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Pharmacokinetic significance of luminal multidrug and toxin extrusion 1 in chronic renal failure rats

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ABSTRACT

Functional and expressional depression of the rat organic cation transporter rOCT2 after 5/6 nephrectomy (Nx) is accompanied by the decreased plasma level of testosterone in the male rats. Though vectorial transport across the tubular epithelial cells is important in the secretion of cationic compounds, there has been no imformation about the luminal organic cation transporter in disease state. In the present study, the role of luminal multidrug and toxin extrusion 1 (rMATE1) was examined using female rats with or without Nx, avoiding the influence of testosterone. The tubular secretion of cimetidine was markedly decreased in female Nx rats as well as male rats. Unlike in the male rats, the plasma level of testosterone and the expression of basolateral rOCT2 were unchanged in the female rats after Nx. On the other hand, the expression of rMATE1 was markedly decreased in both male and female Nx rats, and the level of rMATE1, but not of rOCT2, correlated well with the tubular secretion of cimetidine in the female rats ($r = 0.74$). Immunohistochemical analysis revealed that rMATE1 and Na^+/H^+ exchanger (NHE) 3 were localized at the brush-border membrane of proximal tubules. The level of NHE3 was also markedly depressed in both male and female Nx rats, suggesting that the expression level on the luminal rMATE1 in combination with NHE3 was indicated to be a crucial factor for the tubular secretion of cimetidine.

1. Introduction

The tubular transport of organic substances plays an essential role in the removal of *xenobiotics* including drugs and numerous chemicals contained in our environment and some metabolites. Basolateral types of organic anion and cation transporters, OAT1 (SLC22A6), OAT3 (SLC22A8), OCT1 (SLC22A1) and OCT2 (SLC22A2), have been cloned and characterized [1–4]. These transporters are indicated to mediate the basolateral entry of various anionic or cationic drugs into the proximal tubular epithelial cells [5]. Recently, a renal luminal type of H^+/organic cation antiporter, multidrug and toxin extrusion 1 (MATE1), has been cloned, and considered to be responsible for the final step in the excretion of organic cations [6–8]. However, the pharmacokinetic and pathophysiological role of the luminal MATE1 in combination with the basolateral OCT2 is not clear. Furthermore, there has been no information regarding the change in luminal MATE1 during chronic renal failure.

More gene product of rat (r) OCT2 is found in the male kidney than female kidney, and the expression of rOCT2 in the kidney is regulated by the plasma level of testosterone [9,10]. We previously found that the renal tubular secretion of *para*-aminohippuric acid and cimetidine were markedly decreased in male 5/6 nephrectomized (Nx) rats, the protein expression of rOCT2 but not of rOCT1, rOAT1, or rOAT3 was depressed in male Nx rats [11]. Furthermore, a lowered plasma level of testosterone was likely to be responsible for the depressed rOCT2 expression in chronic renal failure.

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Based on these findings, the female rats were hypothesized to be a good animal model with which to evaluate the pharmacokinetic significance of the luminal organic cation transport system, avoiding the influence of hormonal regulation of the basolateral rOCT2. In the present study, we examined the renal handling of cimetidine, a substrate for the renal organic cation transport system, in rats after Nx. Furthermore, the expression levels of renal organic ion transporers were examined to clarify the responsible factor in the tubular secretion of cationic drugs in the kidney.

2. Materials and methods

2.1. Experimental animals

For ablation of the renal mass, male and female Wistar albino rats (180–200 g) were anesthetized with sodium pentobarbital (50 mg/kg) and the kidneys were exposed under aseptic conditions via a ventral abdominal incision. The right kidney was removed, the posterior and anterior apical segmental branches of the left renal artery were individually ligated, and the abdominal incision was closed with 4-0 silk sutures. In the sham-operated animals, the peritoneal cavity was exposed, and both kidneys were gently manipulated. To examine the effect of the administration of testosterone on drug pharmacokinetics and the renal expression of transporters, the sham-operated and Nx rats were administered a subcutaneous injection of testosterone (0.5 mg testosterone enanthate (T) dissolved in 200 μL corn oil/rat, T(+)) or vehicle (200 μL corn oil/rat, T(−)) at 1, 4, 7, 10 and 13 days after surgery. Except during the subcutaneous administration of testosterone every 3 days, animals were allowed access to water and standard rat chow for 2 weeks.

Rats were maintained in metabolic cages for 24 h before the in vivo experiment, to determine urine output and urinary levels of creatinine. The blood urea nitrogen (BUN) concentration was determined by the urease/indophenol method. The levels of creatinine in plasma and urine were determined with the Jaffé reaction. For measurements, we used assay kits from Wako Pure Chemical Industries (Osaka, Japan). The plasma testosterone and 17β-esrtadiol level was measured with an enzyme immunoassay kit (Cayman Chemical Co., MI, USA). The experiments with animals were performed in accordance with the *Guidelines for Animal Experiments of Kyoto University*.

