Absolute Difference of Hepatobiliary Transporter Multidrug Resistance-Associated Protein (MRP2/Mrp2) in Liver Tissues and Isolated Hepatocytes from Rat, Dog, Monkey, and Human

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ABSTRACT:

We previously reported that hepatobiliary transporter multidrug resistance-associated protein (MRP2/Mrp2) is considered to be the major cause of the interspecies differences detected by efflux of fluorescent substrates in isolated hepatocytes. In the present study, the interspecies differences of MRP2/Mrp2 were first evaluated by quantitative real-time polymerase chain reaction and Western blotting. The mRNA levels were able to distinguish the difference among species with a rank order comparable with the corresponding activities observed, whereas the extents of the differences remained unknown. The cross-reactions of MRP2/Mrp2 protein of different species with anti-human MRP2 polyclonal antibody were found by Western blotting. However, because of the unknown binding affinity of antibody to MRP2/Mrp2 protein across species and lack of purified MRP2/Mrp2 proteins for calibration, the immunoblotting assay was excluded from the absolute quantification of MRP2/Mrp2 protein for multiple species. By using our newly developed liquid chromatography-tandem mass spectrometry quantification method, we were able to measure the absolute amount of MRP2/Mrp2 in liver tissues and isolated hepatocytes across species. Freshly isolated hepatocytes conserved MRP2/Mrp2 protein levels that are comparable with those in the liver tissues. The amount of Mrp2 in rat liver was approximately 10-fold higher than that in other species. Moreover, a significant loss of Mrp2 protein in the membrane fraction of rat cryopreserved hepatocytes was observed. Thus, the absolute differences of MRP2/Mrp2 levels in various species were determined, for the first time, by direct quantification. The results could potentially fill the translational gaps of in vitro/in vivo or preclinical species to human extrapolation of hepatobiliary elimination mediated by MRP2/Mrp2.

Xenobiotics and their metabolites are generally eliminated and detoxified by phase I and phase II enzymatic metabolism, by phase III transporter-mediated drug efflux to bile, or by both mechanisms. The excretion of drugs by hepatocytes into bile is one of the primary elimination routes for xenobiotics and the conjugate metabolites (Arias et al., 1993). In each stage of drug discovery, accurate prediction of human pharmacokinetics for a potential drug candidate is of great value (Mahmood, 1999). Even though the interspecies scaling methods based on physiologically allometric procedures have been successfully applied, particularly into extrapolation of hepatic enzymatic metabolism and urinary excretion (Dedrick et al., 1970; Iwatsubo et al., 1997; Ito et al., 1998), the in vitro or in vivo model for biliary excretion predication is far from being mature (Mahmood and Sahajwalla, 2002). The remarkable interspecies differences in biliary excretion of xenobiotics and drugs/metabolites (Ishizuka et al., 1999; Shilling et al., 2006) may cause significant overestimation of biliary excretion in humans simply by an exponential allometric extrapolation approach (Lave et al., 1999; Pahlman et al., 1999; Ayrton and Morgan, 2001). Therefore, understanding the molecular mechanisms underlying the marked species difference in hepatobiliary elimination of drugs and their metabolites should greatly advance the current allometric scaling models for estimation of human pharmacokinetics.

Several ATP-binding cassette efflux transporters are responsible for the hepatobiliary elimination of therapeutic agents and physiological substances (Keppler and Arias, 1997; Muller and Jansen, 1997; Suzuki and Sugiyama, 1999). Even though the interspecies differences in other transporters involving hepatobiliary elimination remain obscure, the marked species differences in MRP2/Mrp2 transporter activity have been reported in both in vitro and in vivo models (Ishizuka et al., 1999; Ninomiya et al., 2005; Shilling et al., 2006). For example, Ishizuka et al. (1999) found that the in vitro transport of 2,4-dinitrophenyl-S-glutathione into canalicular membrane vesicles was 8-fold higher ($V_{max}/K_m$: 64.2 versus 7.7) in rat than in dog, whereas the in vivo biliary excretion of temocaprilat was 40-fold higher in rat than in dog. Even though species differences in Mrp2-mediated biliary efflux have previously been reported, agreement on the degree of difference remains to be determined, because the absolute differences of transporters in these models remain unknown.

