Glucuronidation of Edaravone by Human Liver and Kidney Microsomes: Biphasic Kinetics and Identification of UGT1A9 as the Major UDP-Glucuronosyltransferase Isoform

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ABSTRACT:

Edaravone was launched in Japan in 2001 and was the first neuroprotectant developed for the treatment of acute cerebral infarction. Edaravone is mainly eliminated as glucuronic conjugate in human urine (approximately 70%), but the mechanism involved in the elimination pathway remains unidentified. We investigated the glucuronidation of edaravone in human liver microsomes (HLM) and human kidney microsomes (HKM) and identified the major hepatic and renal UDP-glucuronosyltransferases (UGTs) involved. As we observed, edaravone glucuronidation in HLM and HKM exhibited biphasic kinetics. The intrinsic clearance of glucuronidation at high-affinity phase (CLint1) and low-affinity phase (CLint2) were 8.4 ± 3.3 and 3.3 ± 0.2 µl·min⁻¹·mg⁻¹, respectively, for HLM and 45.3 ± 8.2 and 1.8 ± 0.1 µl·min⁻¹·mg⁻¹, respectively, for HKM. However, in microsomal incubations contained with 2% bovine serum albumin, CLint1 and CLint2 were 16.4 ± 1.2 and 3.7 ± 0.3 µl·min⁻¹·mg⁻¹, respectively, for HLM and were 78.5 ± 3.9 and 3.6 ± 0.5 µl·min⁻¹·mg⁻¹, respectively, for HKM. Screening with 12 recombinant UGTs indicated that eight UGTs (UGT1A1, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B7, and UGT2B17) expressed in human liver or kidney were selected for kinetic studies. Among them, UGT1A9 exhibited the highest activity (CLmax = 42.4 ± 9.5 µl·min⁻¹·mg⁻¹), followed by UGT2B17 (CLmax = 3.3 ± 0.4 µl·min⁻¹·mg⁻¹) and UGT1A7 (CLmax = 1.7 ± 0.2 µl·min⁻¹·mg⁻¹). Inhibition study found that inhibitor of UGT1A9 (propofol) attenuated edaravone glucuronidation in HLM and HKM. In addition, edaravone glucuronidation in a panel of seven HLM was significantly correlated (r = 0.9940, p = 0.0021) with propofol glucuronidation. Results indicated that UGT1A9 was the main UGT isoform involved in edaravone glucuronidation in HLM and HKM.

Introduction

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, MCI-186; Fig. 1A), a potent free radical scavenger, has been approved in Japan for the treatment of acute cerebral infarction since 2001 (Watanabe et al., 2008; Lapchak, 2010). It is the first neuroprotectant developed for stroke treatment. After being available for more than 10 years, it is still widely used in Japan. The total sale of edaravone reported by Mitsubishi Tanabe for 2008 was approximately $272.6 million. Pharmacologists have expanded its application in other organs (heart, lung, liver, kidney, etc.) with ischemia-reperfusion injury (Watanabe et al., 2008). A phase III clinical trial for amyotrophic lateral sclerosis is ongoing in Japan (Takahashi, 2009), in which edaravone exhibits the potential to be a rather promising drug. It is believed that edaravone is a drug with broad development prospects.

Despite its vast investigation on pharmacological activity, metabolic elimination of edaravone has not been clarified sufficiently. Pharmacokinetic studies showed that edaravone was mainly eliminated as glucuronic conjugate (approximately 70%; Fig. 1B) in humans (Komatsu et al., 1996; Yokota et al., 1997; Shibata et al., 1998). However, no detailed data were available about UDP-glucuronosyltransferase (UGT) isoforms involved in edaravone glucuronidation. Because identification of UGT isoforms helps to predict potential drug-drug interactions mediated by particular enzyme isoforms and polymorphism-related interindividual variability, we thought it necessary to identify the UGT isoforms involved. Generally, liver is considered the major contributor to drug glucuronidation, but extrahepatic tissues or organs may also be involved (Tukey and Strassburg, 2000). Considering that renal disorders have been reported in patients treated with edaravone (Hishida, 2007), investigation of edaravone

