Reduced renal clearance of a zwitterionic substrate cephalexin in Mate1-deficient mice

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Running title: Tubular secretion of cephalexin by MATE1

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The number of text pages: 27
The number of tables: 1
The number of figures: 6
The number of references: 24
The number of words in the Abstract: 208
The number of words in the Introduction: 432
The number of words in the Discussion: 862

Abbreviations: MATE, multidrug and toxin extrusion; SLC, solute carrier; OAT, organic anion transporter; MRP, multidrug resistance-associated protein; ABC, ATP-binding cassette; TEA, tetraethylammonium; MPP, 1-methyl-4-phenylpyridinium; HEK, human embryonic kidney; HPLC, high-performance liquid chromatography; Km,
Michaelis-Menten constant; Vmax, maximum velocity; Ki, inhibition constant; OCT, organic cation transporter

Recommended section: Metabolism, Transport, and Pharmacogenomics
Abstract

Multidrug and toxin extrusion 1 (MATE1/SLC47A1) mediates the transport of not only organic cations but also zwitterions such as cephalexin. However, the contribution of MATE1 to tubular secretion of cephalexin in vivo has not been elucidated. In the present study, we carried out transport experiments of cephalexin via MATE1 and performed pharmacokinetic analyses of cephalexin in Mate1 knockout (Mate1−/−) mice. Cephalexin uptake by human MATE1-expressing HEK293 cells exhibited saturable kinetics (Km = 5.9 ± 0.5 mM) and a bell-shaped pH profile with a maximum at pH 7.0. We confirmed that mouse MATE1 also transported cephalexin. After a single intravenous administration of cephalexin (5 mg/kg), Mate1−/− mice showed higher plasma concentrations of cephalexin than wild-type (Mate1+/+) mice. The urinary excretion of cephalexin for 60 min was significantly reduced, and the renal concentration was markedly increased in Mate1−/− mice compared with Mate1+/+ mice. The renal clearance of cephalexin in Mate1−/− mice was approximately 60% of that in Mate1+/+ mice and appeared to be near the creatinine clearance. In contrast, there were no significant differences between both mice in the pharmacokinetics of anionic cefazolin, which is not a substrate for MATE1. In this study, we demonstrated that MATE1 is responsible for renal tubular secretion of a zwitterionic substrate cephalexin in vivo.
Introduction

At the brush-border membranes of proximal tubular epithelial cells, H+/organic cation antiporter mediates the transport of many organic cations and cationic drugs (Inui et al., 2000). Multidrug and toxin extrusion 1 (MATE1/SLC47A1) and MATE2-K (SLC47A2) have been accepted as molecular entities of H+/organic cation antiport system based on their tissue distribution, membrane localization and functional characteristics (Terada and Inui, 2008). MATEs transport various organic cations such as tetraethylammonium (TEA), 1-methyl-4-phenylpyridinium (MPP), cimetidine, metformin and procainamide (Tanihara et al., 2007). Moreover, we demonstrated that MATE1 plays an essential role in tubular secretion of metformin by pharmacokinetic analysis with Mate1 knockout (Mate1−/−) mice (Tsuda et al., 2009a). Thus, MATE1 has been recognized as important in tubular secretion of cationic drugs.

Many cephalosporin antibiotics are secreted into the proximal tubules via active transport systems (Nightingale et al., 1975; Bergan, 1987). Most cephalosporins exist as anions at the physiological pH and their tubular secretion is mediated mainly by organic anion transport systems, which consist of organic anion transporters (OATs) at the basolateral membranes and multidrug resistance-associated proteins (MRPs) at the brush-border membranes. For example, cefazolin, a representative anionic cephalosporin, was demonstrated to be transported by OAT3 (SLC22A8) (Ueo et al., 2005) and MRP4 (ABCC4) (Ci
Aminocephalosporins such as cephalexin, however, exist as zwitterions at the physiological pH. Cephalexin was shown to be transported by OAT1 (SLC22A6) and OAT3 as well as other anionic cepharosporins (Uwai et al., 2002; Zhang et al., 2010), but not by MRP2 (ABCC2) and MRP4 (Ci et al., 2007; Kato et al., 2008). Therefore, the transport mechanisms for the zwitterionic cephalosporin cephalexin in the brush-border membranes have not been fully elucidated.

