

Original article:

**DISTRIBUTION OF ABCG2 (BCRP) AND ABCC2 (MRP2) MRNAS
IN RAT SMALL INTESTINE AND LIVER**

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ABSTRACT

The purpose of this study is to examine the distribution of Abcg2 and Abcc2 mRNAs in the intestine and liver of male Wistar rats, and any regional differences. Nine-week-old male rats were used, and their intestine divided equally into nine segments. The expression patterns of Abcg2 and Abcc2 mRNAs in the intestine and liver were examined using a reverse transcription-polymerase chain reaction (RT-PCR). PCR products for Abcg2 mRNA were readily detectable in the intestine, but no bands were detected in the liver. In addition, the level of Abcg2 mRNA increased significantly from the duodenum to ileum, indicating a regional difference in Abcg2 mRNA expression in the intestine. This regional difference was comparable to that of Abcb1/mdr1a as reported previously. On the other hand, Abcc2 mRNA was detected at a higher level in the liver than in the intestine. However, there was no regional difference in Abcc2 mRNA expression in the intestine. Collectively, Abcg2 was predominant in the intestine rather than liver in male Wistar rats, whereas Abcc2 was predominant in the liver. These findings will provide useful information for evaluating the gastrointestinal secretion of drugs as well as drug-drug interactions in rats.

Keywords: Abcg2, Abcc2, ABC transporter, Wistar rat, RT-PCR, expression profile

INTRODUCTION

The ATP-binding cassette (ABC) transporters represent a large family of transmembrane proteins that use energy derived from the hydrolysis of ATP to transport various compounds (amino acids, lipids, lipopolysaccharides, inorganic ions, peptides, saccharides, metals, drugs, and proteins) across extra- and intra-cellular membranes (Leslie et al., 2005; Takara et

al., 2006). To date, approximately 50 transporters have been identified in humans and divided into seven subfamilies, ABCA to ABCG, based on a phylogenetic classification (Dean et al., 2001).

Previously, we demonstrated that Abcb1/mdr1a mRNA expression increased gradually from the upper to lower intestine of male Wistar rats (Takara et al., 2003). In addition, the mRNA of the cytochrome P450 (CYP) 3A subfamily was

found to be distributed differently from that of Abcb1/mdr1a, suggesting cooperation among drug clearance pathways (Takara et al., 2003, 2007). Taipalensuu et al. (2001) reported that the level of Abcg2/BCRP or Abcc2/MRP2 in normal human jejunum was higher than that of Abcb1/mdr1 (Taipalensuu et al., 2001). However, the distribution of Abcg2/BCRP in the intestine remains unclear in humans and experimental animals.

The pharmacokinetics of drugs differ between humans and experimental animals (Feng et al., 2000; Lin, 1995). Detailed and systematic studies have revealed marked differences among species in the bioavailability of many drugs (Cao et al., 2006; Chiou and Buehler, 2002; Takahashi et al., 2008). Drug transporters including Abcb1 and Abcg2 are known to contribute to the bioavailability of some drugs. Therefore, the distribution of transporters in the intestine should be clarified in experimental animals as well as humans.

Abcg2/BCRP and Abcc2/MRP2 in humans are referred to as Abcg2 and Abcc2 in rodents, respectively. The present study examined the distribution of Abcg2 and Abcc2 mRNAs in the intestine and liver of male Wistar rats using the semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) technique. Regional differences were also examined.

MATERIALS AND METHODS

Animals

Nine-week-old male Wistar rats (240 to 250 g) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). After an overnight fast with free access to water, the rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (Nembutal injection[®], Dainippon Pharmaceutical Co., Ltd., Osaka, Japan), and sacrificed by exsanguination via the aorta in the abdomen. Animal experimental protocols were carried out in accordance

with the Guidelines for Animal Experimentation at Kyoto Pharmaceutical University.

