Glucuronidation of edaravone by human liver and kidney microsomes: biphasic kinetics and identification of UGT 1A9 as the major UDP-glucuronosyltransferase isoform

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Glucuronidation of edaravone by HLMs and HKMs

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UGT, UDP-glucuronosyltransferase; UDPGA, Uridine 5’-diphosphate-glucuronic acid; LC/MS/MS, liquid chromatography tandem mass sectrometry; HLMs, human liver microsome; HKMs, human kidney microsome; IS, internal standard; BSA, bovine serum albumin
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 glucuronide conjugate in human urine (about 70%), but the mechanism involved in the elimination pathway remains unidentified. We investigated the glucuronidation of edaravone in human liver microsomes (HLMs) and human kidney microsomes (HKMs) and identified the major hepatic and renal UDP-glucuronosyltransferases (UGTs) involved. As we observed, edaravone glucuronidation in HLMs and HKMs exhibited biphasic kinetics. The intrinsic clearance (CL\textsubscript{int1} and CL\textsubscript{int2}) were 8.4±3.3 μl/min/mg and 1.3±0.2 μl/min/mg for HLMs, and 45.3±8.2 μl/min/mg and 1.8±0.1 μl/min/mg for HKMs. However, in microsomal incubations contained with 2% bovine serum albumin (BSA), CL\textsubscript{int1} and CL\textsubscript{int2} were 16.4±1.2 μl/min/mg and 3.7±0.3 μl/min/mg for HLMs, and 78.5±3.9 μl/min/mg and 3.6±0.5 μl/min/mg for HKMs. Screening with twelve recombinant UGTs indicated that eight UGTs (UGT1A1, 1A6, 1A7, 1A8, 1A9, 1A10, 2B7, 2B17) produced significant amount of glucuronide metabolite. Then six UGTs (UGT1A1, 1A6, 1A7, 1A9, 2B7, 2B17) expressed in human liver or kidney were selected for kinetic studies. Among them, UGT1A9 exhibited the highest activity (CL\textsubscript{int1}=42.4±9.5 μl/min/mg), followed by UGT2B17 (CL\textsubscript{int}=3.3±0.4 μl/min/mg) and UGT1A7 (CL\textsubscript{int}=1.7±0.2 μl/min/mg). Inhibition study found that inhibitor of UGT1A9 (propofol) attenuated edaravone glucuronidation in HLMs and HKMs. And edaravone glucuronidation in a panel of seven HLM was significantly correlated (r=0.9340, p=0.0021) with propofol glucuronidation. Results indicated that UGT1A9 was the main UGT isoform involved in edaravone glucuronidation in HLMs and HKMs.
Introduction

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, MCI-186, Fig1A), a potent free radical scavenger, has been approved in Japan for the treatment of acute cerebral infarction since 2001 (Watanabe T et al., 2008; Lapchak PA, 2010). It is the first neuroprotectant developed for stroke treatment. After launching for more than ten years, it is still widely used in Japan. The total sale of edaravone reported by Mitsubishi Tanabe for 2008 was about $272.6 million. Recently, pharmacologist have expanded its application in other organs (heart, lung, liver and kidney, etc.) suffered with ischemia-reperfusion injury (Watanabe T et al., 2008). And a phase III clinical trial for amyotrophic lateral sclerosis are ongoing in Japan (Takahashi R, 2009), in which edaravone exhibits as 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 glucuronide conjugate (about 70%, Fig1B) in human (Komatsu T et al., 1996; Yokota S et al., 1997; Shibata H et al., 1998). However, no detailed data was available about UGT isoforms involved in edaravone glucuronidation. As 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 to be the major contributor to drug glucuronidation, but extrahepatic tissues or organs may also be involved (Tukey RH et al., 2000). Considering that renal disorders has been reported in patients treated with edaravone (Hishida A, 2007), investigation of edaravone glucuronidation by HKMs may help to understand the organ-targeted adverse affect.
As an initial step, edaravone glucuronide, 3-methyl-1-phenylpyrazol-5-yl-β-D-glucuronic acid has been synthesized by our research group (Zhu XR et al., 2010). For the measurement of edaravone glucuronide, an LC/MS/MS method has been built. The main objective of this study is to characterize the glucuronidation of edaravone in HLMs and HKMs, and to identify the hepatic and renal UGTs involved in the metabolism of edaravone.
Materials and Methods