ABBREVIATIONS: UGT, UDP-glucuronosyltransferase; HKM, human kidney microsomes; LC/MS/MS, liquid chromatography/tandem mass spectrometry; UDPGA, UDP-glucuronic acid; MS/MS, tandem mass spectrometry; HLM, human liver microsomes; IS, internal standard; BSA, bovine serum albumin.
glucuronidation by human kidney microsomes (HKM) may help to understand the organ-targeted adverse effect. As an initial step, edaravone glucuronide (3-methyl-1-phenyl-2-pyrazolin-5-one) has been synthesized by our research group (Zhu et al., 2010). For the measurement of edaravone glucuronide, a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method has been built. The main objective of this study is to characterize the glucuronidation of edaravone in human liver microsomes (HLM) and HKM, and to identify the hepatic and renal UGTs involved in the metabolism of edaravone.

Materials and Methods

Chemicals and Materials. Edaravone was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Edaravone glucuronide was synthesized by our research group with purity >95%. UDP-glucuronic acid (UDPGA), α-saccharic acid 1,4-lactone, β-glucuronidase (Escherichia coli), alamethicin, estradiol, propofol, naloxone, androsterone, and phenacetin [internal standard (IS)] were all purchased from Sigma-Aldrich (St. Louis, MO). Pooled HLM and HKM were purchased from Research Institute for Liver Diseases (Shanghai) Co. Ltd. (Shanghai, China). Pooled HLM was prepared from 10 Mongolian donors aged from 24 to 38 years, and pooled HKM was prepared from five white donors aged from 30 to 74 years. Seven individual human livers and a panel of recombinant human UGT Supersomes (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17) expressed in baculovirus-infected insect cells were obtained from BD Biosciences Discovery Labware (Bedford, MA). Milli-Q water (Millipore Corporation, Billerica, MA) was used in all steps, and all other chemicals were of high-performance liquid chromatography grade or the best grade that was commercially available.

LC/MS/MS Determination. The analysis for edaravone glucuronide was performed in a Finnigan Surveyor high-performance liquid chromatography system (Thermo Fisher Scientific, Waltham, MA) coupled with a Finnigan TSQ Quantum Discovery Max system (Thermo Fisher Scientific). Mass spectrometers applied an electrospray ionization source in the positive ion mode. Data acquisition was performed with Xcalibur 1.2 software (Thermo Fisher Scientific). Separation of analytes was achieved with an Agilent ZORBAX Eclipse Plus C18 column (2.1 × 150 mm i.d. 3.5 μm; Agilent Technologies, Santa Clara, CA) at a flow rate of 200 μL/min. The mobile phase consisted of methanol (A) and 0.05% formic acid (B). The following gradient elution was applied: 0 to 2.5 min, 40% A; 2.5 to 3.0 min, increase A to 70%; 3.0 to 7.5 min, 70% A; 7.5 to 8.0 min, decrease A to 40%; run-time, 11 min. The optimized mass conditions were summarized as follows: spray voltage was set at 5 kV.
and capillary temperature was set at 267°C; sheath gas (N₂) and auxiliary gas (N₂) were 35 and 10 Arb, respectively; collision gas (Ar) was 1.5 Pa; specific transitions of edaravone glucuronide (m/z 351.3→175.1), edaravone (m/z 175.1→133.1), and phenacetin as IS (m/z 180.1→110.1) were monitored in the multiple reaction monitoring mode.

