Evaluation of the Role of Breast Cancer Resistance Protein (BCRP/ABCG2) and Multidrug Resistance-Associated Protein 4 (MRP4/ABCC4) in The Urinary Excretion of Sulfate and Glucuronide Metabolites of Edaravone (MCI-186; 3-Methyl-1-phenyl-2-pyrazolin-5-one)

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ABSTRACT:

Edaravone (MCI-186; 3-methyl-1-phenyl-2-pyrazolin-5-one), a free radical scavenger, is used for the treatment of acute cerebral infarction. Edaravone is mainly excreted into the urine after conjugation to glucuronide or sulfate. Previous studies have demonstrated that edaravone sulfate is a good substrate of human organic anion transporter (OAT) 1 (SLC22A6) and human OAT3 (SLC22A8). In this study, we examined the involvement of breast cancer resistance protein (BCRP (ABCG2)) and [multidrug resistance-associated protein 4 MRP4 (ABCC4)] in the luminal efflux in BBM-expressing membrane vesicles compared with control vesicles ($K_m = 16.5 \mu M$). In contrast, edaravone glucuronide, but not edaravone sulfate, exhibited greater ATP-dependent uptake in MRP4-expressing membrane vesicles than that in control vesicles ($K_m = 9.85 \mu M$). Unlike taurocholate uptake, S-methylglutathione had no effect on the ATP-dependent uptake of edaravone glucuronide by MRP4. The functional importance of BCRP and MRP4 in the urinary excretion of edaravone sulfate and edaravone glucuronide, respectively, was investigated using Bcrp and Mrp4 knockout mice. The renal clearance with respect to the kidney concentration of edaravone sulfate was reduced significantly but not abolished in Bcrp knockout mice compared with wild-type mice (3.62 versus 4.85 ml/min/kg b.wt.). The renal clearance of edaravone glucuronide was lower in Mrp4 knockout mice than wild-type mice (2.01 versus 5.06 ml/min/kg BW). Our results suggest that Bcrp and Mrp4 are partly involved in the luminal efflux of edaravone sulfate and edaravone glucuronide, respectively.

Edaravone (MCI-186; 3-methyl-1-phenyl-2-pyrazolin-5-one) is a potent radical scavenger (Yamamoto et al., 1996). It has been suggested that edaravone has protective effects against cerebral and myocardial ischemia-reperfusion injuries in various experimental animal models (Watanabe et al., 1994; Yamamoto et al., 1996). The clinical efficacy of edaravone on ischemic brain attack has been demonstrated by significant improvement in the functional outcome in a randomized, placebo-controlled, double-blind study (Edaravone Acute Infarction Study Group, 2003). Edaravone has been prescribed clinically in Japan for the treatment of acute brain infarction since 2001 (Toyoda et al., 2004). After marketing of the drug, some cases of acute renal failure or renal function disorder were reported in patients following edaravone treatment (Hishida, 2007) although edaravone does not exhibit any nephrotoxicity in animal studies (Dalgard et al., 1997; Okazaki et al., 1997). The mechanism underlying such acute renal failure remains unknown. Because edaravone is mainly excreted into the urine after conjugation to glucuronide or sulfate (Yokota et al., 1997; Shibata et al., 1998), the renal excretion mechanism of edaravone should help provide important information about the clinical cases. The unbound renal clearances of edaravone sulfate and edaravone glucuronide in humans were higher than the glomerular filtration rate (GFR), suggesting that they undergo tubular secretion (Mizuno et al., 2007). Our previous study suggested that edaravone sulfate, but not edaravone glucuronide, is a good substrate for transporters of the organic anion transporter (OAT) family.