Chemicals and materials. Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Edaravone glucuronide have been synthesized by our research group with purity >95%. Uridine 5’-diphosphate-glucuronic acid (UDPGA), D-saccharic acid 1,4-lactone, β-glucuronidase (Escherichia coli), alamethicin, estradiol, propofol, naloxone, androsterone and phenacetin (internal standard) were all purchased from Sigma Chemical (St. Louis, MO, USA). Pooled human liver and kidney microsomes were purchased from Research Institute for Liver Diseases (Shanghai) Co LTD. Pooled HLMs was prepared from ten Mongolian donors aged from 24 to 38 and pooled HKMs was prepared from five Caucasian donors aged from 30 to 74. Seven individual human livers and a panel of recombinant human UGT Supersomes™ (UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15 and 2B17) expressed in baculovirus-infected insect cells were obtained from BD Biosciences (Bedford, MA, USA). Milli-Q water was used in all steps and all other chemicals were of HPLC grade or the best grade that was commercially available.

LC/MS/MS determination. The analysis for edaravone glucuronide was performed in a Finnigan Surveyor™ HPLC system (Thermo Electron, San Jose, CA, USA) coupled with a Finnigan TSQ Quantum Discovery Max system (Thermo Electron, San Jose, CA, USA). Mass spectrometers applied an Electrospray Ionization Source in the positive ion mode. Data acquisition was performed with Xcalibur 1.2 software (Thermo Finnigan, USA). Separation of analytes was achieved with an Agilent ZORBAX Eclipse Plus C18 column (2.1mm×150mm i.d.3.5 µm) 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: 0–2.5 min, 40% A; 2.5–3.0 min,
increase A to 70%; 3.0-7.5 min, 70% A; 7.5-8 min, decrease A to 40%; a run-time, 11 min. The optimized mass conditions were summarized as follow: spray voltage was set at 5kV and capillary temperature was set at 267°C; sheath gas (N₂) and auxiliary gas (N₂) were 35 Arb 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 internal standard (m/z 180.1→110.1) were monitored in the multiple reaction monitoring (MRM) mode.

**Glucuronidation in pooled HLMs and HKMs.** For optimization of the incubation conditions, the linearity of metabolite formation with time (10 to 120 min) and protein (0.05 to 1 mg/ml) in HLMs, and with time (15-90 min) and protein (0.025 to 0.5 mg/ml) in HKMs were 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 terminated by adding 600 μl ice-cold acetonitrile containing 20 ng/ml phenacetin (IS). The mixtures were vortexed and centrifuged (18000 rpm at 4 °C for 10 min) to obtain the supernatants, of which 5 μl was subjected to analysis. Incubations without UDPGA were served as negative controls.

**Glucuronidation by Recombinant UGTs.** A panel of recombinant human UGTs (UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15 and 2B17) was used to screen for the glucuronidation of edaravone at two concentrations (12.5 μM and 500 μM). Incubation conditions were similar to those of HLMs and HKMs except that the protein concentration was 0.2 mg/ml. Kinetic studies for six main UGTs (UGT1A1, 1A6, 1A7, 1A9, 2B7 and 2B17) 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 50-5000 μM for UGT1A1 and 2B7, 10-2500 μM for UGT1A6 and 1A7, 2-2500 μM for UGT1A9 and 5-2500 μM for UGT2B17.

**Chemical Inhibition Study.** Edaravone glucuronidation in HLMs, HKMs and recombinant UGTs (UGT1A1, 1A9, 2B7 and 2B17) were evaluated in the presence or absence of known chemical inhibitors. Estradiol for UGT1A1 (Watanabe Y et al., 2003), propofol for UGT1A9 (Picard N et al., 2005), naloxone for UGT2B7 (Di Marco A et al., 2005) and androsterone for UGT2B17 (Sten T et al., 2009) were used as corresponding inhibitors. Substrate concentrations for HLMs and HKMs were 40 μM and 10 μM, and for recombinant UGTs were around their individual Km values (200 μM for UGT1A1, 10 μM for UGT1A9, 2 mM for UGT2B7 and 100 μM for UGT2B17). Different concentrations of estradiol (10, 20, 50 μM), propofol (100, 400, 1000 μM), naloxone (200, 500, 1000 μM) and androsterone (5, 20, 35 μM) were applied to inhibit edaravone glucuronidation in HLMs, HKMs and recombinant UGTs. Considering that UGT 1A1 are not present in human kidney (Ohno S et al., 2009; Knights KM et al., 2010), inhibition by estradiol was conducted in recombinant UGT 1A1 and HLMs.