2.2. Infusion experiment

Rats were anesthetized with an intraperitoneal administration of 50 mg/kg sodium pentobarbital. Catheters were inserted into the right femoral artery and the left femoral vein with polyethylene tubing (Intramedic PE-50, Becton Dickinson and Co., Parsippany, NJ, USA) filled with a heparin solution (100 U/mL) for blood sampling and drug administration, respectively. Urine was collected from the urinary bladder catheterized with PE-50 tubing. Thereafter, cimetidine was administered as a bolus via the femoral vein and incorporated into the infusion solution as described [12]. The loading and maintenance doses of cimetidine including 4% mannitol were 317 μmol/kg and 21.8 μmol/mL, respectively. The infusion rate was 2.2 mL/h using an automatic infusion pump (Natsume Saisakusho, Tokyo, Japan). Mannitol was used to maintain a sufficient and constant urine flow rate. After a 30-min equilibration period, urine samples were collected three times at 10 min intervals, and blood samples were obtained at the midpoint of urine collection. The plasma was immediately separated from erythrocytes by centrifugation. At the end of the experiment, an adequate volume of blood was collected from the abdominal aorta to examine the plasma protein binding rate, and the kidneys were removed to determine the tissue concentrations of cimetidine and the expression of renal drug transporters. The concentrations of cimetidine in plasma, urine, and the renal homogenate were determined by high performance liquid chromatography [13]. The plasma unbound fraction (fu) of cimetidine was determined by ultrafiltration using a micropartition system (MRS-1, Amicon Inc., Beverly, MA, USA), as described [12]. The free fraction of cimetidine was expressed as the ratio of the concentration in the ultrafiltrate to that in plasma.

2.3. Analytical methods

Pharmacokinetic parameters were calculated using standard procedures for each experimental period. The total plasma clearance (Ctot) was calculated by dividing the infusion rate by the steady-state plasma concentration (Cpss) at the midpoint of urine collection. Renal clearance (Cren) was obtained by dividing the urinary excretion rate by Cpss. The renal clearance of unbound cimetidine (Cr.f) was determined by dividing Cren by the fu of cimetidine. The glomerular filtration rate (GFR) was assumed to be equal to the Cren of creatinine. The renal secretory clearance of unbound cimetidine was calculated by substracting GFR from Cr.f.

2.4. Polyclonal antibody against rMATE1 and Western blot analysis

Polyclonal antibody was raised against the synthetic peptide that corresponded to the C-terminus of MATE1, which is fully conserved in human and rat [8]. The crude plasma membrane fractions were prepared from rat kidneys, as described previously [14]. The crude plasma membrane fractions were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride membranes (Immobilon-P, Millipore, Bedford, MA, USA) by semi-dry electroblotting. The blots were blocked with 5% non-fat dry milk and 5% bovine serum albumin in phosphate-buffered saline (PBS, 137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1 mM KH_2PO_4, and 12 mM K_2HPO_4, pH 7.5) containing 0.5% Tween 20 (PBS-T) for OAT1, OAT3, OCT1, OCT2 and Na^+/K^+-ATPase or 5% non-fat dry milk in Tris-buffered saline (TBS, 20 mM Tris and 137 mM NaCl) containing 0.5% Tween 20 (TBS-T) for MATE1. The blots were then incubated overnight at 4 °C with primary antibody specific for rOAT1[11], rOAT3 [11], rOCT1 [15], rOCT2 [9], rMATE, NHE3 (CHEMICON International Inc., Temecula, CA, USA), Na^+/K^+-ATPase (Upstate Biotechnology Inc., Lake Placid, NY, USA), or with an antibody preabsorbed with the synthetic antigen peptide (20 μg/mL) for rMATE1. The blots were washed three times with PBS-T or TBS-T, and the bound antibody was detected on X-ray film by enhanced chemiluminescence (ECL) with

horseradish peroxidase-conjugated secondary antibodies and cyclic diacylhydrazides (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA). Na^+/K^+-ATPase was examined as a positive control. The relative amounts of the bands in each lane were determined densitometrically using NIH Image 1.61 (National Institutes of Health, Bethesda, MD), and the densitometric ratios relative to each control (Sham or Sham T(−)) were used as the reference and accorded an arbitrary value of 1.0, respectively.

2.5. Immunohistochemical analysis

The animals were anesthetized and the kidneys were perfused via the abdominal aorta, first with saline containing 50 U/mL of heparin and then with 4% paraformaldehyde in PBS. Fixed tissues were embedded in OCT compound (Sakura Finetechnical, Tokyo, Japan) and frozen rapidly in liquid nitrogen. Sections (5 μm thick) were cut and covered with a blocking agent Blocking One® (Nacalai Tesque, Kyoto, Japan) containing 1 mg/mL RNase A (Nacalai Tesque) at 37 °C for 30 min. The covered sections were incubated at 37 °C for 60 min with antiserum specific for MATE1 (1:100 dilution) or anti-NHE3 antibody (1:200). Following two washings each with 3 × PBS and regular PBS, sections were incubated with Cy3-labeled donkey anti-rabbit IgG (CALTAG Laboratory, San Francisco, CA, USA), Alexa 488-Phalloidin (Molecular Probe, Eugene, OR, USA), and 4′,6-diamidino-2-phenylindole (DAPI; Wako, Osaka, Japan) at 37 °C for 60 min. These sections were examined and captured with a BZ-8000 (KEYENCE, Osaka, Japan) at 150× magnification.