ABBREVIATIONS: GFR, glomerular filtration rate; OAT, organic anion transporter; BBM, brush border membrane; ABC, ATP-binding cassette; MRP (Mrp), multidrug resistance associated protein; BCRP (Bcrp), breast cancer resistance protein; DHEAS, dehydroepiandrosterone sulfate; E3040, 6-hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridylmethyl) benzothiazole; MCI-186, methyl-1-phenyl-2-pyrazolin-5-one, edaravone; 4’-Me-MCI-186, 3-methyl-1-(4-methyl-phenyl)-pyrazolin-5-one; $^{13}$C$_2$-MCI-186, 3-$^{13}$C-methyl-1-phenyl-2-[3,4,5-$^{13}$C]pyrazolin-5-one; HPLC, high-performance liquid chromatography; E,S, estrone 3-sulfate; HEK, human embryonic kidney; GFP, green fluorescent fusion protein; h, human; 4-MU, 4-methylumbelliferone.
of the basolateral organic anion transporters, OAT1 (SLC22A6) and OAT3 (SLC22A8) (Mizuno et al., 2007). Vectorial transport across the renal tubules is achieved by an interplay between uptake and efflux transporters at the basolateral membrane and brush border membrane (BBM) for organic ions. The transporters responsible for the luminal efflux need to be identified.

Previous studies have demonstrated the expression of ATP-binding cassette (ABC) transporters, such as multidrug resistance-associated protein (MRP) 2 (MRP2/ABCC4) in the BBM of the proximal tubules of human and rodent kidney (Schaub et al., 1999; van Aubel et al., 2002). Breast cancer resistance protein (BCRP/ABCG2) is also expressed on the BBM of the proximal tubules in rodents (Jonker et al., 2002), whereas it is below the limit of detection in the normal human kidney (Maliepaard et al., 2001). Because the substrate specificities of these ABC transporters are very broad, localization of ABC transporters along the BBM of the proximal tubules has led to considerable interest in their role in the urinary excretion of drugs.

In this study, we focused on BCRP and MRP4. They accept a variety of organic anions as substrates, such as p-aminophenolic acid, 17β-estradiol-17β-glucuronide, dehydroepiandrosterone sulfate (DHEAS), and methotrexate (van Aubel et al., 2002; Suzuki et al., 2003; Zelcer et al., 2003; Smeets et al., 2004). For BCRP, in vivo studies using Bcrp knockout mice have demonstrated that BCRP is responsible for the urinary excretion of some organic anions, such as methotrexate and the sulfate conjugate of E3040 (Breedveld et al., 2004; Mizuno et al., 2004). Recently, Hasegawa et al. (2007) and Imaoka et al. (2007) demonstrated using Mpr4 knockout mice that Mpr4 plays a role in the luminal efflux of diuretics, hydrochlorothiazide and furosemide, and antiviral drugs, adefovir and tenofovir.

In this investigation, an in vitro transport study using BCRP- and MRP4-expressing membrane vesicles was performed to examine whether edaravone sulfate and glucuronide are transported by BCRP or MRP4. Furthermore, the functional involvement of Mpr4 and Bcrp in the urinary excretion of edaravone conjugates was examined by comparing their in vivo pharmacokinetics using their corresponding gene knockout mice.

Materials and Methods

Materials. Edaravone, edaravone sulfate, edaravone glucuronide, and [14C]edaravone (516 MBq/mmol) were synthesized in Mitsubishi Pharma Corporation. [35S]Edaravone sulfate ([35S]edaravone) and [3H]edaravone prepared in Mitsubishi Pharma Corporation (Osaka, Japan); [14C]edaravone (516 MBq/mmol) were synthesized in Mitsubishi Pharma Corporation. [13C4]MCI-186 (internal standard) and extracted by dichloromethane–methanol (3:7, v/v), and then the organic layer was obtained. For measurement of edaravone sulfate and glucuronide in plasma, plasma was added to each tube of ice-cold buffer containing 10 mM Tris-HCl, 250 mM sucrose and 0.1 M NaCl, pH 7.4. The mixture was vacuum dried and then dissolved with 100 μl of a membrane vesicle suspension (5 μl of protein). 18F-Na2H[14C]urate (186 GBq/mmol; PerkinElmer Life and Analytical Sciences, Boston, MA) in a final volume of 1 ml. After incubation for 2 h at 37°C, both reactions were terminated by adding 4 ml of ice-cold methanol. After centrifugation, the supernatant was subsequently evaporated under N2 and injected into an HPLC system. The chromatographic conditions were as follows: column, CAPCELL PAK C18MG (5 μm, 4.6 mm × 150 mm; Shiseido, Tokyo, Japan); mobile phase, methanol–0.1 M ammonium acetate (pH 5.5), 35:65 (v/v); flow rate, 0.8 ml/min; and UV detection at 254 nm. Purification was conducted by comparing the retention time of each standard compound. The radiochemical purities of [14C]edaravone and [13C]edaruvo sulfate prepared by this method were found to be 99.5 to 99.9% and 99.8%, respectively. [35S]HDEAS (2738 GBq/mmol) and [3H]estrone 3-sulfate (E3S) (1610 GBq/mmol) were purchased from PerkinElmer Life and Analytical Sciences. All other chemicals were of analytical grade and commercially available.