**Correlation analysis.** A correlation analysis was carried out between the activities of edaravone glucuronidation versus propofol glucuronidation (UGT1A9), estradiol 3-glucuronide (UGT1A1) or seroquine glucuronidation (UGT1A6) in seven donor human liver microsomes. Glucuronidation activity of edaravone was measured when substrate and protein concentrations were 40 μM and 0.1 mg/ml, respectively. Glucuronidation activities of estradiol-3β-glucuronidation (UGT1A1) and propofol (UGT1A9) glucuronidation were provided by the manufacturer. Serotonin (UGT1A6) glucuronidation activity was measured based on a previously reported method (Krishnaswamy S et al., 2003). Correlation analysis was conducted and p value less than 0.05 was considered statistically significant.
Enzyme Kinetic Data Analysis. First of all, data were transformed and Eadie-Hofstee curves were plotted, which help to identify kinetic models (Hutzler JM et al., 2002). Then kinetic parameters were obtained by fitting velocity data to the appropriate kinetic models (equation 1 and 2) using GraphPad Prism 5.0 software (CA,USA).

Michaelis-Menten model (Iwuchukwu OF et al., 2008):

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

\text{(1)}

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

Biphasic kinetic model (Iwuchukwu OF et al., 2008):

\[ v = \frac{V_{max1} \cdot [S]}{K_{m1} + [S]} + \frac{V_{max2} \cdot [S]}{K_{m2} + [S]} \]  

\text{(2)}

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. It was sure that edaravone did not influence the detection of its glucuronide (Fig 2). As the incubation mixtures contained high concentration of edaravone, edaravone was not detected in the final method. The MS/MS spectra of edaravone glucuronide was presented in Fig 3.

**Edaravone glucuronidation by HLMs and HKMs.** Researchers have found the “albumin effect” in drug glucuronidation, and glucuronidation clearance was recently investigated in the presence of 2% BSA or fatty acid-free human serum albumin (Rowland A et al., 2007; Rowland A et al., 2008). We investigated edaravone glucuronidation in HLMs and HKMs, in the presence or absence of 2% BSA. As showed 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, twelve commercially available recombinant UGTs were evaluated for their ability to metabolize edaravone, and a low concentration of 12.5 µM (close to $K_{m1}$ values of HLMs and HKMs) and a high concentration of 500 µM (close to $K_{m2}$ values of HLMs and HKMs) were used, see Fig 5. Incubations with low concentration indicated that detectable edaravone glucuronides were generated in five UGTs and they were ordered as UGT1A9, 1A7, 2B17, 1A6 and 1A8. 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, 1A6, 1A9, 1A7, 2B7, 2B17, 1A1 and 1A10.

**Kinetics of edaravone glucuronidaion by recombinant UGTs.** Among the eight UGTs with high activity, UGT1A8 and UGT1A10 were mainly expressed in gastrointestinal tract (Strassburg CP et al., 1999; Bowalgaha K et al., 2005; Ohno S et al., 2009). This study focused primarily on the hepatic and renal glucuronidation of edaravone, so the kinetic studies were performed in UGT1A1, 1A6, 1A7, 1A9, 2B7 and 2B17. The curves are presented in Fig 6-1 and Fig 6-2 and the kinetic parameters are listed in Table 2. The kinetic profiles of UGT1A6, 1A7, 2B7 and 2B17 were fitted to the Michaelis-Menten model, and those of UGT1A1 and 1A9 were fitted to the biphasic kinetics model. The fitting for UGT1A1 was weighted by $1/X^2$. With the linear increased at higher concentration, the apparent $K_m2$ and $V_{max2}$ for UGT1A1 could not be estimated. Of those UGTs studied, UGT1A9 showed significant high affinity and activity with a $K_m1$ of 6.0±2.0 μM and a $CL_{int1}$ of 42.4±9.5 μl/min/mg.