2.6. Statistical analysis

All data are expressed as means ± S.E. Data from the Western blot analysis are expressed in arbitrary units of densitometry/25 μg protein. Comparisons were made using the unpaired *t*-test. $P < 0.05$ was considered significant.

3. Results

3.1. Renal functional data after administration of testosterone in female rats with chronic renal failure

As shown in Table 1, body weight tended to decline and 24 h urine volume was markedly increased in female Nx rats. The levels of BUN and creatinine in plasma (Pcr) were significantly increased, and the creatinine clearance (Ccr) was markedly decreased in female Nx rats in comparison with sham-operated controls. These parameters were consistent with our previous report using male Nx rats [11]. Unlike in the male rats, plasma level of testosterone was comparable between female sham-operated rats and Nx rats. Although an elevation in the plasma level of testosterone on the administration of testosterone was confirmed to occur both in sham-operated and in Nx rats, the plasma concentration of 17β-estradiol was not significantly changed. In addition, none of the renal functional data changed significantly after the administration of testosterone in female rats.

3.2. Pharmacokinetics of cimetidine after administration of testosterone in female rats with chronic renal failure

Table 2 shows the pharmacokinetic parameters of cimetidine in female rats with or without treatment with testosterone. The steady-state plasma concentration (Cpss) of cimetidine was markedly elevated in the Nx rats in comparison with sham-operated controls. The total plasma clearance (Ctot) of cimetidine was significantly decreased in female Nx rats compared with the controls. The renal clearance (Cren) of cimetidine in female sham-operated rats was about 60% of that in male sham-operated rats according to our previous report [11]. In female rats, the Cren of cimetidine was markedly decreased by Nx to 43% of that in the sham-operated controls. The ratio between the renal concentration and Cpss of cimetidine (Kp) was shown to be significantly decreased in Nx females compared to the sham-operated controls. Similar to the renal functional data in Table 1, there was no significant influence on the pharmacokinetic parameters of cimetidine in female rats with or without the administration of testosterone.

As shown in Fig. 1, the tubular secretory clearance (Csec) of cimetidine in female sham-operated rats was about 50% of that in the sham-operated males. In male and female Nx rats, it was markedly decreased. After the administration of testosterone, the Csec of cimetidine in male Nx rats recovered significantly to be 80% of that in male controls, but that in female Nx rats did not change at all.

3.3. Gender difference in protein expression of rOCT2, rMATE1 and NHE3

At first, a primary band with a size of 66 kDa was detected using the antibody raised against rMATE1 (Fig. 2(A)). The

Table 1 – Renal functional data and plasma testosterone and 17β-estradiol levels after the administration of testosterone in female rats

	Body weight (g)	Urine volume (mL/24 h)	Pcr (mg/dL)	Ccr (mL/min kg)	BUN (mg/dL)	Testosterone (ng/mL)	17β-Estradiol (pg/mL)
Sham T(−)	212 ± 10	18.2 ± 2.2	0.52 ± 0.08	6.0 ± 1.0	16.7 ± 0.7	0.1 ± 0.0	51.3 ± 10.1
Sham T(+)	228 ± 9	17.8 ± 2.0	0.55 ± 0.07	5.2 ± 0.8	18.1 ± 0.9	5.5 ± 0.7**	30.1 ± 14.4
Nx T(−)	199 ± 7	30.3 ± 5.4**	1.20 ± 0.12**	2.3 ± 0.3**	59.4 ± 5.6**	0.2 ± 0.0	58.6 ± 14.4
Nx T(+)	207 ± 6	31.7 ± 3.3**	1.12 ± 0.11**	2.4 ± 0.3**	58.7 ± 5.3**	5.0 ± 0.6**	38.7 ± 8.3

Values are means ± S.E. for 6–10 rats; Pcr, plasma creatinine; Ccr, creatinine clearance; BUN, blood urea nitrogen; Sham T(−), sham-operated rats administered vehicle; Sham T(+), sham-operated rats administered testosterone, Nx T(−), 5/6 nephrectomized rats administered vehicle; Nx T(+), 5/6 nephrectomized rats administered testosterone. **$P < 0.01$, significantly different from Sham T(−) rats.