Animals. Female Mpr4 knockout and wild-type C57BL/6J mice (10–12 weeks of age) and Bcrp knockout and wild-type FVB/Ncx1 mice (9–15 weeks of age) were used in the present study. Mpr4 knockout mice and Bcrp knockout mice had been established previously (Jonker et al., 2002; Leggas et al., 2004). All animals were treated humanely. Mice were housed and handled according to the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Membrane Vesicle Study. Membrane vesicles were prepared from HEK293 cells infected with recombinant adenovirus that contained human MRP4 or BCRP as described previously (Kondo et al., 2004). As negative controls, cells were infected with a virus that contained green fluorescence protein (GFP) cDNA. Membrane vesicles were isolated by the hypotonic method described in detail previously (Kondo et al., 2004). They were then frozen in liquid nitrogen and stored at −80°C until required.

Transport studies were performed using a rapid filtration technique (Suzuki et al., 2003). In brief, 15 μl of transport medium (10 mM Tris-Cl, 250 mM sucrose, and 10 mM MgCl2, pH 7.4) containing radiolabeled compounds, with or without unlabeled substrate, was preincubated at 37°C for 3 min and then rapidly mixed with 5 μl of a membrane vesicle suspension (5 μg of protein). The reaction mixture contained 5 mM ATP or AMP along with the ATP-regenerating system (10 mM creatine phosphate and 100 μg/μl creatine phosphokinase). The reaction was terminated by the addition of 1 ml of ice-cold buffer containing 10 mM Tris-Cl, 250 mM sucrose and 0.1 M NaCl, pH 7.4. The stopped reaction mixture was passed through a 0.45-μm nitrocellulose membrane filter (Millipore Corporation, Billerica, MA) and then washed twice with 5 ml of stop solution. The radioactivity retained on the filter was determined by liquid scintillation counting after the addition of scintillation cocktail (ACS II; GE Healthcare Bio-Sciences, Little Chalfont, Buckinghamshire, UK).

Kinetic parameters were obtained using the equation \( v = \frac{V_{\text{max}} \times S}{K_s + S} \), where \( v \) is the uptake velocity of the substrate (picomoles per minute per milligram), \( S \) is the substrate concentration in the medium (micromolar), \( K_s \) is the Michaelis-Menten constant (micromolar), and \( V_{\text{max}} \) is the maximum uptake rate (picomoles per minute per milligram). Fitting was performed by the nonlinear least-squares method using WinNonlin (version 4.1; Pharsight Corporation, Mountain View, CA).