Removal of tissue and extraction of RNA

The removal of tissue from rats and the extraction of total RNA from the tissue samples were carried out as reported previously (Takara et al., 2003, 2007). That is, the intestine was excised quickly after exsanguination, and rinsed in an ice-cold 0.9 % NaCl solution. The intestine (total of ca. 90 cm) was divided into three parts; upper (almost corresponding to the duodenum, ~30 cm immediately distal to the pyloric valve), middle (almost corresponding to the jejunum, the 30 cm between the upper and lower sites), and lower (almost corresponding to the ileum, the last 30 cm before the cecum). Each part was re-divided into three (about 10 cm each), and the centers (1 cm distal) were cut out to obtain nine segments. These segments were designated S1, S2, S3, S4, S5, S6, S7, S8, and S9, in the direction from the duodenum to ileum. For hepatic samples, the liver was excised quickly after exsanguination and cut into parts (3 mm squares). Tissue samples of both intestine and liver were collected from three rats, immersed quickly into RNAlater[™] (Sigma-Aldrich Corp., St. Louis, MO), and stored at -80 °C until the extraction of RNA.

For the extraction of RNA, aliquots (3 mm squares) of the tissue samples were homogenized in RNAlater[™] using a pestle, and total RNA was extracted using a GenElute[™] Mammalian Total RNA kit (Sigma-Aldrich) according to previous reports (Takara et al., 2003, 2007).

Table 1: Gene-specific oligonucleotide primers used in the PCR for detecting mRNA

Gene		Sequence (5' to 3')	Fragment size ^{a)}
Abcg2	Forward	AGT CCG GAAAC AGC TGA GA	234
	Reverse	CCC ATC ACAACG TCATCT TG	
Abcc2	Forward	CTG GTT GGAAAC TTG GTC GT	172
	Reverse	CAA CTG CCA CAATGT TGG TC	
GAPDH	Forward	CGA TCC CGC TAA CAT CAAAT	391
	Reverse	GGATGC AGG GAT GAT GTT CT	

^{a)}Area amplified refers to sequences deposited in GenBank with accession numbers as follows: NM181381 (Abcg2), D86086 (Abcc2) and AF106860 (GAPDH). The primer sequences for Abcg2 were designed by Primer3 software (Rozen and Skaletsky, 2000). The primer sequences for Abcc2 and GAPDH were cited from a previous report (Tavelin et al., 2003; Vos et al., 1998).

RT-PCR

The RT-PCR analysis was performed as described (Takara et al., 2003, 2007). Aliquots (0.3 µg) of total RNA were used for reverse transcription with an RNA PCR kit (AMV) ver. 2.1 (TakaraBio Co., Ltd., Shiga, Japan). The RT reaction was conducted in 15 µL of RT reaction mixture, which was incubated at 30 °C for 10 min and subsequently at 42 °C for 30 min in a thermal cycler (iCycler Thermal Cycler, Bio-Rad Laboratories, Inc., CA). The reaction was terminated by heating at 99 °C for 5 min, followed by cooling at 5 °C for 5 min in a thermal cycler (Bio-Rad), giving the RT product.

PCR primer sequences for the amplification of Abcg2, Abcc2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are listed in Table 1 (Takara et al., 2003, 2007; Tavelin et al., 2003; Vos et al., 1998), and were synthesized by GeneDesign, Inc. (Osaka, Japan). The amplification of cDNA was performed in a total volume of 25 µL including 5 µL of RT product using an RNA PCR kit (AMV) ver. 2.1 (TakaraBio). After initial denaturation at 95 °C for 1 min, the three cDNAs were amplified for 22 cycles of 30 sec at 94 °C for denaturation, 1 min at 60 °C for annealing, and 1 min at 72 °C

for extension, with a final extension period of 10 min at 72 °C, using a thermal cycler (Bio-Rad). The PCR products were separated on Tris-acetate-EDTA 3 % agarose gels containing 100 ng/mL ethidium bromide, and photographed under ultraviolet illumination at 312 nm with a Polaroid camera. Band densities were measured using the computer program NIH Image ver. 1.63 (National Institutes of Health, Bethesda, MD), and the ratio of band density (target gene /GAPDH) was calculated.