Table 2 – Pharmacokinetic parameters of cimetidine in infusion experiments after the administration of testosterone in female rats

	Cpss (μmol/L)	fu	Ctot (mL/min kg)	Cren (mL/min g kid)	Kp
Sham T(−)	254 ± 12	0.74 ± 0.04	15.5 ± 0.9	1.4 ± 0.1	1.6 ± 0.0
Sham T(+)	260 ± 20	0.76 ± 0.01	13.8 ± 0.6	1.4 ± 0.2	1.6 ± 0.1
Nx T(−)	409 ± 17**	0.70 ± 0.05	9.5 ± 0.3**	0.6 ± 0.1**	0.9 ± 0.1**
Nx T(+)	405 ± 30**	0.78 ± 0.04	9.4 ± 0.7**	0.6 ± 0.2**	1.0 ± 0.1**

Each value represents the mean ± S.E. of six rats; Cpss, steady-state plasma concentration; Ctot, total clearance; Cren, renal clearance; fu, plasma unbound fraction; Kp, tissue to plasma concentration ratio. Sham T(−), sham-operated rats administered vehicle; Sham T(+), sham-operated rats administered testosterone, Nx T(−), 5/6 nephrectomized rats administered vehicle; Nx T(+), 5/6 nephrectomized rats administered testosterone. **$P < 0.01$, significantly different from Sham T(−) rats.

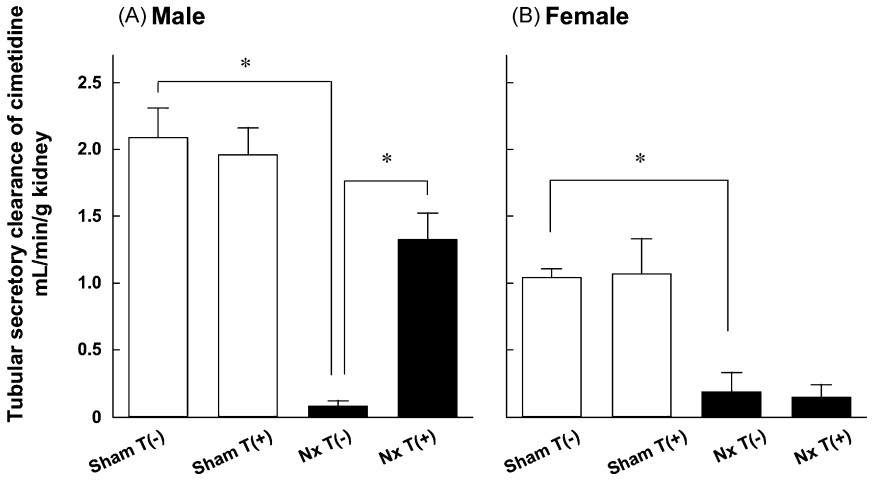

Fig. 1 – (A and B) Renal secretory clearance of unbound cimetidine. Cimetidine (21.8 μmol/mL) was infused at a rate of 2.2 mL/h using an automatic infusion pump. The renal secretory clearance was calculated by substracting GFR from Cr.f. Each column represents the mean ± S.E. for six rats. *Statistically significant difference. Sham T(−), sham-operated rats administered vehicle; Sham T(+), sham-operated rats administered testosterone enanthate; Nx T(−), 5/6 nephrectomized rats administered vehicle; Nx T(+), 5/6 nephrectomized rats administered testosterone enanthate.

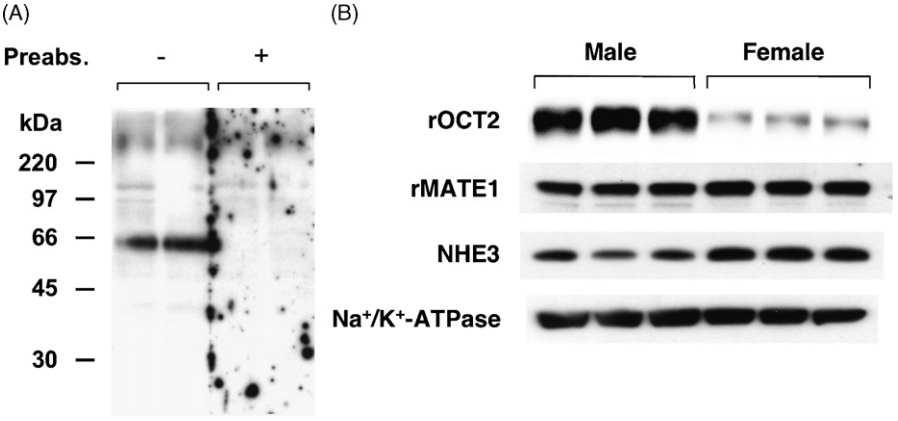

Fig. 2 – Protein expression of rOCT2, rMATE1, NHE3 and Na^+/K^+-ATPase in male and female rats. Crude plasma membrane fractions (25 μg) from total kidneys were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10%) and blotted onto Immobilon® membranes. (A) The antisera (1:1000 dilution) for rMATE1 was preabsorbed (Preab.) with (+) or without (−) antigen peptide (20 μg/mL) of rMATE1. (B) Antisera specific for rOCT2, rMATE1, NHE3 and Na^+/K^+-ATPase (1:1000–10,000 dilution) were used as primary antibodies.