**Chemical inhibition results.** The inhibitory effects of estradiol (UGT1A1), propofol (UGT1A9), naloxone (UGT2B7) and androsterone (UGT2B17) on edaravone glucuronidation were investigated in recombinant UGTs, HLMs and HKMs. As showed in Fig 7, glucuronidation activities in recombinant UGT isoforms were all reduced by inhibitors with a concentration-dependent manner. However, in HLMs and HKMs, it was found that (1) propofol inhibited edaravone glucuronidation in a concentration-dependent manner, (2) naloxone and androsterone had no effect on edaravone glucuronidation, (3) high concentration of estradiol had a slight inhibitory effect in HLMs. Results from these indicated that UGT1A9 might contribute mainly to edaravone glucuronidation in HLMs and HKMs, and in high substrate concentration, UGT1A1 might be involved in edaravone glucuronidation in HLMs.
**Correlation results.** To further assess the contribution of UGT isoforms, correlation analysis were conducted with alamethicin-activated HLM from seven individual human donor livers at a substrate concentration of 40 µM. As displayed 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 seronine 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 M et al., 2003). In this study we explored the comparative role of HLMs and HKMs in edaravone glucuronidation and identified the main UGT isoforms responsible for edaravone glucuronidaiton by investigating reactions in pooled HLMs and HKMs, 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 HLMs and HKMs. Clinical studies showed that the maximal plasma concentration of therapeutic dose (0.5 mg/kg) of edaravone was around 6 μM (Yokota S et al., 1997), which was less than K_{m1} values of HLMs and HKMs and much less than K_{m2} value of them. It is likely that high affinity phase contributed mainly to the edaravone glucuronidation in vivo. In high affinity phase, HKMs exhibited 5.4- and 4.8- fold higher CL_{int1} than HLMs, 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 CL_{int1} in HLMs and HKMs. Until now, enhancement of UGTs activity by albumin was only found in UGT1A9 and UGT2B7, but not in UGT1A1 and UGT1A6 (Tsoutsikos P et al., 2004; Rowland A et al., 2007; Rowland A et al., 2008; Kilford PJ et al., 2009).

Screening with twelve recombinant UGT isoforms showed that eight UGTs produced significant amount of glucuronide metabolite. And among them, six UGTs (UGT1A1, 1A6, 1A7, 1A9, 2B7 and 2B17) expressed in human liver and kidney were selected for kinetic study.
UGT1A9 showed significant high affinity and activity ($K_{m1}=6.0\pm2.0 \mu M$, $CL_{int1}=42.4\pm9.5 \mu l/min/mg$), making it the focus of interest. Notably, the $K_{m1}$ and $CL_{int1}$ values for UGT1A9 were close to the values for HKMs ($K_{m1}=7.4\pm1.8 \mu M$ and $CL_{int1}=45.3\pm8.2 \mu l/min/mg$). A substrate which is solely metabolized by a single UGT isoform should have a similar $K_m$ value compared with that by pooled microsomes (Court MH et al., 2003). It seems that UGT1A9 may be the predominant UGT isoform for edaravone glucuronidation in HKMs.

Chemical inhibition study demonstrated that UGT1A9 was the main contributor to edaravone glucuronidation in HLMs and HKMs. And correlation study in seven HLM also demonstrated that UGT1A9 was the most important one. Correlation assay in HKM has failed to conduct as different human kidney microsomes were not available at present. However, we found in previous reports that the mRNA expression of UGT1A6 vs UGT1A9 in human liver was 0.39/1 and in human kidney was 0.13/1 (Ohno S et al., 2009). Since human kidney expressed lower ratio of UGT1A6 vs UGT1A9, we deduced that UGT1A6 may not be the major isoform for the glucuronidation of edaravone in human kidney.