A LC-MS/MS-based absolute quantitative method (also known as
AQUA), has been widely used in protein quantification in biological and clinical samples (John et al., 2004). However, the application for the measurement of membrane proteins has been delayed because of their hydrophobic nature and extremely low expression level (Barnidge et al., 2003). In a recent study, a strategy to absolutely quantify MRPP2/Mrp2 across species has been developed in our laboratory and has been found to be a highly sensitive, selective, accurate, and precise method (Li et al., 2008b). In the present study, the absolute amount of MRPP2/Mrp2 was measured in membrane fractions of liver tissues and isolated hepatocytes from various species. To our knowledge, coupled with the quantitative measurement of mRNA levels and immunologic based protein detection, this was the first comprehensive report of the absolute differences of MRPP2/Mrp2 protein levels across species. The findings could provide fundamental support for in vitro/vivo correlation and preclinical animal to human pharmacokinetics prediction and further promote the progression of pharmaceutical practice in drug discovery.

Materials and Methods

Chemicals and Reagents. High-performance liquid chromatography-grade acetonitrile and water were purchased from Burdick & Jackson (Muskegon, MI) and EMD Chemicals, Inc. (Gibbstown, NJ), respectively. Formic acid and anti-human MRPP2 polyclonal antibody were obtained from Sigma-Aldrich (St. Louis, MO). The protein quantification BCA kit and the in-solution digestion kit were purchased from Pierce Biotechnology (Rockford, IL). The ProteoExtract Native Membrane Protein Extraction Kit was purchased from Calbiochem (Temecula, CA). PrecaT Tris-HCl SDS-polyacrylamide gel electrophoresis gradient gel, Coomasie Blue R-250, and the destaining buffer were purchased from Bio-Rad (Hercules, CA). The enhanced chemiluminescence ECL plus kit was purchased from GE Healthcare (Little Chalfont, Buckinghamshire, UK). The RNeasy and RNase-free DNase kits were purchased from Qiagen (Valencia, CA). SYBR SuperMix was purchased from Applied Biosystems (Foster City, CA).

Snap-Frozen Liver Tissues. Snap-frozen liver tissues from 15 normal human donors aged from 1 to 78 years (7 males and 8 females), 6 beagle dogs, 4 rhesus monkeys, and 7 cynomolgus monkeys were obtained from the Pfizer Animal Care and Use Program, Inc., Darmstadt, Germany. The animal care and use program is fully accredited by the St. Louis Pfizer Institutional Animal Care and Use Committee. After 30 min of incubation at 4°C with rotating, the supernatant containing cytosolic protein was removed, and the pellets were resuspended in extraction buffer II with the proper amount of protease inhibitors. After 30 min of incubation at 4°C with rotating, the suspension was centrifuged at 14,000 rpm for 15 min at 4°C. The supernatant containing cytosolic protein was removed, and the pellets were resuspended in extraction buffer II with the proper amount of protease inhibitors. After 30 min of incubation at 4°C with rotating, the suspension was centrifuged at 14,000 rpm for 15 min at 4°C. The supernatant-containing membrane protein was collected and stored at −80°C for future analysis. Protein concentrations of the membrane fraction obtained were determined with a BCA protein assay kit (Pierce Biotechnology).

Western Blotting Analysis. An aliquot of the membrane extraction (30 μg of protein) was mixed with an equal volume of Laemmli buffer (Bio-Rad) and incubated at room temperature for 5 min. The denaturated samples were then fractionated on 4 to 20% gradient SDS gel (Bio-Rad) and electrophoretically transferred on to a nitrocellulose membrane (Bio-Rad). After incubation with blocking reagent, the membrane was washed and incubated with anti-human MRPP2 polyclonal antibody at 1:500 dilution overnight at 4°C. Bound antibody was detected with horseradish peroxidase-conjugated anti-rabbit antibody and visualized with the enhanced chemiluminescence ECL plus kit. Coomassie Blue staining of the gel served as a loading control.