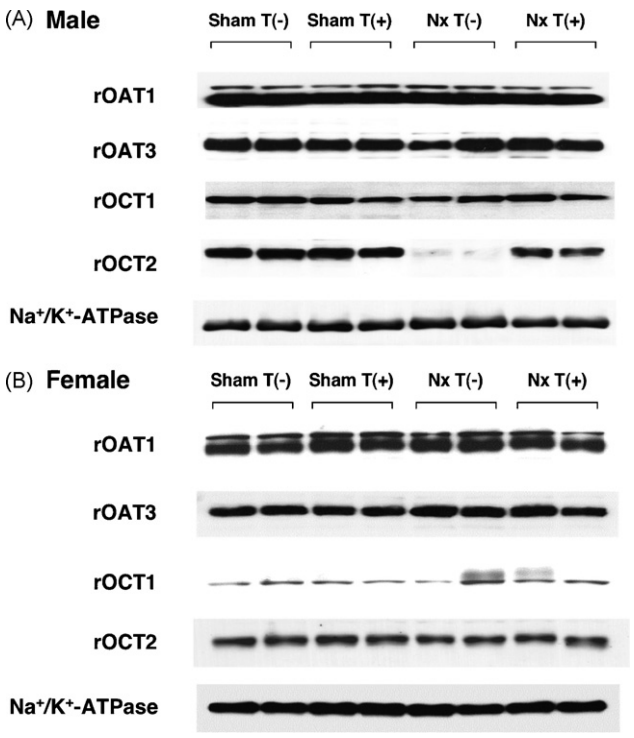

Fig. 3 – Protein expression of organic ion transporters in sham-operated and Nx rats after treatment with testosterone. Crude plasma membrane fractions (25 μg) from total kidneys were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10%) and blotted onto Immobilon® membranes. The expression levels of various transporters in male (A) and female (B) rats. Antisera specific for rOAT1, rOAT3, rOCT1, rOCT2, and Na^+/K^+-ATPase (1:1000–10,000 dilution) were used as primary antibodies. Sham T(−), sham-operated rats administered vehicle; Sham T(+), sham-operated rats administered testosterone enanthate; Nx T(−), 5/6 nephrectomized rats administered vehicle; Nx T(+), 5/6 nephrectomized rats administered testosterone enanthate.

preabsorption of antibody with antigen peptide abolished this band, showing the presence of rMATE1 protein in the rat kidney. The level of rOCT2 in female rats was about 25% of that in male rats (Fig. 2(B)). On the other hand, the expression level of rMATE1 was comparable between sexes. The level of NHE3 was slightly, but not significantly, higher in female rats compared to male rats.

3.4. *Protein expression of basolateral organic ion transporters in Nx rats*

Consistent with our previous findings [11], the protein expression of rOCT2 was markedly depressed in male Nx rats, and recovered to around the control level with the administration of testosterone (T(+)) (Fig. 3(A)). In addition, there was no influence of Nx on the administration of testosterone on the expression levels of rOAT1, rOAT3, rOCT1 and Na^+/K^+-ATPase. In female rats, the levels of the basolateral organic ion transporters, rOAT1, rOAT3, rOCT1 and rOCT2, and Na^+/K^+-ATPase were not affected by Nx with or without the administration of testosterone (Fig. 3(B)).

Although the level of rOCT2 protein correlated well with the Csec of cimetidine in the male rats, no significant correlation was observed in the female rats (Fig. 4).

3.5. *Expressional change and pharmacokinetic significance of luminal rMATE1 and NHE3 in Nx rats*

Next, we examined the expressional change of the luminal H^+/organic cation antiporter rMATE1 and Na^+/H^+ exchanger 3 (NHE3), which generates an inward H^+-gradient at the brush-border membranes. Western blot analysis revealed that the levels of rMATE1 and NHE3 were markedly decreased by Nx in both male and female rats (Fig. 5).

The coefficient of correlation between the Csec of cimetidine and the level of rMATE1 was 0.72 ($P = 0.0037$) in male rats, whereas the data was relatively scattered (Fig. 6(A)). In contrast, the Csec of cimetidine showed better correlation