Results from Ohno (Ohno S et al., 2009) indicated that the mRNA expression of UGT1A9 in HKMs was 4.9-fold higher than in HLMs, which was consistent with our observation that edaravone glucuronidation by HKMs was 5.4-fold higher than HLMs in high affinity phase. Apart from this, works from other researchers also reported that human kidney microsomes showed higher catalytic activity towards mycophenolic acid and propofol, two typical substrates for UGT1A9, than human liver microsomes (McGurk KA et al., 1998; Bowalgaha K et al., 2001), and metabolic renal clearance of propofol accounted for almost one-third of the total body clearance of this drug (Hiraoka H et al., 2005). As for edaracone, the total contribution of human kidney for the metabolism of edaravone should be investigated in the future.
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 glucuronide, while in human urine, glucuronide excreted more than sulfate (Komatsu T et al., 1996). This species difference may due to differential expression of UGT1A9 in kidneys of these species. Compared with HKMs, dog kidney microsomes (DKMs) exhibited limited glucuronidation activity, and only bilirubin was metabolized by DKMs and no activity was observed for propofol (Soars MG et al., 2001). Rat UGT1A9 was considered to be a pseudogene and do not code for functional enzyme (Emi Y et al., 1995; Shelby MK et al., 2003; Shiratani H et al., 2008). These may have a bearing on the choice of animal species for the pharmacokinetic as well as toxicology assessment, as renal disorders has been reported in patients treated with edaravone (Hishida A, 2007). Glucuronidation of endogenous renal mediators in human kidney has been implicated into the maintenance of renal homeostasis (Knights KM et al., 2010). Whether or not extensive glucuronidation in human kidney have some thing to do with nephrotoxicity remains to be explored in the future.

Furthermore, interactions induced by edaravone with other medicinal or dietary components primarily catalyzed by UGT1A9 should be given attention in clinical applications. $K_{m1}$ values of edaravone towards UGT1A9 is 6.0±2.0 μM, which is less than $K_m$ values of propofol (41 μM) and mycophenolic acid (160 μM) (Picard N et al., 2005; Rowland A et al., 2008). Thus, edaravone has a high affinity towards 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 DR et al., 2005; Baldelli S et al., 2007; Sanchez-Fructuoso AI et al., 2009). The relationships between UGT1A9 polymorphisms and edaravone metabolism have never been mentioned before, which
should be paid more attention.

In conclusion, our study indicated that (1) edaravone glucuronidation in HLMs and HKMs exhibited biphasic kinetics; (2) HKMs showed higher activity than HLMs, 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 HLMs and HKMs.
Acknowledgements

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Authorship Contributions.

Participated in research design: Ma, Sun, Peng, Zhang, Cheng, G.J.Wang

Conducted experiments: Ma, J.P.Zhu, X.J.Wang, Y.C.Zhu, Wan, Feng, Wu

Contributed new reagents: Shao, Hu

Performed data analysis: Ma, Sun, Peng, Zhang

Contributed to the writing of the manuscript: Ma, Sun, Peng
Reference


Footnotes

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**Legends for figures**

Fig 1 Structure of Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) (A) and its glucuronide (B).

Fig 2 Representative chromatogram of edaravone (m/z 175.1 → 133.1), edaravone glucuronide (m/z 351.3 → 175.1) and phenacetin (m/z 180.1 → 110.1, internal standard) in incubation mixtures.

Fig 3 MS/MS spectra of edaravone glucuronide.

Fig 4 Atypical kinetic profiles for formation of edaravone glucuronide in the absence of 2% BSA in HLMs (A) and HKMs (B), and in the presence of 2% BSA in HLMs (C) and HKMs (D). Inset, Eadie-Hofstee plots for each of the profiles are shown. In the absence of 2% BSA, incubations were carried out across 5 to 2500 µM substrate concentration range for HLMs and 2.5 to 2500 µM for HKMs. In the presence of 2% BSA, incubations were carried out across 1 to 5000 µM substrate concentration range for HLMs and HKMs. Each data points represented mean±SD of triplicate determinations.

Fig 5 Formation of edaravone glucuronide by recombinant human UGT isoforms. 12.5 μM and 500 μM edaravone were incubated with recombinant human UGT isoforms (UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15, and 2B17) at the protein concentration of 0.2 mg/ml and other incubation conditions are similar with HLMs and HKMs. Each bar is the mean±S.D. of triplicate determinations.