In Vivo Infusion Study in Mice. After anesthesia with i.p. sodium pentobarbital (78 mg/kg), the bladder was catheterized for urine collection. The doses of edaravone (0.21 μmol/min/kg) including 4% mannitol were infused via the tail vein. Mannitol was used to maintain an adequate, constant urinary flow rate (Gotoh et al., 2002). Blood samples were collected from the retro-orbital sinus at 15, 45, 75, 105, and 120 min after administration and centrifuged. Urine was collected at 0 to 30, 30 to 60, 60 to 90, and 90 to 120 min by washing the bladder with 0.2 ml of saline using a tube containing 50 μl of McIlvaine buffer (44 mM citric acid and 111 mM Na2HPO4, pH 5.4) containing 0.04% 4- Me-MCI-186 (w/v). Plasma and urine samples were immediately extracted. At the end of the experiment, the kidneys were removed and stored at −80°C until required for measurement. The levels of creatinine in plasma and urine were determined using assay kits (L-Type Wako creatinine F; Wako Pure Chemical Industries, Osaka, Japan).

Liquid Chromatography/Tandem Mass Spectrometry Analysis. The quantification of edaravone and its conjugates was performed as described previously (Yokota et al., 1997). For measurement of edaravone in plasma, plasma was added to McIlvaine buffer containing 4-Me-MCI-186 and [14C]MCI-186 (internal standard) and extracted by dichloromethane–n-pentane (3:7, v/v), and then the organic layer was obtained. For measurement of edaravone sulfate and glucuronide in plasma, plasma was added to each tube containing 4-Me-MCI-186 and [14C]MCI-186. After the addition of 3 mol/liter HCl, edaravone sulfate was hydrolyzed at room temperature for 10 min (mild acid treatment), and both edaravone sulfate and glucuronide were hydrolyzed at 100°C for 10 min (heat-acid treatment) (Böttcher et al., 1984). After the neutralization by saturated sodium acetate and the addition of McIlvaine buffer, edaravone released from its sulfate or glucuronide conjugate would be converted into edaravone in the presence of NaCl and 10 mM Tris base. The reaction mixture was then extracted with chloroform–methanol (3:7, v/v). The organic phase was dried under N2, and the residue was dissolved in the mobile phase and analyzed by LC–MS/MS.
†††, using the electrospray interface. The following transitions were monitored: acetate, pH 5.5 (40:60, v/v) at a flow rate of 0.2 ml/min. The eluate was ionized to Shiseido) at 50°C. Elution was performed with methanol-10 mM ammonium gluronide (B) by membrane vesicles (5 g). The effect of contained 5 mM ATP (closed symbols) or AMP (open symbols). Each point represents the mean ± S.E. (n = 3–4).

Pharmacokinetic Analysis. Total body clearance (CLrenal), renal clearance with respect to the plasma concentration (CLrenal,p), and renal clearance with respect to the kidney concentration (CLrenal,k) were calculated from the following equations: CLrenal = I/ Cplasma, CLrenal,p = Vcren/Cplasma, and CLrenal,k = (Vcren - fup × GFR × Cplasma)/(Cplasma - Vcren), where I, Cplasma, Vcren, fup, and furen represent the infusion rate (nanomoles per minute per kilogram), plasma concentrations at 105 min (micromolar), urinary excretion rate from 90 to 120 min (nanomoles per minute per kilogram), plasma unbound fraction, and kidney concentration at 120 min (micromolar), respectively. GFR was assumed to be equal to the creatinine clearance (CLCRE) calculated from the equation CLCRE = Ucren × Vuren, where Ucren, Vcren, and furen represent urine creatinine concentration, urine flow rate, and plasma creatinine concentration (Li et al., 2002). fup was determined by ultrafiltration. Briefly, serum that was obtained from C57BL/6j mice was incubated with edaravone, edaravone sulfate, and edaravone glucuronide for 5 min at 37°C, followed by ultrafiltration using an Amicon ultrafiltration membrane (Centriconfree; Amicon, Bedford, MA). The free fraction of edaravone, edaravone sulfate, or edaravone glucuronide was expressed as the ratio of the concentration in the ultrafiltrate to that in serum.

Statistical Analysis. Statistical differences were analyzed by using Student’s t test to identify significant differences between two sets of data and by one-way analysis of variance with Dunnett’s test for multiple pairwise comparisons.