Tryptic Digestion and Sample Preparation. The membrane protein samples were diluted to a working concentration of 2 μg/μL and 20 μL of the extracted membrane protein (40 μg of protein) was subsequently reduced with 10 mM dithiothreitol and alkylated with iodoacetamide in 50 mM ammonium bicarbonate digestion buffer. After addition of 50 fmol of stable isotope-labeled (SIL) MRPP2 peptide serving as the internal standard (Li et al., 2008b), the protein samples were digested by trypsin in a final volume of 40 μL at 37°C for 4 h and then at 30°C for 14 h. The optimal ratio of trypsin and protein was 1:50. At the end of digestion, the samples were acidic with equal amounts of 50% acetonitrile in H2O containing 0.1% formic acid and centrifuged at 3000 rpm for 20 min before LC-MS/MS analysis. A 16-mer synthetic peptide corresponding to MRPP2/Mrp2 tryptic fragment (LTIQPDPILFSGSLR) and the SIL internal standard (LTIQPDPILFSGSLR16C43N30JR) were obtained from Celtek Bioscience (Nashville, TN) and Sigma-Aldrich, respectively. The calibration curve was prepared at a range of concentrations of the synthetic AQUA peptide, respectively, of 31.25, 62.5, 125, 250, 500, 1000, and 2500 pM with the SIL internal standard at a fixed concentration of 2500 pM. Data were processed by integrating the appropriate peak areas generated from the reconstructed ion chromatograms for the 16-mer analyte peptide and the SIL internal standard peptide by Analyst 1.4.1 (Applied Biosystems). The ratio of the peak area of the AQUA peptide to the SIL peptide (y) was plotted against the concentration of the synthetic native peptide (x) for constructing the regression analysis.
LC-MS/MS Quantitative Measurement of MRP2/Mrp2 Protein. Sample quantification was conducted by coupling a triple quadruple mass spectrometer (API 4000; Applied Biosystems) to a Shimadzu LC (SCL-10A) system (Shimadzu, Wooddale, IL) and HTS PAL Leap Autosampler (Leap Technologies, Carrboro, NC). The LC column used for peptide separation and elution was a 2.1 × 100 mm C18 column containing 5-μm size beads with 300 Å pore size (Vydac EVEREST). The mobile phase A was water with 0.1% v/v formic acid, whereas mobile phase B was acetonitrile with 0.1% v/v formic acid. A linear gradient was used to achieve the chromatographic separation starting from 5% B and progressing to 35% B over a period of 30 min. A sample volume of 20 μl was injected onto the LC column at a flow rate of 0.4 ml/min. The parent-to-product transition for the AQUA peptide monitored represented the doubly charged parent (LTIIPQDPILFSGSLR)2H+ and was selected for quantitation as below: declustering potential, 50 V; collision energy, 38 V; entrance potential, 10 V; collision cell exit potential, 11 V. The instrument settings of the API4000 triple quadruple mass spectrometer were as follows: ion spray voltage, 4 kV; temperature, 400°C; declustering potential, 50 V; collision energy, 38 V; entrance potential, 10 V; and collision cell exit potential, 11 V.

Data Analysis. Data are representative of a minimum of two experiments performed on different days. The MRP2/Mrp2 protein amount across species was statistically analyzed using one-way ANOVA. Comparison of MRP2/Mrp2 expression between isolated hepatocytes and frozen liver tissues or within the same species was performed with Student’s t test. A value of p < 0.05 was regarded as statistically significant.

Results
Quantitative mRNA detection as a surrogate protein measurement has been very popular for investigation of the differences in transporter protein levels in in vitro or in vivo models (Goh et al., 2002; Hilgendorf et al., 2007). Approaching on that front, we examined the mRNA level in liver tissues and isolated hepatocytes by using qRT-PCR. Table 1 lists the primers for MRP2/Mrp2 and the control housekeeping gene GAPDH of four tested species. In freshly isolated hepatocytes and livers, the highest amount of Mrp2 mRNA among the tested species was expressed in rats, whereas the least was expressed in monkey. The mRNA level ranked as rat > human > dog > monkey (p < 0.05 by ANOVA) (Fig. 1, A and B). In cryopreserved hepatocytes, the mRNA level of Mrp2 in monkey was the lowest among the tested species, whereas no significant differences were found among human, rat, and dog (Fig. 1C).

Immunoblotting-based protein detection (e.g., Western blotting) has been a widely used assay for protein detection and quantification for decades. By using the commercially available anti-human MRP2 polyclonal antibody, we performed Western blotting assay to detect the MRP2/Mrp2 protein in membrane extractions across species. As shown in Fig. 2, anti-human polyclonal antibody was cross-reacted with the MRP2/Mrp2 proteins extracted from liver tissue (Fig. 2A), freshly isolated (Fig. 2B), or cryopreserved hepatocytes (Fig. 2C) of various tested species. Apparently, the MRP2/Mrp2 protein of human and monkey exhibited the highest cross-reactivity with the antibody, whereas the dog exhibited the least. However, because of the lack of the purified MRP2/Mrp2 proteins as calibration standard for each species, we were not able to compare the protein level across species. In addition, the results were not consistent with the mRNA levels of MRP2/Mrp2 and also not in the agreement with the differential transport activities of MRP2/Mrp2 reported previously (Ishizuka et al., 1999; Mahmood and Sahajwalla, 2002; Shilling et al., 2006; Li et al., 2008a).