Glucuronidation in Pooled HLM and HKM. For optimization of the incubation conditions, the linearity of metabolite formation with time (10–120 min) and protein (0.05–1.00 mg/ml) in HLM and with time (15–90 min) and protein (0.025–0.5 mg/ml) in HKM was evaluated in advance. All incubation mixtures contained microsomes treated with 25 µg/ml alamethicin at 4°C for 20 min, 10 mM magnesium chloride, 5 mM D-saccharic acid 1,4-lactone, and edaravone in 200 µl of 100 mM phosphate buffer, pH 7.4. Drug was dissolved in methanol, and the final concentration of methanol in the reaction was 1% (v/v). The reactions were initiated by the addition of 2 mM UDPGA, incubated at 37°C for 30 min, and then were terminated by adding 600 µl of ice-cold acetonitrile containing 20 ng/ml phenacetin (IS). The mixtures were vortexed and centrifuged (18,000 rpm at 4°C for 10 min) to obtain the supernatants, of which 5 µl was subjected to analysis. Incubations without UDPGA served as negative controls.

Glucuronidation by Recombinant UGTs. A panel of recombinant human UGTs (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17) was used to screen for the glucuronidation of edaravone at two concentrations (12.5 and 500 µM). Incubation conditions were similar to those of HLM and HKM except that the protein concentration was 0.2 mg/ml. Kinetic studies for six main UGTs (UGT1A1, UGT1A6, UGT1A7, UGT1A9, UGT2B7, and UGT2B17) were conducted with protein concentration of 0.1 mg/ml and incubation time of 30 min. The ranges of edaravone concentrations used to obtain kinetic profiles were 50 to 5000 for UGT1A1 and UGT2B7, 10 to 2500 for UGT1A6 and UGT1A7, 2 to 2500 for UGT1A9, and 5 to 2500 µM for UGT2B17.

Chemical Inhibition Study. Edaravone glucuronidation in HLM, HKM, and recombinant UGTs (UGT1A1, UGT1A9, UGT2B7, and UGT2B17) was evaluated in the presence or absence of known chemical inhibitors. Estradiol for UGT1A1 (Watanabe et al., 2003), propofol for UGT1A9 (Picard et al., 2005), naloxone for UGT2B7 (Di Marco et al., 2005), and androsterone for UGT2B17 (Sten et al., 2009) were used as corresponding inhibitors. Substrate concentrations for HLM and HKM were 40 and 10 µM, respectively, and those...
for recombinant UGTs were around their individual \( K_m \) values (200 \( \mu M \) for UGT1A1, 10 \( \mu M \) for UGT1A9, 2 \( mM \) for UGT2B7, and 100 \( \mu M \) for UGT2B17). Different concentrations of estradiol (10, 20, and 50 \( \mu M \)), progesterol (100, 400, and 1000 \( \mu M \)), naloxone (200, 500, and 1000 \( \mu M \)), and androstenedione (5, 20, and 35 \( \mu M \)) were applied to inhibit edaravone glucuronidation in HLM, HKM, and recombinant UGTs. Considering that UGT1A1 is not present in human kidney (Ohno et al., 2009; Knights and Miners, 2010), inhibition by estradiol was found in recombinant UGT1A1 and HLM.

**Correlation Analysis.** A correlation analysis was performed between the activities of edaravone glucuronidation versus propofol (UGT1A9) glucuronidation, estradiol-3-glucuronide (UGT1A1), and serotonin (UGT1A6) glucuronidation in seven donor HLM. Glucuronidation activity of edaravone was measured when substrate and protein concentrations were 40 \( \mu M \) and 0.1 mg/ml, respectively. Glucuronidation activities of estradiol-1\( \beta \)-glucuronidation (UGT1A1) and propofol (UGT1A9) glucuronidation were provided by the manufacturer. Serotonin (UGT1A6) glucuronidation activity was measured on the basis of a method reported previously (Krishnaswamy et al., 2003). Correlation analysis was performed, and \( p \) values less than 0.05 were considered statistically significant.

**Enzyme Kinetic Data Analysis.** First, data were transformed, and Eadie-Hofstee curves were plotted, which help to identify kinetic models (Hutzler and Tracy, 2002). Then kinetic parameters were obtained by fitting velocity data to the appropriate kinetic models (eqs. 1 and 2) using GraphPad Prism 5.0 software (GraphPad Software Inc., San Diego, CA).