Fig 6. Kinetic profiles for formation of edaravone glucuronide in UGT1A1 (A), 1A6 (B), 1A7 (C), 1A9 (D), 2B7 (E), 2B17 (F). Inset, Eadie-Hofstee plots for each of the profiles are shown. Data for UGT1A1 represented mean ± S.D. of duplicate determinations and for others represented mean±S.D. of triplicate determinations.

Fig 7 Inhibitory effects of typical substrates for the formation of edaravone glucuronide in recombinant human UGT isoforms, HLMs and HKMs. Estradiol (UGT1A1), isoprofol (UGT1A9), naloxone (UGT2B7) and androsterone (UGT2B17) were used as inhibitors. And each data point represents mean±SD of triplicate incubations.
Fig 8 Correlation analysis between edaravone glucuronidation in seven individual HLM and the typical UGT1A9 substrate (propofol), UGT1A1 substrate (Estradiol) and UGT1A6 substrate (Serotonin). p value less than 0.05 was considered statistically significant. Each data point represented mean±SD of triplicate incubations.
Table 1 Kinetic parameters of edaravone glucuronidation in HLMs and HKMs

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<td>HKMs</td>
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<td>$K_m$ (µM)</td>
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<td>$CL_{int}$ (µl/min/mg)</td>
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Table 2 Kinetic parameters of edaravone glucuronidation in UGT1A1, 1A6, 1A7, 1A9, 2B7, 2B17.

<table>
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<th>Enzyme</th>
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<th>(CL_{int}) ((\mu)l/min/mg)</th>
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<td>UGT1A1</td>
<td>(K_{m1}=284.8\pm199.6)</td>
<td>(V_{max1}=45.2\pm28.0)</td>
<td>(CL_{int1}=0.2\pm0.0)</td>
</tr>
<tr>
<td>UGT1A6</td>
<td>777.7\pm37.0</td>
<td>1244.0\pm24.5</td>
<td>1.6\pm0.0</td>
</tr>
<tr>
<td>UGT1A7</td>
<td>432.3\pm62.4</td>
<td>740.2\pm34.6</td>
<td>1.7\pm0.2</td>
</tr>
<tr>
<td>UGT1A9</td>
<td>(K_{m1}=6.0\pm2.0)</td>
<td>(V_{max1}=254.5\pm33.0)</td>
<td>(CL_{int1}=42.4\pm9.5)</td>
</tr>
<tr>
<td></td>
<td>(K_{m2}=419.4\pm183.4)</td>
<td>(V_{max2}=340.4\pm31.4)</td>
<td>(CL_{int2}=0.8\pm0.4)</td>
</tr>
<tr>
<td>UGT2B7</td>
<td>2384.0\pm376.6</td>
<td>1769.0\pm129.1</td>
<td>0.7\pm0.1</td>
</tr>
<tr>
<td>UGT2B17</td>
<td>128.6\pm17.1</td>
<td>428.7\pm14.5</td>
<td>3.3\pm0.4</td>
</tr>
</tbody>
</table>
Fig 1

A

B
Fig 7

(A) UGT1A1 and HLMs

Percent Activity (%)

Estradiol (μM)

0 10 20 50

(B) UGT1A9, HLMs, HKMs

Percent Activity (%)

Propofol (μM)

0 100 400 1000

(C) UGT2B7, HLMs, HKMs

Percent Activity (%)

Naloxone (μM)

0 200 500 1000

(D) UGT2B17, HLMs, HKMs

Percent Activity (%)

Androsterone (μM)

0 5 20 25
Fig 8

A. Estradiol-3-glucuronidation (pmol/min/mg protein) vs. V (pmol/min/mg protein)
- UGT1A1: r = 0.2934, p = 0.5230

B. Serotonin glucuronidation (pmol/min/mg protein) vs. V (pmol/min/mg protein)
- UGT1A6: r = -0.1997, p = 0.6677

C. Propotal glucuronidation (pmol/min/mg protein) vs. V (pmol/min/mg protein)
- UGT1A9: r = 0.9340, p = 0.0021