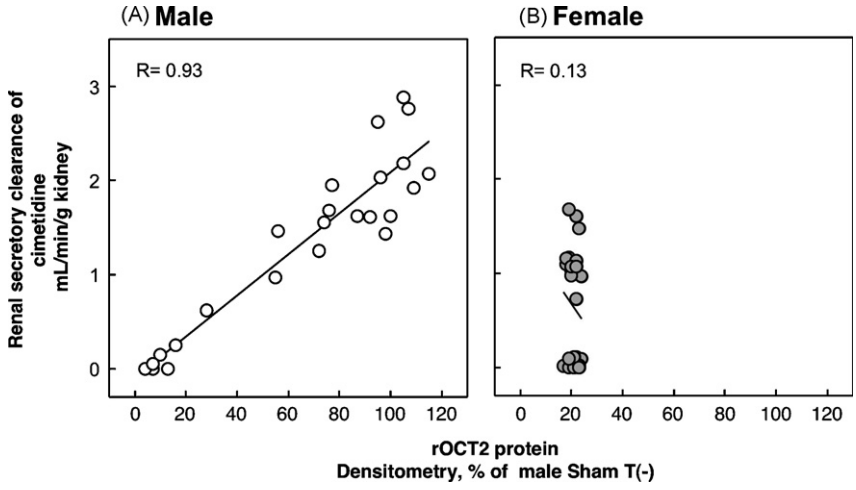

Fig. 4 – Correlation between rOCT2 expression and the renal clearance of cimetidine in rats. Correlation between the rOCT2 protein expression and renal secretory clearance of unbound cimetidine in male (A, open circles) and female rats (B, closed circles). The rOCT2 protein level was determined as outlined in Section 2. Sham T(−), sham-operated rats administered vehicle.

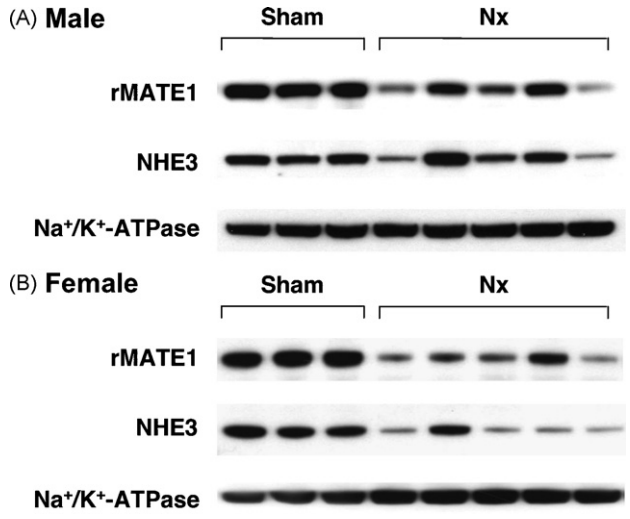

Fig. 5 – (A and B) Protein expression of rMATE1 and NHE3 in sham-operated and Nx rats. Crude plasma membrane fractions (25 μg) from total kidneys were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10%) and blotted onto Immobilon® membranes. Antisera specific for rMATE1, NHE3, and Na^+/K^+-ATPase (1:1000 dilution) were used as primary antibodies. Sham, sham-operated rats; Nx, 5/6 nephrectomized rats.

with the level of rMATE1 protein in female rats ($r = 0.74$, $P = 0.0036$) (Fig. 6(B)).

3.6. *Immunohistochemical analysis of rMATE1 and NHE3*

An immunohistochemical analysis was performed to examine the localization of rMATE1 and NHE3 in female rats. Positive staining for rMATE1 and NHE3 was detected in the brush-border membranes of proximal tubules (Fig. 7(A) and (B)). Both rMATE1 and NHE3 was abundant in the renal cortex. In addition, it seemed that the localization of rMATE1 and NHE3 was not affected by Nx (Fig. 7(C)–(F)).

4. Discussion

Functional changes in renal organic ion transporters are of clinical relevance, particularly to the use of drugs with that are highly toxic and have a narrow therapeutic index. Serious kidney disease, such as chronic renal failure, will influence the renal disposition of organic ions and the expression of drug transporters. Our previous study demonstrated that the mRNA expression levels of OAT-K1 and OAT-K2 were markedly diminished after Nx, and the renal clearance of methotrexate was markedly decreased in Nx rats compared with sham-operated rats [16]. Furthermore, the renal secretion of cimetidine and the level of basolateral rOCT2 was also decreased under chronic renal failure in male rats, and the reduced plasma level of testosterone was considered to cause these phenomena [11]. Although two steps of transmembrane transport were thought to be involved in the vectorial secretion of cationic drugs, there has been no report about the luminal MATE1 in renal disease state. Sex-hormonal levels differ markedly between the genders, and recently transporters such as rOCT2, rOAT2 and oatp1 have been reported to exhibit gender differences in their expression in the rat kidney [9,17,18]. Based on these findings, we hypothesized that female rats would show significantly different changes in the urinary excretion of cimetidine and the expression of organic ion transporters during chronic renal failure, avoiding the influence of testosterone. In the present study, to obtain more information about the pharmacokinetic significance of cationic drug excretion systems focusing on luminal transporter, rMATE1, we have examined the renal excretion of the cationic substrate cimetidine and the expression of organic ion transporters in male and female Nx rats.