Michaels-Menten model (Iwuchukwu and Nagar, 2008):

\[
v = \frac{V_{\text{max}} \times [S]}{K_m + [S]}
\]

where \( v \) is the rate of metabolite formation, \( K_m \) is the Michaelis-Menten constant (substrate concentration at 0.5 \( V_{\text{max}} \)), and \([S]\) is the substrate concentration.

Biphasic kinetic model (Iwuchukwu and Nagar, 2008):

\[
v = \frac{V_{\text{max1}} \times [S]}{K_m1 + [S]} + \frac{V_{\text{max2}} \times [S]}{K_m2 + [S]}
\]

where \( K_m1 \) represents the high-affinity component and \( K_m2 \) represents the low-affinity component (\( K_m1 < K_m2 \)).

**Results**

**LC/MS/MS Determination of Edaravone Glucuronide:** Initially, an LC/MS/MS method for the determination of edaravone and its glucuronide conjugate has been developed. The separation of edaravone with its glucuronide was achieved with our liquid conditions. We were sure that edaravone did not influence the detection of its glucuronide (Fig. 2). Because the incubation mixtures contained a high concentration of edaravone, edaravone was not detected in the final method. The tandem mass spectrometry (MS/MS) spectra of edaravone glucuronide are presented in Fig. 3.

**Edaravone Glucuronidation by HLM and HKM.** Researchers have found the “albumin effect” in drug glucuronidation, and glucuronidation clearance was investigated in the presence of 2% BSA or fatty acid-free human serum albumin (Rowland et al., 2007, 2008). We investigated edaravone glucuronidation in HLM and HKM, in the presence or absence of 2% BSA. As shown in Fig. 4, edaravone glucuronidation displayed biphasic kinetics in all four conditions. The enzyme kinetic parameters derived from these data are listed in Table 1.

**Screening by Recombinant UGTs.** As an initial screen, 12 commercially available recombinant UGTs were evaluated for their ability to metabolize edaravone, and a low concentration of 12.5 \( \mu M \) (close to \( K_m \) values of HLM and HKM) and a high concentration of 500 \( \mu M \) (close to \( K_m \) values of HLM and HKM) were used, see Fig. 5. Incubations with a low concentration indicated that detectable edaravone glucuronides were generated in five UGTs and they were ordered as UGT1A9, UGT1A7, UGT2B17, UGT1A6, and UGT1A8.

Incubation with high substrate concentration showed that all isoforms but UGT1A4 generated detectable edaravone glucuronide. Among them, eight UGTs produced significant amount of metabolite, and they were ordered as UGT1A8, UGT1A6, UGT1A9, UGT1A7, UGT2B7, UGT2B17, UGT1A1, and UGT1A10.

**Kinetics of Edaravone Glucuronidation by Recombinant UGTs.** Among the eight UGTs with high activity, UGT1A8 and UGT1A10 were mainly expressed in gastrointestinal tract (Strassburg