Previously, we found that cephalexin and another aminocephalosporin cephradine were transported via H⁺/organic cation antiporter in uptake experiments with rat renal brush-border membrane vesicles (Inui et al., 1985). As well as H⁺/organic cation antiporter, MATE1 can also transport cephalexin and cephradine (Tanihara et al., 2007). Therefore, MATE1 is predicted to be a candidate transporter responsible for the efflux of cephalexin and cephradine in the proximal tubules. Furthermore, recent studies showed that zwitterionic drugs such as fexofenadine and fluoroquinolones were transported by MATE1 (Matsushima et al., 2009; Ohta et al., 2009). These in vitro findings suggested that MATE1 contributes to tubular secretion of not only cationic drugs but also zwitterionic drugs.

Therefore, in the present study, to elucidate the involvement of MATE1 in tubular secretion of zwitterions in vivo, pharmacokinetic analyses of cephalexin using Mate1⁻/⁻ mice were carried out. In addition, we investigated the effect of
*Matel* deficiency on the pharmacokinetics of cefazolin, which is not transported by MATE1.
Methods

Materials. Cephalexin (Shionogi & Co., Ltd., Osaka, Japan) and cefazolin (Astellas Pharma Inc., Tokyo, Japan) were kindly provided by the respective suppliers. All other chemicals used were of the highest purity available.

Isolation of mouse MATE1 cDNA. The mouse (m) MATE1 cDNA was cloned by RT-PCR from Mouse Kidney Marathon-Ready cDNA (Clontech Laboratories, Inc., Mountain View, CA). Primers specific for mMATE1 were designed on the basis of the sequence information of the NCBI reference sequence NM_026183. The mMATE1 cDNA was cloned using the following primers: forward 5’-GGGGTACCCCACGGAGGCCACATGGAAC-3’ and reverse 5’-CGCTCGAGTCCACTCCAGAGCATCTCCT-3’. The PCR product was subcloned into pFLAG-CMV-6 expression vector (Sigma-Aldrich Co., St. Louis, MO) and sequenced using a multicapillary DNA sequencer RISA384 system (Shimadzu Co., Kyoto, Japan).

Cell culture, transfection and uptake experiments. HEK293 cells stably expressing human MATE1 (HEK-hMATE1 cells) and mock cells (HEK-pcDNA cells) were cultured according to our previous report (Tanihara et al., 2007). pFLAG plasmid vector DNA containing mMATE1 cDNA was transfected into HEK293 cells using LipofectAMINE 2000 Reagent (Invitrogen,
Carlsbad, CA) as described previously (Urakami et al., 2002; Terada et al., 2006). At 48 h after the transfection, the cells were used for uptake experiments. The uptake experiments of cephalexin were carried out as described previously (Ueo et al., 2005; Tanihara et al., 2007).

**Animals.** Animal experiments were conducted in accordance with the *Guidelines for Animal Experiments of Kyoto University*. All protocols were approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University. Male *Mate1*+/+ and *Mate1*−/− mice (13-18 weeks of age, C57BL/6 genetic background) were used in the present study.