Consistent with previous findings, the decreased expression level of the renal rOCT2 and the Csec of cimetidine were recovered by the administration of testosterone in the male Nx

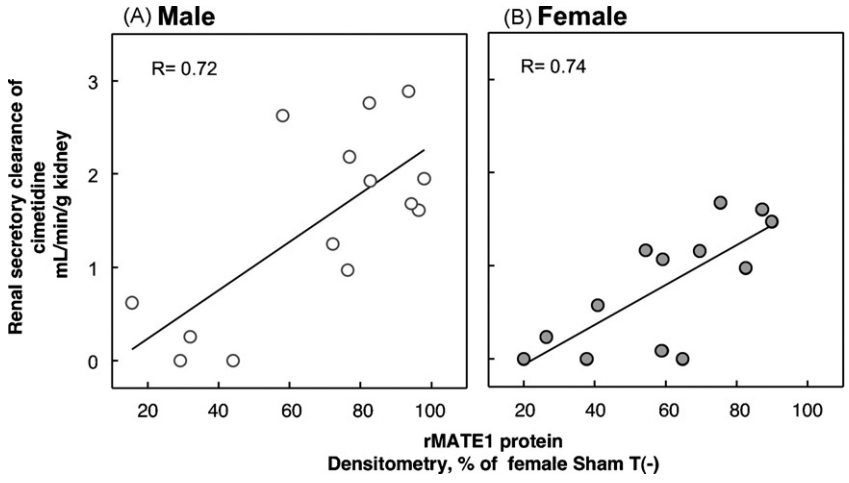

Fig. 6 – Correlation between rMATE1 expression and the renal clearance of cimetidine in rats. Correlation between the rMATE1 protein expression and renal secretory clearance of unbound cimetidine in male (A, open circles) and female rats (B, closed circles). The rMATE1 protein level was determined as outlined in Section 2. Sham T(−), sham-operated rats administered vehicle.