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HLM - BSA</th>
<th>HLM + BSA</th>
<th>HKM - BSA</th>
<th>HKM + BSA</th>
</tr>
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<tr>
<td>( K_m ), ( \mu M )</td>
<td>36.7 ± 22.7</td>
<td>7.4 ± 1.8</td>
<td>8.4 ± 1.9</td>
<td>8.7 ± 1.0</td>
</tr>
<tr>
<td>( V_{\text{max}} ), pmol ( \cdot ) min(^{-1} \cdot ) mg(^{-1} )</td>
<td>309.4 ± 89.1</td>
<td>336.5 ± 27.3</td>
<td>137.9 ± 25.7</td>
<td>696.0 ± 59.9</td>
</tr>
<tr>
<td>( CL_{\text{int}} ), ( \mu l \cdot ) min(^{-1} \cdot ) mg(^{-1} )</td>
<td>8.4 ± 3.3</td>
<td>45.3 ± 8.2</td>
<td>16.4 ± 1.2</td>
<td>78.5 ± 3.9</td>
</tr>
<tr>
<td>( K_m ), ( \mu M )</td>
<td>5876.0 ± 2181.0</td>
<td>1363.0 ± 128.5</td>
<td>1348.0 ± 208.0</td>
<td>1466.0 ± 323.8</td>
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<td>( V_{\text{max}} ), pmol ( \cdot ) min(^{-1} \cdot ) mg(^{-1} )</td>
<td>7429.0 ± 1721.0</td>
<td>2396.0 ± 73.6</td>
<td>4904.0 ± 433.1</td>
<td>5265 ± 595.3</td>
</tr>
<tr>
<td>( CL_{\text{int}} ), ( \mu l \cdot ) min(^{-1} \cdot ) mg(^{-1} )</td>
<td>1.3 ± 0.2</td>
<td>1.8 ± 0.1</td>
<td>3.7 ± 0.3</td>
<td>3.6 ± 0.5</td>
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Fig. 5. Formation of edaravone glucuronide by recombinant human UGT isoforms. A total of 12.5 (A) and 500 (B) \( \mu M \) edaravone were incubated with recombinant human UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17) at a protein concentration of 0.2 mg/ml, and other incubation conditions are similar with HLM and HKM. Each bar represents the mean ± S.D. of triplicate determinations.
et al., 1999; Bowalgaha et al., 2005; Ohno and Nakajin, 2009). This study focused primarily on the hepatic and renal glucuronidation of edaravone, so the kinetic studies were performed in UGT1A1, UGT1A6, UGT1A7, UGT1A9, UGT2B7, and UGT2B17. The curves are presented in Fig. 6, and the kinetic parameters are listed in Table 2. The fitting for UGT1A1 was weighted by \(1/X^2\). With the linear increase at higher concentration, the apparent \(K_m\) and \(V_{\text{max}}\) for UGT1A1 could not be estimated. Of those UGTs studied, UGT1A9 showed significant high affinity and activity with a \(K_m\) of 6.0 ± 2.0 \(\mu\text{M}\) and a \(CL_{\text{int}}\) of 42.4 ± 9.5 \(\mu\text{l} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}\).

**Chemical Inhibition Results.** The inhibitory effects of estradiol (UGT1A1), propofol (UGT1A9), naloxone (UGT2B7), and androstenedione (UGT2B17) on edaravone glucuronidation were investigated in

![Fig. 6. Kinetic profiles for formation of edaravone glucuronide in UGT1A1 (A), UGT1A6 (B), UGT1A7 (C), UGT1A9 (D), UGT2B7 (E), and UGT2B17 (F). Inset, Eadie-Hofstee plots for each of the profiles are shown. Data for UGT1A1 represents mean ± S.D. of duplicate determinations and for others represents mean ± S.D. of triplicate determinations.](image-url)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>(K_m) ((\mu\text{M}))</th>
<th>(V_{\text{max}}) (pmol min(^{-1}) mg(^{-1}))</th>
<th>(CL_{\text{int}}) ((\mu\text{l} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}))</th>
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<tr>
<td>UGT1A1</td>
<td>284.8 ± 199.6</td>
<td>45.2 ± 28.0</td>
<td>0.2 ± 0.0</td>
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<tr>
<td>UGT1A6</td>
<td>777.7 ± 37.0</td>
<td>1244.0 ± 24.5</td>
<td>1.6 ± 0.0</td>
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<tr>
<td>UGT1A7</td>
<td>432.3 ± 62.4</td>
<td>740.2 ± 34.6</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>UGT1A9</td>
<td>6.0 ± 2.0</td>
<td>254.5 ± 33.0</td>
<td>42.4 ± 9.5</td>
</tr>
<tr>
<td>UGT2B7</td>
<td>419.4 ± 183.4</td>
<td>340.4 ± 31.4</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>UGT2B17</td>
<td>128.6 ± 17.1</td>
<td>1769.0 ± 129.1</td>
<td>3.3 ± 0.4</td>
</tr>
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</table>

