-1})	0.036±0.005	0.010±0.003***
$t_{1/2\alpha}$ (min)	6.6±0.2	5.9±3.7
$t_{1/2\beta}$ (min)	60.1±8.1	221.3±69.1**

Each value represents the mean±SD from 4 rats. *P<0.05, **P<0.01, and ***P<0.001, vs. sham-operated rats.

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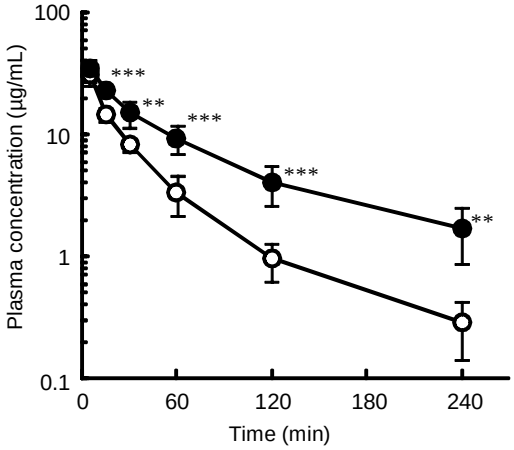

Fig. 1

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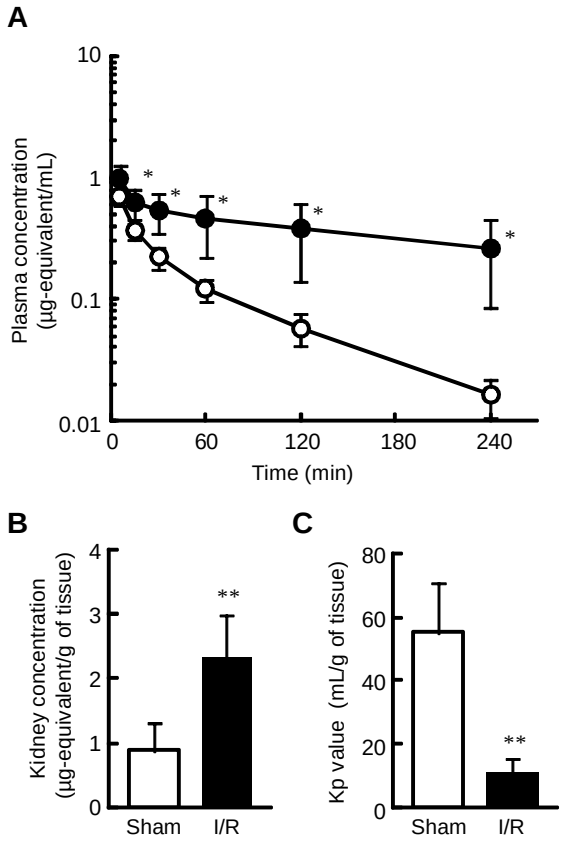

Fig. 2

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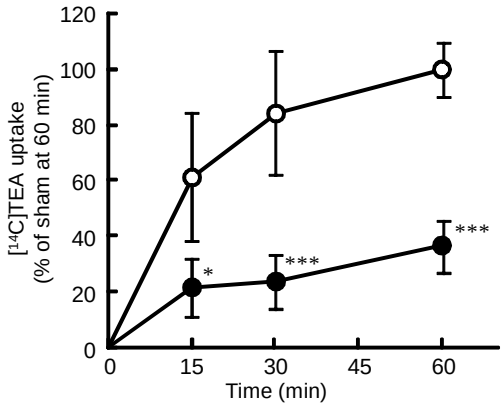

Fig. 3

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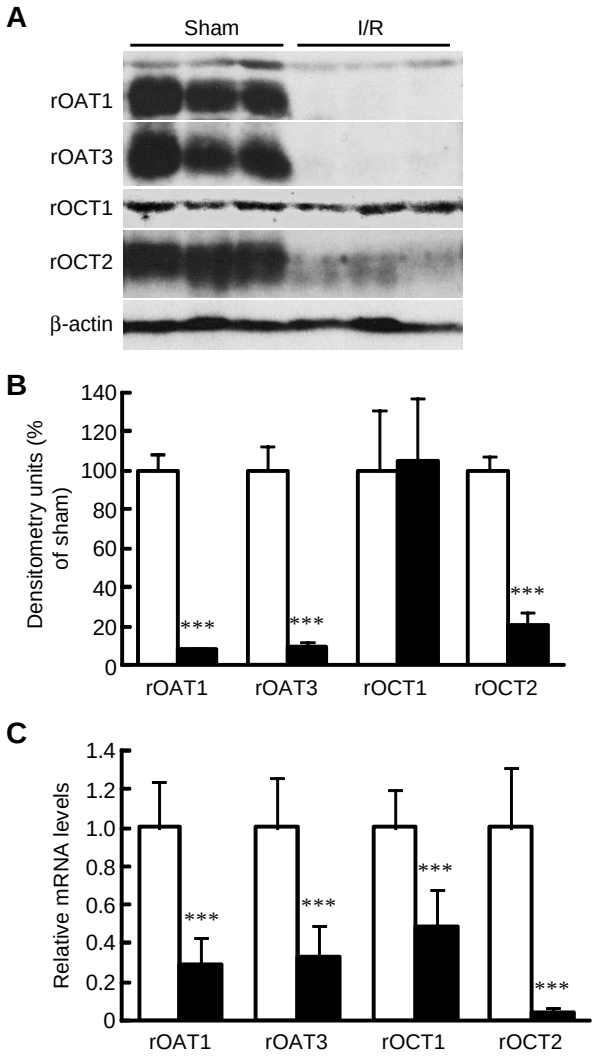

Fig. 4

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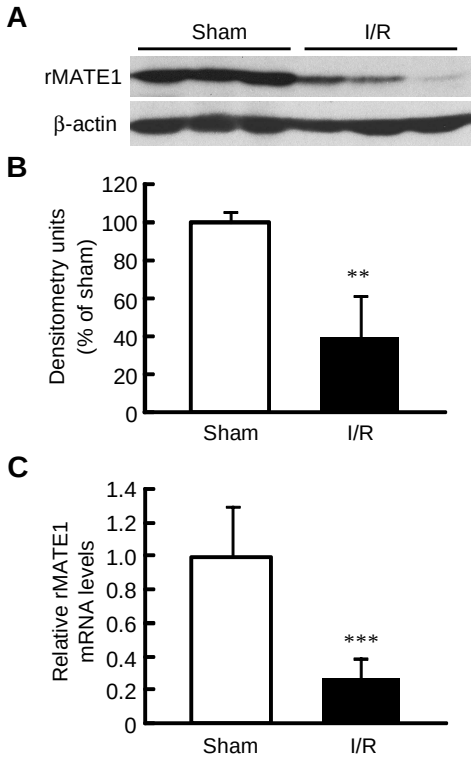

Fig. 5