Results

ATP-Dependent Transport of Edaravone Sulfate and Glucuronide by BCRP- and MRP4-Expressing Membrane Vesicles. Membrane vesicles were prepared from HEK293 cells infected with recombinant adenovirus harboring hBCRP, hMRP4, or GFP. E1S and DHEAS, typical substrates of hBCRP and hMRP4, were used as positive controls. The uptakes of E1S by hBCRP after a 2-min
FIG. 3. Time profiles and concentration dependence of [14C]edaravone glucuronide transport by human MRP4. A, uptake of [14C]edaravone glucuronide (2 μM) by membrane vesicles (5 μg of protein) was examined at 37°C in medium containing 5 mM ATP (closed symbols) or AMP (open symbols). Circles and triangles represent the uptakes in MRP4- and GFP-expressing membrane vesicles, respectively. B, concentration dependence of MRP4-mediated transport of [14C]edaravone glucuronide was determined for 1 min. The MRP4-dependent transport was calculated by subtracting the transport of edaravone sulfate in the presence of 5 mM AMP in GFP-expressing vesicles from that in MRP4-expressing vesicles. Each point represents the mean ± S.E. (n = 3–4).

FIG. 4. Time profiles of the plasma concentration (A, C, and E) and urinary excretion (B, D, and E) of edaravone (A and B), edaravone sulfate (C and D), and edaravone glucuronide (E and F) in Bcrp knockout (open symbols) and wild-type mice (closed symbols). Edaravone was infused i.v. at 0.21 μmol/min/kg. Results are means ± S.E. (n = 6). *, P < 0.05 versus wild-type mice.
incubation at 37°C in the presence of ATP and AMP were 574 ± 15
and 28.3 ± 8.4 μl/mg protein, respectively. The uptake of DHEAS
by hMRP4 after a 5-min incubation at 37°C in the presence of ATP
and AMP were 919 ± 52 and 10.3 ± 13.6 μl/mg protein, respectively.
These values were comparable to previously reported values (Hase-
gawa et al., 2007; Imaoka et al., 2007).

The uptake of edaravone sulfate and glucuronide by hBCRP-
and hMRP4-expressing membrane vesicles were determined (Fig. 1).
The ATP-dependent uptake of edaravone sulfate by hBCRP-expressing
vesicles, but not by hMRP4-expressing vesicles, was markedly higher
than that by GFP-expressing vesicles (Fig. 1A). In contrast, increased
ATP-dependent uptake of edaravone glucuronide was observed in
hMRP4-expressing vesicles but not in hBCRP-expressing vesicles
compared with GFP-expressing vesicles (Fig. 1B). Glutathione has
been reported to stimulate the ATP-dependent uptake of taurocholate
by MRP4 (Rius et al., 2003). However, this stimulatory effect was not
observed for edaravone glucuronide (Fig. 1C). Edaravone did not
exhibit ATP-dependent transport in any membrane vesicles (data not
shown). The ATP-dependent uptake of edaravone sulfate by hBCRP
and that of edaravone glucuronide by hMRP4 were increased with
time (Figs. 2A and 3A) and saturable (Figs. 2B and 3B). The
\( K_{\text{m}} \) and \( V_{\text{max}} \) values of edaravone sulfate for hBCRP
were found to be 16.5 ± 1.6 μM and 852 ± 40 pmol/min/mg, respectively. The corresponding
parameters of edaravone glucuronide for hMRP4 were found to be
9.85 ± 0.81 μM and 3512 ± 137 pmol/min/mg, respectively.

Renal Excretion of Edaravone and Its Conjugates in Bcrp
Knockout and Wild-Type Mice. Edaravone was administered by
constant i.v. infusion and the parent compound and sulfate and gluc-
uronide conjugates in plasma, urine, and kidney were measured (Fig.
4). The pharmacokinetic parameters are summarized in Table 1. Creatinine clearance in Bcrp knockout mice is similar to that in
wild-type mice as previously reported (Table 1) (Mizuno et al., 2004).
Urinary recovery of edaravone sulfate was significantly reduced in
Bcrp knockout mice compared with wild-type mice, whereas the
kidney concentrations of edaravone sulfate were similar in the two
strains (Table 1). \( CL_{\text{renal, p}} \) and \( CL_{\text{renal, k}} \) of edaravone sulfate were
reduced significantly in Bcrp knockout mice (Table 1). Knockout of
Bcrp did not affect any pharmacokinetic parameters of edaravone
and edaravone glucuronide (Table 1).