In a recent study, a LC-MS-MS-mediated MRP2/Mrp2 protein quantification method has been developed in our laboratory. The method has been validated as a highly sensitive and selective quantitative approach with great accuracy and precision (Li et al., 2008b). In the present study, the LC-MS/MS method was applied to absolutely measure MRP2/Mrp2 protein in membrane fraction extracted from freshly isolated/cryopreserved hepatocytes and frozen liver samples across species. The strategy that we used was to select the proteotypic peptide representing MRP2/Mrp2 protein. The process includes a combination of comprehensive MS/MS verification of the candidate peptides and in silico prediction of the tryptic digested fragments by using the online software http://prospector.ucsf.edu/. To obtain the selective peptide exclusively representing MRP2/Mrp2 across species, a further genome-wide BLAST search was conducted to ensure the specificity. Figure 3 shows the alignment of the MRP2/Mrp2 sequence across species and highlights the position of the proteotypic peptide. A synthetic peptide (LTIIPQDPIFSGLSLR) representing the fragment cleaved from tryptic proteolysis of MRP2/Mrp2 serves as the calibration standard for LC-MS/MS quantification. Figure 4 exhibits the examples of reconstituted ion chromatograms representing the MRP2/Mrp2 peptide produced by tryptic digestion of liver membrane proteins from various species and the internal standard, SIL peptide added before tryptic digestions. A quality control study was performed to ensure the performance of the MRP2/Mrp2 quantitative method by LC-MS/MS. Both the accuracy (relative error) and precision (coefficient of variation) of all quality control results were less than 15% (Table 2), at two known concentrations (0.625 and

**TABLE 1**

<table>
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<tr>
<th>Species</th>
<th>Gene</th>
<th>GenBank No.</th>
<th>Sequence</th>
<th>Size bp</th>
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<tr>
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<td></td>
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<td></td>
<td>TAGGGGGACAGGCTCTTCTA</td>
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</tr>
<tr>
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Carrboro, NC). The LC column used for peptide separation and elution was a 2.1 × 100 mm C18 column containing 5-μm size beads with 300 Å pore size (Vydac EVEREST). The mobile phase A was water with 0.1% v/v formic acid, whereas mobile phase B was acetonitrile with 0.1% v/v formic acid. A linear gradient was used to achieve the chromatographic separation starting from 5% B and progressing to 35% B over a period of 30 min. A sample volume of 20 μl was injected onto the LC column at a flow rate of 0.4 ml/min. The parent-to-product transition for the AQUA peptide monitored represented the doubly charged parent ion (LTIIPQDPILFSGSLR)2H+ and was selected for quantitation as below: declustering potential, 50 V; collision energy, 38 V; entrance potential, 10 V; and collision cell exit potential, 11 V.
1.64 nM) of synthetic AQUA peptide spiked in the biological matrix prepared from liver samples. In addition, the great recovery rate also indicated that the potential variations caused by the discrepancy of biological matrix from different species could be omitted (Table 2). In the present study, snap-frozen liver tissues of 15 human donors aged from 1 to 78 years old, 5 rats, 6 dogs, and 11 monkeys were subjected to LC-MS/MS quantitative analysis. MRP2/Mrp2 protein measurement was also conducted for the pooled samples from three lots of cryopreserved hepatocytes of human and rats and two lots for dog and monkey. The absolute amount of MRP2/Mrp2 protein in freshly isolated hepatocytes was averaged from the lots prepared from three individual donors for human and monkey and two donors for rat and dog. Figure 5A summarizes the quantitative results of MRP2/Mrp2 in liver tissues and freshly isolated and cryopreserved hepatocytes of human, rat, dog, and monkey. In liver tissue, the MRP2/Mrp2 protein level ranked rat/dog/human with the average ranging from 0.6 to 5.5 fmol/g of membrane protein. A comparable amount of MRP2/Mrp2 was detected in freshly isolated hepatocytes of all tested species. The absolute amount of Mrp2 protein in rat was 10-fold higher in liver tissue than that in human. Impressively, a greater variation of MRP2 expression was observed in human liver donors (6-fold, ranging from 0.2 to 1.2 fmol/µg protein) and the animals (monkey and dog, ~4-fold, ranging from 0.6 to 2.7 and 0.5 to 1.7 fmol/µg protein, parallel, respectively) that have been tested with discovery compounds before tissue collecting, compared with less than 2-fold variation in rat (ranged from 4.6 to 6.1 fmol/µg protein).