**Pharmacokinetic experiments.** Pharmacokinetic experiments were carried out according to our previous report (Tsuda et al., 2009a) with a slight modification. Briefly, after a catheter was inserted into the right jugular vein, 5 mg/kg cephalexin and 146 mg/kg mannitol were administered as a bolus injection. At indicated times, plasma and urine were collected and analyzed. At the end of experiments, the kidney and liver were removed, and excised tissues were gently washed, weighed and homogenized. In the case of cefazolin, the same experimental procedures were applied. For the determination of cephalexin and cefazolin in renal and hepatic tissues, homogenates (100 μl) were loaded onto an Oasis HLB cartridge (Waters Corporation, Milford, MA) preconditioned with 1 ml each of methanol and water. The column was washed with 1 ml of water, and
Cephalexin and cefazolin were eluted from the column with 1 ml of methanol. The eluate was evaporated to dry at 45-50°C and resuspended in 200 µl of each mobile phase buffer. The solutions were filtered through a Millipore filter (SGJVL, 0.45 µm) and analyzed. The concentrations of drugs in plasma, urine, the renal homogenate and the hepatic homogenate were determined by high-performance liquid chromatography (HPLC). The levels of creatinine in plasma and urine at 60 min were determined with the Jaffé reaction using an assay kit from Wako Pure Chemical Industries (Osaka, Japan).

**Determination of pharmacokinetic parameters.** A conventional two-compartmental analysis was used to investigate the plasma concentration-time profiles of cephalexin and cefazolin after the intravenous administration in mice using WinNonlin version 5.2.1 (Pharsight Corporation, Mountain View, CA). Pharmacokinetic parameters, the area under the blood concentration-time curve from time zero to infinity (AUC$_\infty$), total body clearance (CL$_{tot}$), central volume of distribution (V$_1$), intercompartmental clearance (Q) and volume of distribution at steady-state (Vd$_{ss}$), were calculated by the nonlinear least-squares method. The AUC until 60 min (AUC$_{0-60}$) was determined by the trapezoidal rule. Renal clearance (CL$_{ren}$) of cephalexin and cefazolin was obtained by dividing the amounts of each drug eliminated into urine during 60 min by the AUC$_{0-60}$. The nonrenal clearance (CL$_{nr}$) of each drug was calculated by subtracting CL$_{ren}$ from CL$_{tot}$. The kidney-to-plasma concentration ratio (Kp,
kidney) and liver-to-plasma concentration ratio ($K_p$, liver) were calculated by dividing the tissue concentration by plasma concentration at 60 min of each drug.

**Analytical methods.** Cephalexin and cefazolin levels were analyzed with a high-performance liquid chromatograph (LC-10AT, Shimadzu Co.) equipped with a UV spectrophotometric detector (SPD-10AV, Shimadzu Co.) and an integrator (Chromatopac C-R8A, Shimadzu Co.). The level of cephalexin in uptake experiments was determined according to a previous report (Inui et al., 1983). The conditions for measurements of cephalexin and cefazolin in the pharmacokinetic experiments were as follows: column, Zorbax ODS 4.6 mm inside diameter × 250 mm (Agilent Technologies, Inc., Santa Clare, CA) for cephalexin and cefazolin; mobile phase, 30 mM phosphate buffer (pH 3.0) containing 1 mM SDS in methanol at 57:43 for cephalexin, 30 mM phosphate buffer (pH 5.0) in methanol at 83:17 for cefazolin; flow rate, 1.0 ml/min; wavelength, 262 nm for cephalexin, 270 nm for cefazolin; injection volume, 50 μl for plasma and liver samples, 20 μl for urine and kidney samples; column temperature, 40°C.

**Statistical analysis.** All data were expressed as the mean ± S.D. Data from pharmacokinetic analyses were analyzed statistically using the unpaired $t$-test.
Results

The ionic species of cephalexin and cefazolin. To investigate the ionic species of cephalexin and cefazolin at the physiological pH, the percentages were calculated by using each pKa value according to the Henderson-Hasselbalch equation. Cephalexin has a carboxyl group (pKa₁ = 3.1) and an amino group (pKa₂ = 6.8) (Fig. 1A) (Mrestani et al., 1998). As shown in Fig. 1C, 20% and 80% of cephalexin existed as zwitterionic and anionic species, respectively, at pH 7.4. As cefazolin only has a carboxyl group (pKa = 2.1) (Fig. 1B) (Foye et al., 1995), however, it is an anion at pH 7.4 (Fig. 1D).