Renal Excretion of Edaravone and Its Conjugates in Mrp4
Knockout and Wild-Type Mice. The plasma concentrations and
urinary excretion rates of edaravone and its conjugates after i.v.
infusion of edaravone in Mrp4 knockout mice and wild-type mice are
shown in Fig. 5. The pharmacokinetic parameters are summarized in
Table 2. There was no difference between creatinine clearance of
Mrp4 knockout mice and that of wild-type mice (Table 2). The plasma
concentration and urinary excretion rate were similar for edaravone
and its conjugates in the two strains (Fig. 5). Compared with the
wild-type mice, the kidney/plasma concentration ratio (\( K_{\text{p, kidney}} \)
value of edaravone glucuronide was 2.8-fold greater in Mrp4 knock-
out mice, although this was not statistically significant. The \( CL_{\text{renal, k}} \)
of edaravone glucuronide, but not edaravone sulfate, was significantly
reduced in Mrp4 knockout mice. The \( f_{\text{p}} \) values of edaravone, edara-
vone sulfate, and edaravone glucuronide were 0.099 ± 0.007, 0.018 ±
0.001, and 0.72 ± 0.03, respectively.

Discussion

In the present study, we have demonstrated that edaravone sulfate
and glucuronide are substrates of BCRP and MRP4, respectively,
using membrane vesicles. In in vivo studies using gene knockout
mice, we have demonstrated that BCRP and MRP4 play significant
roles in the urinary excretion of edaravone sulfate and glucuronide,
respectively.

It was found that edaravone sulfate, but not edaravone glucuronide,
was a substrate of human BCRP (Figs. 1A and 2). This result is
consistent with the previous finding of Suzuki et al. (2003), who
demonstrated that 4-MU sulfate and E3040 sulfate were extensively
transported by BCRP, but 4-MU glucuronide and E3040 glucuronide
were transported to a lesser extent. On the contrary, MRP4 accepts
only edaravone glucuronide (Figs. 1B and 3). Rius et al. (2003)
demonstrated that the ATP-dependent uptake of taurocholate by
MRP4 was stimulated by cotransport of reduced glutathione (GSH)
or its S-methyl derivative. However, this is not the case with edaravone
glucuronide (Fig. 1C). In vitro transport studies suggest that BCRP
and MRP4 are candidate transporters involved in the luminal efflux
of edaravone sulfate and glucuronide, respectively, in the kidney.

This possibility was investigated by comparing pharmacokinetic
profiles in knockout mice and wild-type mice. The renal clearances
of edaravone sulfate and glucuronide are much greater than the
\( f_{\text{p}} \times GFR \), suggesting that tubular secretion accounts for the major part of
their urinary excretion. In contrast, the renal clearance of edaravone
is comparable with the \( f_{\text{p}} \times GFR \), suggesting that glomerular filtration
is mainly responsible for its urinary excretion.