**Table 2.** Kinetic parameters of edaravone glucuronidation in UGT1A1, UGT1A6, UGT1A7, UGT1A9, UGT2B7, and UGT2B17
recombinant UGTs, HLM, and HKM. As shown in Fig. 7, glucuronidation activities in recombinant UGT isoforms were all reduced by inhibitors with a concentration-dependent manner. However, in HLM and HKM, it was found that 1) propofol inhibited edaravone glucuronidation in a concentration-dependent manner, 2) naloxyone and androsterone had no effect on edaravone glucuronidation, and 3) high concentration of estradiol had a slight inhibitory effect in HLM. Results from these studies indicated that UGT1A9 might contribute mainly to edaravone glucuronidation in HLM and HKM, and in high substrate concentration, UGT1A1 might be involved in edaravone glucuronidation in HLM.

Correlation Results. To further assess the contribution of UGT isoforms, correlation analyses were performed with alamethicin-activated HLM from seven individual human donor livers at a substrate concentration of 40 μM. As shown in Fig. 8, there was a significant correlation between edaravone glucuronidation and propofol glucuronidation, a typical substrate for UGT1A9 (r = 0.9340, p = 0.0021). However, no correlation was found with estradiol-3-glucuronidation (r = 0.2934, p = 0.5230) and serotonin glucuronidation (r = −0.1997, p = 0.6677), typical substrates for UGT1A1 and UGT1A6, respectively.

Discussion
UGTs catalyze the conjugation of glucuronic acid from UDPGA with a large variety of lipophilic substrates, facilitating their detoxification and excretion from the body (Ouzzine et al., 2003). In this study, we explored the comparative role of HLM and HKM in edaravone glucuronidation and identified the main UGT isoforms responsible for edaravone glucuronidation by investigating reactions in pooled HLM and HKM, in individual HLM and recombinant UGTs. As for measurement of edaravone glucuronide, a sensitive and selective LC/MS/MS method was developed.

As we observed, edaravone glucuronidation exhibited biphasic kinetics in pooled HLM and HKM. Clinical studies showed that the maximal plasma concentration of a therapeutic dose (0.5 mg/kg) of edaravone was approximately 6 μM (Yokota et al., 1997), which was less than Km1 values of HLM and HKM and much less than Km2 values of them. It is likely that the high-affinity phase contributed mainly to the edaravone glucuronidation in vivo. In the high-affinity phase, HKM exhibited 5.4- and 4.8-fold higher CLint1 than HLM, in the absence and presence of 2% BSA. Hence, human kidney might play an important role in extrahepatic clearance of edaravone in patients treated with this drug. Interestingly, we found that the addition of 2% BSA resulted in 2.0- and 1.7-fold increase of CLint1 in HLM and HKM. Until now, enhancement of UGT activity by albumin was found only in UGT1A9 and UGT2B7, but not in UGT1A1 and UGT1A6 (Tsoutsikos et al., 2004; Rowland et al., 2007, 2008; Kilford et al., 2009).

Screening with 12 recombinant UGT isoforms showed that eight UGTs produced a significant amount of glucuronide metabolite. In addition, among them, six UGTs (UGT1A1, UGT1A6, UGT1A7, UGT1A9, UGT2B7, and UGT2B17) expressed in human liver and kidney were selected for kinetic study. UGT1A9 showed significant high affinity and activity (Km1 = 6.0 ± 2.0 μM, CLint1 = 42.4 ± 9.5 μl · min⁻¹ · mg⁻¹), making it the focus of interest. It is noteworthy that the Km1 and CLint1 values for UGT1A9 were close to the values for HKM (Km1 = 7.4 ± 1.8 μM and CLint1 = 45.3 ± 8.2 μl · min⁻¹ · mg⁻¹). A substrate that is solely metabolized by a single UGT isoform should have a similar Km value compared with that by pooled microsomes (Court et al., 2003). It seems that UGT1A9 may be the predominant UGT isoform for edaravone glucuronidation in HKM.