Uptake experiments by HEK-hMATE1 cells. The transport characteristics of cephalexin via hMATE1 have not been fully elucidated. Therefore, we carried out uptake experiments by HEK-hMATE1 cells. Cephalexin uptake by HEK-hMATE1 cells exhibited saturable kinetics (Fig. 2A), and an apparent Michaelis-Menten constant (Km) of 5.9 ± 0.5 mM and maximum velocity (Vmax) of 12.6 ± 1.1 nmol/mg protein/min were calculated from three separate experiments. When the extracellular pH was changed from 6.0 to 8.5, a bell-shaped pH profile of cephalexin uptake via hMATE1 was observed, and the uptake was greatest at pH 7.0 and lowest at pH 6.0 (Fig. 2B).

Uptake experiments by HEK293 cells transiently expressing mMATE1. We then examined whether cephalexin is transported by
mMATE1 as well as hMATE1. As shown in Fig. 3, cephalexin was markedly transported by mMATE1.

Pharmacokinetics of cephalexin and cefazolin in \textit{Mate1}^{+/+} and \textit{Mate1}^{-/-} mice. We compared pharmacokinetic profiles of cephalexin and cefazolin in \textit{Mate1}^{+/+} and \textit{Mate1}^{-/-} mice. In the case of cephalexin, the plasma concentration was significantly elevated in \textit{Mate1}^{-/-} mice compared with \textit{Mate1}^{+/+} mice (Fig. 4A). The urinary excretion of cephalexin for 60 min after the intravenous administration was significantly reduced in \textit{Mate1}^{-/-} mice (Fig. 5A). The renal concentration was markedly increased in \textit{Mate1}^{-/-} mice, but the hepatic concentration did not differ significantly between \textit{Mate1}^{+/+} and \textit{Mate1}^{-/-} mice (Figs. 6A and 6B). The pharmacokinetic parameters of cephalexin and cefazolin are summarized in Table 1. The CL\textsubscript{tot} of cephalexin was significantly decreased in \textit{Mate1}^{-/-} mice compared with \textit{Mate1}^{+/+} mice. The CL\textsubscript{ren} of cephalexin in \textit{Mate1}^{-/-} mice was approximately 60\% of that in \textit{Mate1}^{+/+} mice, whereas the CL\textsubscript{nr} was not significantly changed. The K\textsubscript{p, kidney} was 4.5-fold higher in \textit{Mate1}^{-/-} mice than in \textit{Mate1}^{+/+} mice. The V\textsubscript{1} value of cephalexin was significantly decreased in \textit{Mate1}^{-/-} mice compared with \textit{Mate1}^{+/+} mice. In contrast, there were no significant differences in the plasma concentration (Fig. 4B), urinary excretion (Fig. 5B), renal concentration (Fig. 6C) and hepatic concentration (Fig. 6D) of cefazolin between \textit{Mate1}^{+/+} and \textit{Mate1}^{-/-} mice.
Furthermore, the pharmacokinetic parameters of cefazolin showed no significant changes between $Mate1^{+/+}$ and $Mate1^{-/-}$ mice (Table 1).
**Discussion**

MATE1 is an important transporter involved in tubular secretion of cationic drugs. In addition, our previous studies using renal brush-border membrane vesicles and heterologous expression systems of MATE1 demonstrated that cephalexin, a zwitterionic drug, was transported by H⁺/organic cation antiporter (Inui et al., 1985; Terada et al., 2006; Tanihara et al., 2007), suggesting the involvement of MATE1 in tubular secretion of cephalexin.

In the present pharmacokinetic study, we revealed that urinary excretion of cephalexin for 60 min after the intravenous administration and were significantly decreased in *Mate1⁻/⁻* mice compared with *Mate1⁺/+* mice (Fig. 5A). The renal concentration of cephalexin and *Kp, kidney* value were markedly elevated in *Mate1⁻/⁻* mice (Fig. 6A and Table 1). The *V₁* value of cephalexin was significantly decreased in *Mate1⁻/⁻* mice compared with *Mate1⁺/+* mice (Table 1). Furthermore, the *CL₉* of cephalexin in *Mate1⁻/⁻* mice was also significantly decreased and appeared to be near the creatinine clearance (Ccr) (Table 1). These results are consistent with those of our previous *in vitro* transport studies. This is the first demonstration that MATE1 is responsible for renal tubular secretion of cephalexin *in vivo*.