The kinetic parameters of urinary excretion of edaravone, edarav-
vone sulfate, and its glucuronide were determined in wild-type, Bcrp
knockout, and Mrp4 knockout mice (Tables 1 and 2). The total body
clearance of edaravone was unchanged in Bcrp and Mrp4 knockout
mice. Considering that the major elimination pathway of edaravone
is conjugation with glucuronide or sulfate, the conjugation activities
will be similar between wild-type and Bcrp knockout and Mrp4 knockout
mice. The renal clearances with respect to the plasma concentration
and to the kidney concentration of edaravone sulfate were reduced by
45 and 25%, respectively, in Bcrp knockout mice compared with
wild-type mice (Table 1). This result suggests that Bcrp is involved in
the tubular secretion of edaravone sulfate. Although the luminal efflux
clearance of edaravone sulfate was decreased in Bcrp knockout mice,
the \( K_{\text{p, kidney}} \) exhibited no change. Therefore, it is possible that lumii-
nal efflux mediated by Bcrp is not the major sequestration pathway in the kidney. Because it was demonstrated that edaravone sulfate undergoes deconjugation in human kidney (illustrated in Fig. 6) (Yokota et al., 1997), metabolism by sulfatase could be another elimination pathway from the proximal tubules. Otherwise, it is possible that edaravone sulfate undergoes basolateral efflux. In the case of Mrp4 knockout mice, the $K_p$, kidney value of edaravone glucuronide was increased 2.8-fold, and the luminal efflux of edaravone glucuronide was reduced by 60% of control values in Mrp4 knockout mice (Table 2), suggesting that Mrp4 is partly involved in the urinary excretion of edaravone glucuronide.

The renal clearances of edaravone sulfate and its glucuronide were decreased but not abolished in Bcrp and Mrp4 knockout mice, suggesting the involvement of other efflux transporters. MRP2 is expressed on the BBM of the kidney proximal tubules (Schaub et al., 1999). We were unable to observe any MRP2-dependent transport of edaravone glucuronide in membrane vesicles expressing human MRP2 (data not shown). Edaravone sulfate and glucuronide are barely excreted into the bile in rats although they are formed mainly in the liver (Komatsu et al., 1996). Therefore, Mrp2 may not be involved in the tubular secretion of edaravone glucuronide. However, the possibility of species difference cannot be excluded and in vivo studies in Mrp2 knockout mice could be helpful to evaluate the contribution of Mrp2 to their luminal efflux.

In addition to the kidney, MRP4 is expressed on the sinusoidal membranes of hepatocytes and plays a role in the efflux of GSH and bile acids from hepatocytes into blood (Rius et al., 2003). In addition, it has been demonstrated that the basolateral excretory clearance of acetaminophen sulfate, 4-MU sulfate, and harmol sulfate is reduced in the livers of Mrp4 knockout mice (Zamek-Gliszczynski et al., 2006), suggesting that Mrp4 participates in the hepatic basolateral excretion of these drugs. In contrast, there was no significant difference in the plasma concentrations of edaravone glucuronide between Mrp4 knockout mice and wild-type mice. Thus, it is likely that MRP4 makes only a minor contribution to the sinusoidal efflux of edaravone glucuronide in the liver.

Edaravone glucuronide is the main metabolite in human urine: approximately 70% of the dose was excreted into urine as the gluc-
Edaravone sulfate and glucuronide in Mrp4 knockout and wild-type mice

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<th>Wild-type</th>
<th>Mrp4−/−</th>
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<tr>
<td><strong>Cl_{CRE}</strong> (ml/min/kg)</td>
<td>8.63 ± 1.50</td>
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<td><strong>Cl_{total}</strong> (ml/min/kg)</td>
<td>105 ± 5</td>
<td>104 ± 8</td>
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<tr>
<td>Edaravone (ml/min/kg)</td>
<td>0.915 ± 0.114</td>
<td>0.818 ± 0.129</td>
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<td>Sulfate</td>
<td>7.33 ± 0.43</td>
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<td>Glucuronide</td>
<td>22.1 ± 1.8</td>
<td>19.6 ± 2.7</td>
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<tr>
<td><strong>Cl_{total,k}</strong> (ml/min/kg)</td>
<td>4.92 ± 0.83</td>
<td>2.86 ± 0.53</td>
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<td>Glucuronide</td>
<td>5.06 ± 1.18</td>
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<td>C_{kidney} (nmol/g kidney)</td>
<td>7.79 ± 2.16</td>
<td>14.1 ± 2.7</td>
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<td>Sulfate</td>
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<td>K_s (kidney)</td>
<td>3.39 ± 1.08</td>
<td>6.82 ± 1.73</td>
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<td>Glucuronide</td>
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<td>15.0 ± 6.0</td>
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<td>Urinary recovery (g/24h)</td>
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<td>0.730 ± 0.069</td>
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<td>Edaravone (%) (dose)</td>
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<tr>
<td>Sulfate</td>
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<td>26.6 ± 3.2</td>
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</table>

* P < 0.05, significantly different from wild-type mice using Student’s t-test.