Chemical inhibition study demonstrated that UGT1A9 was the main contributor to edaravone glucuronidation in HLM and HKM, and correlation study in seven HLM also demonstrated that UGT1A9
triplicate incubations. Considered statistically significant. Each data point represents mean ± S.D. of triplicate incubations.

was the most important one. Correlation assay in HKM has failed to conduct because different HKM were not available. However, we found in previous reports that the mRNA expression of UGT1A6 versus UGT1A9 in human liver was 0.39:1 and in human kidney was 0.13:1 (Ohno and Nakajin, 2009). Because human kidney expressed lower ratio of UGT1A6 versus UGT1A9, we deduced that UGT1A6 may not be the major isoform for the glucuronidation of edaravone in human kidney.

Results from Ohno and Nakajin (2009) indicated that the mRNA expression of UGT1A9 in HKM was 4.9-fold higher than that in HLM, which was consistent with our observation that edaravone glucuronidation by HKM was 5.4-fold higher than that by HLM in the high-affinity phase. In addition, works from other researchers also reported that HKM showed higher catalytic activity toward mycophenolic acid and propofol, two typical substrates for UGT1A9, than HLM (McGurk et al., 1998; Bowalgaha and Miners, 2001), and metabolic renal clearance of propofol accounted for almost one third of the total body clearance of this drug (Hiraoka et al., 2005). As for edaravone, the total contribution of human kidney to the metabolism of edaravone should be investigated.

Metabolism study of edaravone indicated that species difference existed in urinary excretion of edaravone metabolites. In rats and dogs, urinary excretion rates of sulfate were higher than those of glucuronide, whereas in human urine, glucuronide was excreted more than sulfate (Komatsu et al., 1996). This species difference may be due to differential expression of UGT1A9 in kidneys of these species. Compared with HKM, dog kidney microsomes exhibited limited glucuronidation activity, only bilirubin was metabolized by dog kidney microsomes, and no activity was observed for propofol (Soars et al., 2001). Rat UGT1A9 was considered to be a pseudogene and does not code for functional enzyme (Emi et al., 1995; Shelby et al., 2003; Shiratani et al., 2008). These findings may have a bearing on the choice of animal species for the pharmacokinetic and toxicological assessment, because renal disorders have been reported in patients treated with edaravone (Hishida, 2007). Glucuronidation of endogenous renal mediators in human kidney has been implicated into the maintenance of renal homeostasis (Knights and Miners, 2010). Whether extensive glucuronidation in human kidney has something to do with nephrotoxicity remains to be explored.

Furthermore, interactions induced by edaravone with other medicinal or dietary components primarily catalyzed by UGT1A9 should be given attention in clinical applications. $K_{m}$ value of edaravone toward UGT1A9 is $6.0 \pm 2.0 \mu M$, which is less than $K_{m}$ values of propofol (41 $\mu M$) and mycophenolic acid (160 $\mu M$) (Picard et al., 2005; Rowland et al., 2008). Thus, edaravone has a high affinity toward UGT1A9 and may have the potential to inhibit the glucuronidation of UGT1A9 substrates. In addition, genetic polymorphisms of UGT1A9 have been implicated in the interindividual variation of mycophenolic acid metabolism (Kuypers et al., 2005; Baldelli et al., 2007; Sánchez-Fructuoso et al., 2009). The relationships between UGT1A9 polymorphisms and edaravone metabolism have never been mentioned before, but they should be paid more attention.

In conclusion, in our study, 1) edaravone glucuronidation in HLM and HKM exhibited biphasic kinetics; 2) HKM showed higher activity than HLM, indicating an important role of human kidney in edaravone glucuronidation in vivo; 3) kinetic study by using recombinant UGTs, coupled with inhibition study and correlation analysis, indicated that UGT1A9 was the main contributor to edaravone glucuronidation in HLM and HKM.

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References