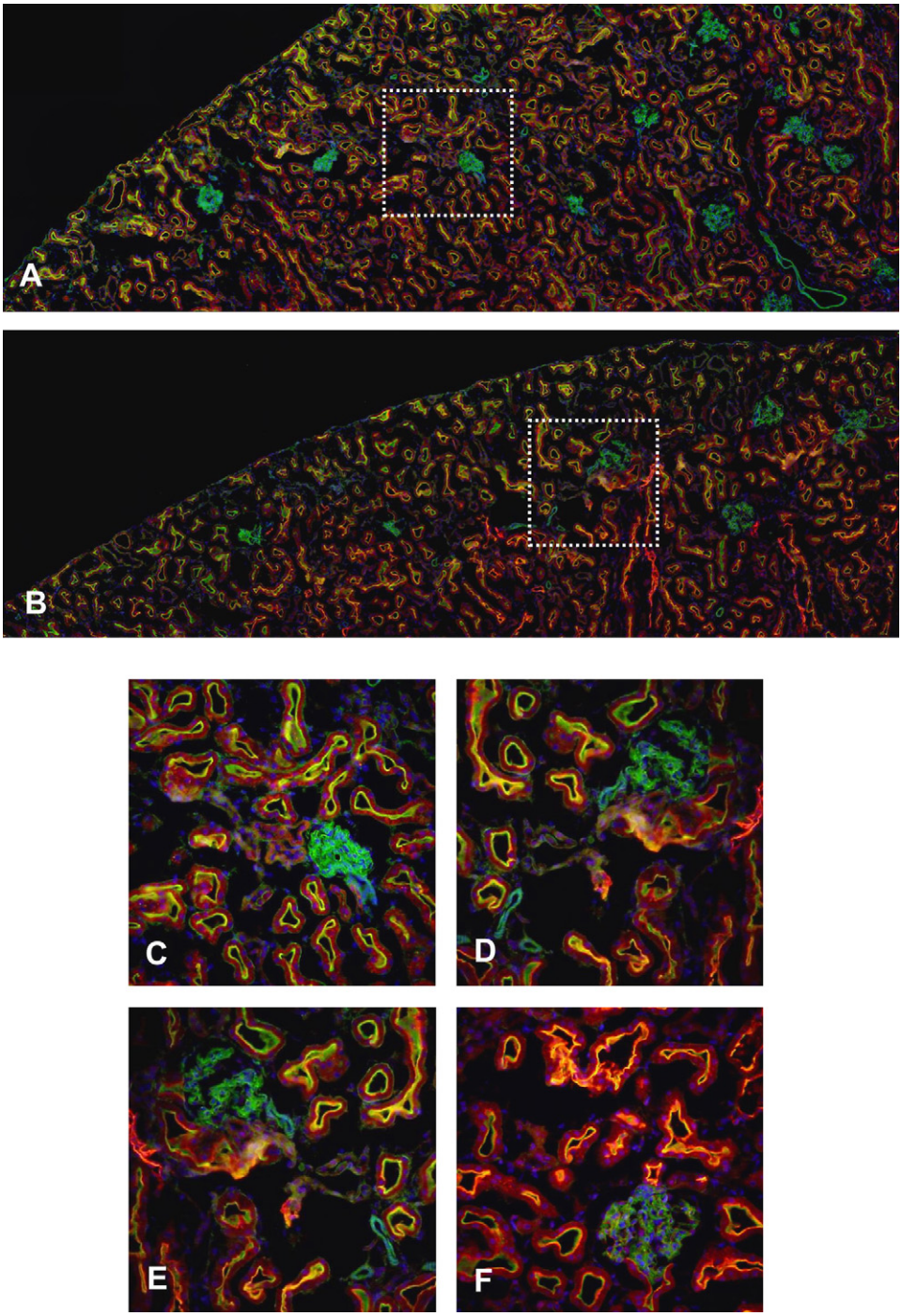

Fig. 7 – Immunohistochemistry of rMATE1 and NHE3 in female sham-operated and Nx rats. The rMATE1 or NHE3 (red), F-actin (green), and DAPI (blue) signals were merged in the same section. The yellow signals consisting of rMATE1 or NHE3 and F-actin were concentrated in the brush-border membranes of proximal tubules. The localization of rMATE1 (A) and NHE3 (B) in sham-operated rats were relatively condensed around the cortex. The luminal localization of rMATE1 (C and D) and NHE3 (E and F) in sham-operated (C and E) and in Nx (D and F) rats were confirmed. Magnification: 150×.

rats (Figs. 1(A) and 3(A)). In the female rats, the renal Csec of cimetidine was about 50% of that in the male rats (Fig. 1(B)). The expression levels of basolateral transporters were not affected by Nx and testosterone. In addition, no correlation was observed between the renal level of rOCT2 and the Csec of cimetidine (Fig. 4(B)). In infusion experiments, tissue to plasma concentration ratio (Kp) was significantly decreased in Nx rats. In Nx rats, the level of the tubular sodium transporters were markedly decreased and the plasma levels of sodium and potassium were significantly changed [19]. This suggested that the difference in the membrane potential at the basolateral side of renal epithelial cells were also changed and

the uptake of cationic drugs in epithelial cells was reduced, though the expressional change of rOCT2 was not observed. Considering these results, only the level of basolateral rOCT2 in the kidney could not explain the entire vectorial secretion of the cationic drugs.

Recently, the luminal H^+/organic cation antiporters, human (h) MATE1, hMATE2-K, rMATE1 and mouse MATE1 have been cloned and characterized [6–8,20]. rMATE1 was mainly expressed in the kidney and placenta, and suggested to be a major contributor to the tubular H^+/organic cation antiport activity in rats [7]. In contrast, hMATE2-K was isolated as a second member of the MATE family in the human kidney, despite no counterpart gene having been identified in rats [8]. Most recently, a platinum agent oxaliplatin was found to be a superior substrate for hMATE2-K rather than hMATE1, and therefore, the extensive tubular secretion of oxaliplatin via hMATE2-K were suggested to be a mechanism behind the lowered renal toxicity of the drug [21].

In the female as well as male rats, the level of rMATE1 was markedly depressed by the Nx (Fig. 5). In contrast to the level of basolateral rOCT2, the level of rMATE1 correlated well with the Csec of cimetidine in the female rats. Considering that there was no significant alteration to the expression of the basolateral transporters (rOAT1, rOAT3, rOCT1 and rOCT2) in the female rats following Nx, the luminal rMATE1 should play a crucial role in the tubular secretion of cimetidine in the female rats.

In the rat renal tubular brush-border membranes, the luminal secretion of cimetidine as well as tetraethyl ammonium was mediated by an electroneutral H^+/organic cation antiport system driven by an inward H^+-gradient, and that H^+-gradient was mainly created by NHE [22–24]. At the brush-border membranes of proximal tubules, the type 3 NHE are considered dominant among the NHE family [25,26]. In the present study, we also examined the expression level and membrane localization of NHE3 as well as rMATE1 in the female rats. The immunohistochemical analysis using antibodies specific for rMATE1 or NHE3 strongly suggested that both transporters cooperatively function on the luminal side of the proximal tubules, especially in the superficial cortex (Fig. 7(A) and (B)), i.e., rMATE1-mediated tubular secretion of cationic compounds could be driven by the NHE3-created inward H^+-gradient.

The expression of NHE3 was depressed after Nx in both the male and female rats (Fig. 5). Kwon et al. [19] showed that the level of the tubular sodium transporters including NHE3 were significantly decreased in Nx rats. Furthermore, they demonstrated that the localization of NHE3 was also found at the brush-border membranes in the Nx rats, which was comparable with our present results. Therefore, the decreased renal tubular secretion of cimetidine was not only due to the decreased in the expression of rMATE1, but also the functional loss of this transporter via a lowered H^+-gradient at the brush-border membrane, caused by the decrease in NHE3.

In conclusion, we have advanced our findings on the renal handling of cationic drugs, demonstrating that the level of luminal rMATE1 was markedly decreased during chronic renal failure, and rMATE1 as well as the basolateral rOCT2 played a crucial role in the vectorial transport of organic cations as detoxicating factors. In addition, the expression of NHE3 was also depressed in Nx rats, and the localization of NHE3 was similar to that of rMATE1, suggesting the expression level of NHE3 might affect rMATE1 activity by creating an inward H^+-gradient.

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