Edaravone, whereas the sulfate is the main metabolite in human plasma (Yokota et al., 1997; Shibata et al., 1998). Unlike humans, in rats and dogs the main metabolite in the urine and plasma is the sulfate. Edaravone sulfate could undergo deconjugation and, in turn, form a glucuronide conjugate in the human kidney (Fig. 6) (Yokota et al., 1997). The conversion activity of edaravone sulfate by sulfatase in human kidney homogenate was higher than that in rats (data not shown), and the expression of BCRP protein is undetectable in human kidney (Maliepaard et al., 2001). These factors possibly produce the species difference in the ratio of conjugates in urine. Taking the results obtained so far into consideration, we suggest that edaravone should be taken up by the human kidney mainly as the sulfate form via OAT1 and OAT3, converted from the sulfate to the glucuronide in the human kidney, and excreted into the urine mainly as the glucuronide form partly via MRP4 (Fig. 6). Consideration of the metabolism and transport of each metabolite is necessary to properly understand the renal handling of edaravone.

Generally, accumulation of drugs in the kidney is associated with their nephrotoxicity. OAT1-mediated uptakes of adefovir, an antiviral drug, and cephapirin, a β-lactam antibiotic, into the kidney are associated with the nephrotoxicity of these drugs. Increased cytotoxicity of these drugs toward OAT1-transfected cells compared with control cells has been observed (Jariyawat et al., 1999; Takeda et al., 1999; Ho et al., 2000). In contrast, in the case of edaravone, although edaravone sulfate is efficiently transported by OAT1, edaravone and edaravone sulfate did not exhibit any cytotoxic effects on either OAT1-transfected HEK293 cells or control cells even at 1 mM (Mizuno et al., 2007). In addition, they both showed little cytotoxicity for human renal proximal tubule epithelial cells and normal human cortical epithelial cells (Iwase et al., 2004). Thus, edaravone itself seems not to be directly associated with nephrotoxicity. The mechanism of acute renal failure in patients after edaravone treatment remains currently unknown. A retrospective review of the clinical cases indicated that approximately 40% of patients with acute renal failure were given edaravone in combination with cephem antibiotics (data not shown). In consideration of this fact, the package insert of edaravone was revised to describe the possibility of increased renal failure by coadministration of edaravone and antibiotics such as cefazolin and cefotiam. In addition, in vivo study in rats showed that a higher dose (100 and 200 mg/kg) of edaravone aggravated nephrotoxicity induced by cefalotin and glycerol, especially under conditions of dehydration in rats, although edaravone alone did not cause any nephrotoxicity (Ohyma et al., 2004). Recently, it has been reported that several cephalosporins (cefotaxime, cefazolin, cefmetazole, and cefotaxime) are substrates of MRP4 (Ci et al., 2007). If the kidney concentrations of edaravone glucuronide at the clinical dose (30 mg/man) can inhibit MRP4, it can be speculated that edaravone may increase kidney concentrations of some cephalosporins. Further studies are necessary to examine this speculation, because when MRP4-mediated transport is not major elimination pathway of cephalosporins in the kidney, the kidney concentrations do not always change. In addition, acute renal failures were found in patients who were dehydrated, had an infection, or were of advanced age (Hishida, 2007), suggesting that complicated factors may be involved. Further investigation and careful discussion are required to understand the clinical cases.

In conclusion, Bcrp and Mrp4 are partly involved in the luminal efflux of edaravone sulfate and glucuronide, respectively, but other unknown transporter(s) are also involved.

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References


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