Discussion

MRP2/ABCC2, belonging to the ATP-binding cassette transporter superfamily, is one of the major efflux transporters localized on the hepatic canalicular membrane in liver and has been demonstrated to be responsible for interspecies difference in hepatobiliary secretion (Niinuma et al., 1997; Ishizuka et al., 1999; Shitara et al., 2005). Species differences in MRP2/Mrp2 activity have been reported in both in vivo and in vitro models (Ishizuka et al., 1999; Shilling et al., 2006) and are considered to be one of the major causes of the failure in interspecies scaling from preclinical animal to human. As a general rule, two major factors are commonly used to characterize transporter-mediated drug clearance: $V_{\text{max}}$ and $K_m$. Although the $K_m$ is the unique property of a certain transporter substrate and usually is a fixed parameter, $V_{\text{max}}$ is determined by the expression level of a given transporter in a tissue or the model being applied. The $V_{\text{max}}$ varies by tissues and individual species and is sensitive to treatment with inducers and suppressors. During the past decade, various in vitro systems have been developed to determine the transport kinetics or modulate recognition in a variety of species. However, direct comparisons of the absolute amount of MRP2/Mrp2 protein across species...
have not been addressed. In addition, as the in vitro “golden tool” for
drug metabolism research, freshly isolated and cryopreserved hepa-
tocytes are most commonly and widely used as in vitro liver models
in drug discovery. The retention of hepatobiliary transporters remains
unknown, when the polarized structure of hepatocytes is disrupted
during the hepatocyte isolation/cryopreservation process. In a recent
study, we demonstrated that the elimination rate of an MRP2/Mrp2-
specific substrate was 4- to 6-fold faster in isolated rat hepatocytes
than in human hepatocytes (Li et al., 2008a). However, the extrapolation
of transporter-mediated drug clearance from in vitro to in vivo
still remains a challenge because the assumption was made that the
amount of transporters remains unchanged or underwent a similar
degree of loss during the hepatocyte preparation from each species. As
another example, the internalization of Mrp2 and P-glycoprotein in
freshly isolated rat hepatocytes has recently been reported (Bow et al.,
2008). The report could be expected to produce obvious discrepancies
in the functional efflux observed in isolated hepatocytes (Oude Elf-
erink et al., 1990; Lam and Benet, 2004; Li et al., 2008a). Therefore,
investigation into the absolute amount of transporter proteins existing
in isolated or cultured hepatocytes would be of great value for under-
standing the protein-activities relationship.

Several attempts were made in our laboratory to elucidate the molec-
ular mechanisms underlying the species differences of MRP2/Mrp2 ac-
tivities in isolated hepatocytes (Li et al., 2008a). As mRNA quantification
has been used widely as an alternate measurement of protein, we first
evaluated the species difference of MRP2/Mrp2 in mRNA expression.
In both liver tissues and isolated hepatocytes, the rank order and the
extent of difference in mRNA levels among the species were not
consistent with the corresponding activities reported previously (Li et
al., 2008a) or the uptake $V_{\text{max}}$ of MRP2 substrate, 2,4-dinitrophenyl-
S-glutathione, into canalicular membrane vesicles prepared from rat
and human liver (1.9 versus 0.23 nmol/min/mg protein) (Ishizuka et
al., 1999). The results revealed that the mRNA levels of MRP2/Mrp2
in liver tissue or isolated hepatocytes might not directly reflect the
amount of functional protein existing on plasma membrane. In fact,
despite some examples of the concordance between mRNA and protein
expression of the drug transporters published in literature, skepticism
regarding mRNA as a surrogate protein measurement is frequently
a concern (Behrens et al., 2004; Taipalensuu et al., 2004; Jones et al., 2005). It has been reported that the translation of rat Mrp2
mRNA is differentially regulated by the upstream open reading
frames in the 5'-untranslated region (Zhang et al., 2007) and results in
the disconnection between mRNA level and protein amounts. Fur-
thermore, post-transcriptional mechanisms may play a more promi-
nent role in lipopolysaccharide-induced regulation of human MRP2
and bile salt export pump compared with the counterparts in rat
(Elferink et al., 2004). Thus, one must exercise caution with regard to
using qRT-PCR as an alternative approach to absolute quantification
of MRP2/Mrp2 protein in liver or isolated hepatocytes. The different
mRNA expression profile observed in cryopreserved hepatocytes
(both in individual measurements and rank order) compared with that
in liver tissues and fresh isolated hepatocytes was postulated to result
from the cryopreservation procedure, which was further continued by
the absolute measurement of MRP2/Mrp2 protein discussed below.
Because of the discrepancy between the mRNA expression level and
the corresponding transport activities of MRP2/Mrp2, a more relevant
caracterization, i.e., direct quantification, of interspecies differences
in hepatobiliary transporters should be considered. Therefore, in the
present study, we also attempted to quantify the proteins by immu-
noblotting assay. However, given the unknown affinity of this anti-
body to MRP2/Mrp2 protein of different species, purified MRP2/
Mrp2 proteins are essential as the calibration standard for protein
quantification. In this context, the immunoblotting-based approach for
protein quantification of MRP2/Mrp2 was excluded owing to lack of
the purified proteins.