Previously, it was reported that renal elimination of cephalexin was significantly inhibited by the coadministration of cimetidine, a cationic drug, in healthy subjects (van Crugten et al., 1986). This report suggested that organic cation transport systems were involved in tubular secretion of cephalexin in
humans. We recently demonstrated that cimetidine at the clinical plasma concentration inhibited apical hMATE1 more strongly than human organic cation transporter 2 (hOCT2/SLC22A2) (Tsuda et al., 2009b). Therefore, it is likely that MATE1 is responsible for the drug interaction between cimetidine and cephalexin in tubular secretion. Furthermore, fexofenadine and fluoroquinolones such as levofloxacin, which are transported by MATE1, caused the drug interaction with cimetidine in healthy volunteers (Fish and Chow, 1997; Yasui-Furukori et al., 2005). These studies suggested that MATE1 is also important in tubular secretion of zwitterionic drugs in humans.

In this study, there were no significant differences in the pharmacokinetic profiles of cefazolin between Mate1+/+ and Mate1−/− mice (Figs. 4B, 5B, 6C, 6D and Table 1). Hence, these results indicated that MATE1 does not contribute to tubular secretion of cefazolin in vivo. Previous study showed that MRP4 is involved in tubular secretion of cefazolin in the brush-border membranes (Ci et al., 2007). On the other hand, we demonstrated in this pharmacokinetic analysis that MATE1 plays a key role in tubular secretion of cephalexin. Thus, there is a distinct difference in the efflux transporter between cephalexin and cefazolin, although both drugs are transported by OATs at the basolateral membranes. This may be attributed to the difference in charge states of each drug.

It was reported that the uptake of TEA, a typical organic cation, by hMATE1 was increased when the extracellular pH was changed from 6.0 to 8.5
under the intracellular acidified conditions (Tanihara et al., 2007). Since the intracellular pH of HEK293 cells is temporarily reduced to 6.0 - 6.5 by pretreatment with NH₄Cl (Lang et al., 2003), it was considered that TEA uptake from the extracellular pH 6.0 to 8.5 was activated by an increase in the oppositely directed H⁺ gradient. On the other hand, cephalexin uptake via hMATE1 showed a bell-shaped curve with a maximum at pH 7.0 (Fig. 2B). The uptake of cephalexin as well as organic cations was increased from pH 6.0 to 7.0, because the oppositely directed H⁺ gradient was increased. However, it was markedly decreased from pH 7.0 to 8.5 even though the oppositely directed H⁺ gradient was increased. As shown in Fig. 1C, when the extracellular pH was changed from 7.0 to 8.5, the anionic and zwitterionic forms of cephalexin were increased and decreased, respectively. Furthermore, a previous report showed that a zwitterionic fluoroquinolone, norfloxacin, was transported by rat MATE1, and that the pH profile was correlated with the percentage of the zwitterionic form of norfloxacin (Ohta et al., 2009). These findings suggested that MATE1 prefers the zwitterionic form of drugs, rather than the anionic form.

Cephalexin uptake via hMATE1 exhibited saturable kinetics, and the apparent Km value was calculated to be 5.9 ± 0.5 mM (Fig. 2A). This Km value showed greater than the Km or inhibition constant (Ki) for hMATE1 of various organic cations, for example TEA (Km = 0.38 mM), MPP (Km = 0.10 mM) and metformin (Km = 0.78 mM, Ki = 0.67 mM) (Tanihara et al., 2007; Tsuda et al., 2009b). These results suggest some differences in the interaction
with hMATE1 between organic cations and cephalexin. One possibility is that a negatively charged carboxyl group of cephalexin interferes with the interaction between cephalexin and hMATE1. Alternatively, cephalexin has a charged free amino group, whereas other typical cationic substrates of hMATE1 have a charged nitrogen atom constituting a secondary or tertiary amine. It is likely that structural features of the cationic moiety also contribute to substrate affinity for hMATE1.