Coupled to one- or two-dimensional gel electrophoresis, LC-MS/MS
has been increasingly used for a variety of biomarker evaluations and
quantification (Wu et al., 2002; Barnidge et al., 2004; Kuhn et al., 2004).
In a recent study, we have overcome serial obstacles in developing a
method to absolutely quantify MRP2/ABCC2 for multiple species by
LC-MS/MS (Li et al., 2008b). In the present study, we were able to
demonstrate the absolute amount of MRP2/Mrp2 protein ranked as rat
rail monkey > human = dog in liver tissues and isolated hepatocytes.
Although the level of MRP2/Mrp2 protein in the membrane fraction of
freshly isolated hepatocytes was conserved comparable with that of
whole liver tissue, a significant loss (40%) of Mrp2 was found in rat
cryopreserved hepatocytes at 3.24 versus 5.71 or 5.45 fmol/g blackened in fresh
isolated hepatocytes and frozen liver tissues, respectively (Fig. 5A). Even
though the causes of the Mrp2 decrease in rat cryopreserved hepatocytes
were not explored here, it is speculated that the cryopreservation process
might alter the membrane density of Mrp2 protein. Significan-

cantly, the remarkable difference in Mrp2 protein level between rat cryopreserved

\[ \text{Fig. 3. Schematic representation of the membrane topology of } \text{MRP2/Mrp2 and protein alignment across species. The selected AQUA peptide is highlighted in gray. The} \]
and freshly isolated hepatocytes correlated with the previous finding that the elimination half-life of glutathione conjugates of 5-chloromethylfluorescein diacetate (a MRP2/Mrp2 substrate) was significantly longer in rat cryopreserved hepatocytes than in the freshly isolated counterpart (Li et al., 2008a). Under the notion that there could be absolute differences of MRP2/Mrp2 in liver tissues, Shilling et al. (2006) addressed the species difference of uptake clearance of leukotriene C4 (a MRP2/Mrp2 substrate) in canalicular membrane vesicles among different species. The results suggest that the absolute amount of MRP2/Mrp2 could serve as the key in the biliary excretion activities.

In the present study, a larger variation of the amount of MRP2 was observed in human samples. However, MRP2 protein level in livers among human donors varies in an age-independent manner (Fig. 5B), and there was also no significant difference between male and female (data not shown). Previous studies showed that multiple mechanisms underlie the regulation of MRP2/Mrp2 expression. For example, the MRP2 level in human placenta was affected by gestational age with an increased MRP2 protein level at later stages of pregnancy (Meyer zu Schwabedissen et al., 2005). In rat, Mrp2 protein started to be detected in livers of 16- and 20-day-old fetuses and tend to increase

**TABLE 2**

Quality control of synthetic proteotypic peptide in membrane protein matrix across species

<table>
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<th>Statistic Analysis</th>
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<td></td>
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<tr>
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<tr>
<td>Monkey 2</td>
<td>1.64</td>
<td>3</td>
<td>1.69</td>
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QC, quality control; RE, relative error; CV, coefficient of variation.
The amount of MRP2 protein and the age of the individual donor. Freshly isolated hepatocytes and liver tissues from different species and the differences in Mrp2 level among rat cryopreserved hepatocytes and human in liver tissue. Coupled with the determination of in vitro transport kinetic parameters (e.g., $K_m$), the absolute comparison of MRP2/Mrp2 protein across species could facilitate the interspecies scaling of pharmacokinetic parameters in drug discovery. Further investigation on protein synthesis and degradation of hepatic efflux transporter MRP2/Mrp2 could improve understanding of the marked differences in biliary excretion across species.

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**References**