In conclusion, we demonstrated for the first time that MATE1 plays an important role in the renal clearance of cephalexin in vivo. It is possible that MATE1 is involved in tubular secretion of zwitterionic substrates as well as organic cations, because MATE1 was shown to mediate the transport of other zwitterionic drugs.
References


Footnotes

This work was supported in part by the Ministry of Education, Culture, Sports, Science and Technology of Japan [Grant-in-Aid for Scientific Research]. Masahiro Tsuda is a Research Fellow of the Japan Society for the Promotion of Science.
Legends for Figures

Fig. 1. A and B, structure of cephalexin (A) and cefazolin (B). C and D, the percentages of the ionic species of cephalexin (C) and cefazolin (D) as a function of pH. The percentages of cationic, zwitterionic and anionic species of cephalexin and cefazolin were calculated by using each pKa value according to the Henderson-Hasselbalch equation.

Fig. 2. Transport of cephalexin by HEK-hMATE1 cells. A, concentration-dependence of cephalexin uptake by HEK-hMATE1 cells. HEK-hMATE1 cells were preincubated with 30 mM NH₄Cl (pH 7.4) for 20 min. Then, the preincubation medium was removed, and the cells were incubated with various concentrations of cephalexin (pH 7.4) in the absence (●) or presence (○) of 10 mM MPP for 1 min at 37°C. Each point represents the mean ± S.D. for three monolayers. This figure is representative of three separate experiments. B, effect of the extracellular pH on cephalexin uptake by HEK-hMATE1 and HEK-pcDNA cells. HEK-hMATE1 cells (●) and HEK-pcDNA cells (○) were preincubated with 30 mM NH₄Cl (pH 7.4) for 20 min. Then, the preincubation medium was removed, and the cells were incubated with 1 mM of cephalexin (indicated pH) for 1 min at 37°C. Each point represents the mean ± S.D. for three monolayers.
Fig. 3.  Uptake of cephalexin by HEK293 cells transiently expressing mMATE1.  The cells were preincubated with 30 mM NH₄Cl (pH 7.4) for 20 min.  Then, the preincubation medium was removed, and the cells were incubated with 1 mM of cephalexin (pH 7.4) for 30 sec at 37°C.  Each column represents the mean ± S.D. for three monolayers.

Fig. 4.  Plasma concentration profiles of cephalexin (A) and cefazolin (B) in Mate1+/+ (○) and Mate1−/− (●) mice.  Cephalexin at 5 mg/kg and mannitol at 146 mg/kg were administered as a bolus injection via the jugular vein.  Then, 1% mannitol was administered to maintain a sufficient and constant urine flow rate by continuous infusion at 0.35 ml/h using an automatic infusion pump.  Thereafter, blood samples were collected at the time points indicated.  In the case of cefazolin, the same experimental procedures were applied.  The plasma concentrations of cephalexin and cefazolin were determined by HPLC.  Each point represents the mean ± S.D. for five or six mice of each genotype.  *P < 0.05, **P < 0.01, significantly different from Mate1+/+ mice.

Fig. 5.  Urinary excretion of cephalexin (A) and cefazolin (B) in Mate1+/+ (open column) and Mate1−/− (closed column) mice.  Urine was collected for 60 min after the drug administration.  Cephalexin and cefazolin levels in the urine
samples were determined by HPLC. Each column represents the mean ± S.D. for five or six mice. **P < 0.01, significantly different from Mate1+/+ mice.

Fig. 6. Tissue distribution of cephalexin (A, B) and cefazolin (C, D) in Mate1+/+ (open column) and Mate1-/− (closed column) mice. The kidney (A, C) and liver (B, D) were removed to determine the tissue concentration of cephalexin or cefazolin at 60 min after the drug administration. Cephalexin and cefazolin levels in the tissue samples were determined by HPLC. Each column represents the mean ± S.D. for five or six mice. ***P < 0.001, significantly different from Mate1+/+ mice.
**Table 1.** Pharmacokinetic parameters of cephalexin and cefazolin, and creatinine clearance (Ccr) in *Mate1*+/+ and *Mate1*−/− mice.

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<th>parameters</th>
<th>Cephalexin</th>
<th>Cefazolin</th>
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<tr>
<td>AUC&lt;sub&gt;0-60&lt;/sub&gt; (μg·min/ml)</td>
<td>351 ± 74</td>
<td>429 ± 97</td>
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<td>AUC&lt;sub&gt;∞&lt;/sub&gt; (μg·min/ml)</td>
<td>414 ± 106</td>
<td>621 ± 160&lt;sup&gt;∗&lt;/sup&gt;</td>
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<td>CL&lt;sub&gt;tot&lt;/sub&gt; (ml/min/kg)</td>
<td>12.8 ± 3.1</td>
<td>8.7 ± 2.9&lt;sup&gt;∗&lt;/sup&gt;</td>
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<td>CL&lt;sub&gt;ren&lt;/sub&gt; (ml/min/kg)</td>
<td>11.0 ± 3.0</td>
<td>6.5 ± 2.8&lt;sup&gt;∗&lt;/sup&gt;</td>
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<tr>
<td>CL&lt;sub&gt;nr&lt;/sub&gt; (ml/min/kg)</td>
<td>1.8 ± 1.9</td>
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<td>K&lt;sub&gt;p, kidney&lt;/sub&gt;</td>
<td>3.1 ± 0.5</td>
<td>13.8 ± 4.0&lt;sup&gt;∗∗∗&lt;/sup&gt;</td>
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<tr>
<td>K&lt;sub&gt;p, liver&lt;/sub&gt;</td>
<td>1.5 ± 0.2</td>
<td>1.7 ± 0.8</td>
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<td>Q (ml/min/kg)</td>
<td>28.0 ± 11.3</td>
<td>21.6 ± 3.2</td>
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<td>V&lt;sub&gt;1&lt;/sub&gt; (ml/kg)</td>
<td>183 ± 15</td>
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<td>V&lt;sub&gt;dss&lt;/sub&gt; (ml/kg)</td>
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<td>Ccr (ml/min/kg)</td>
<td>5.8 ± 1.5</td>
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AUC<sub>0-60</sub>, area under the blood concentration-time curve until 60 min; AUC<sub>∞</sub>, AUC from time 0 to infinity; CL<sub>tot</sub>, total body clearance; CL<sub>ren</sub>, renal clearance; CL<sub>nr</sub>, nonrenal clearance; K<sub>p, kidney</sub>, kidney-to-plasma concentration ratio; K<sub>p, liver</sub>, liver-to-plasma concentration ratio; Q, intercompartmental clearance; V<sub>1</sub>, central volume of distribution; V<sub>dss</sub>, volume of distribution at steady state. The CL<sub>ren</sub> of cephalexin and cefazolin was obtained by dividing the amounts of each drug eliminated into urine during 60 min by the AUC<sub>0-60</sub>. The CL<sub>nr</sub> of cephalexin...
and cefazolin was calculated by subtracting CL_{ren} from CL_{tot}. Each value represents the mean ± S.D. for five or six mice. *P < 0.05, **P < 0.01, ***P < 0.001 significantly different from Mate1^{+/+} mice.
Fig. 2

A

B

UPTAKE (nmol/mg protein/min)

CONCENTRATION (mM)

UPTAKE (nmol/mg protein/min)

pH

0 2 4 6 8 10

0 1 2 3 4

2.5 5.0 7.5 10

6.0 6.5 7.0 7.5 8.0 8.5
Fig. 3

![Graph showing uptake comparison between pFLAG and mMATE1](image-url)
Fig. 5

**A**

Excretion in Urine (% of Dose)

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**B**

Excretion in Urine (% of Dose)

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