Statistical analysis

Results are expressed as percentages of the ratio of Abcc2 or Abcg2 mRNA to GAPDH mRNA in the S1 region, and represent the mean ± S.E. for three rats. All results except those for the liver were compared with the S1 region by a non-repeated measures one-way analysis of variance followed by the Bonferroni test. *p* values of less than 0.05 (two-tailed) were considered significant.

RESULTS

Expression of Abcg2 and Abcc2 mRNAs in the intestine and liver of male rats

Figure 1 shows representative electrophoretograms of PCR products derived from the *Abcg2* and *Abcc2* mRNAs. *Abcg2* mRNA was detected in the intestine (Figure 1A), but not in the liver. *Abcc2* mRNA was expressed strongly in the liver and weakly in the intestine (Figure 1B).

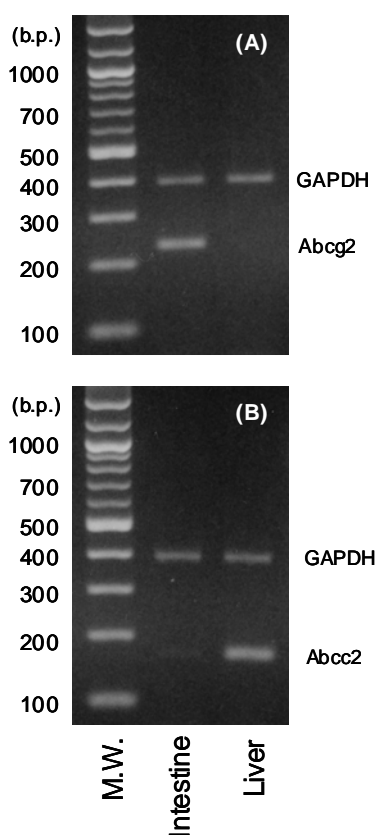


Figure 1: Expression for *Abcg2* (A) and *Abcc2* (B) mRNAs in the intestine and liver of male Wistar rats determined by RT-PCR. Rats were sacrificed, and their tissues were quickly collected into RNA later^{TM} . Total RNA was extracted from the tissue using a GenElute $^{\text{TM}}$ Mammalian Total RNA kit. PCR products were separated on a 3% agarose gel stained with 100 ng/mL ethidium bromide, to visualize the DNA bands. M.W., Molecular weight marker (100 bp DNA ladder).

Distribution of Abcg2 mRNA in the intestine

Regional differences in the expression of *Abcg2* mRNA in the intestine were evaluated by examining the PCR products (234 b.p.) derived from the mRNA (Figure 2). The level of *Abcg2* mRNA increased gradually from the upper to lower intestine, with the level of expression markedly higher in the lower region than upper or middle region.

Distribution of Abcc2 mRNA in the intestine

The PCR product (172 b.p.) derived from *Abcc2* mRNA in the intestine increased slightly in the direction from S1 (the upper region) to S4 (the middle region), but subsequently decreased in the S5 region (Figure 3). The expression from S5 to S9 was almost stable, and not significantly different from that in the S1 region.

DISCUSSION

In the present study, the distribution of *Abcg2* and *Abcc2* mRNAs was examined in the intestine and liver of male Wistar rats using the semi-quantitative RT-PCR technique. The findings were then compared with those for *Abcb1/mdr1a* (Takara et al., 2003).

Abcg2 mRNA was observed in the intestine but not in the liver (Figure 1A). Tanaka et al. (2005) detected some *Abcg2* mRNA in the liver using the branched DNA hybridization assay (bDNA), but found that levels were higher in the intestine (Tanaka et al., 2005). Therefore, *Abcg2* was suggested to predominate in the intestine. In addition, the level of *Abcg2* mRNA increased gradually from the upper to lower intestine (Figure 2). This expression profile was similar to that of *Abcb1/mdr1a* mRNA (Takara et al., 2003), but different from the distribution of the cytochrome P450 (CYP) 3A family in the intestine of normal male rats (Ta-

kara et al., 2003, 2007). Therefore, Abcg2 as well as Abcb1/mdr1a may cooperate with the CYP3A family in drug clearance. So, Abcg2 was considered to play an important role in the pharmacokinetics, including intestinal absorption and bioavailability, of substrates for Abcg2 drugs.

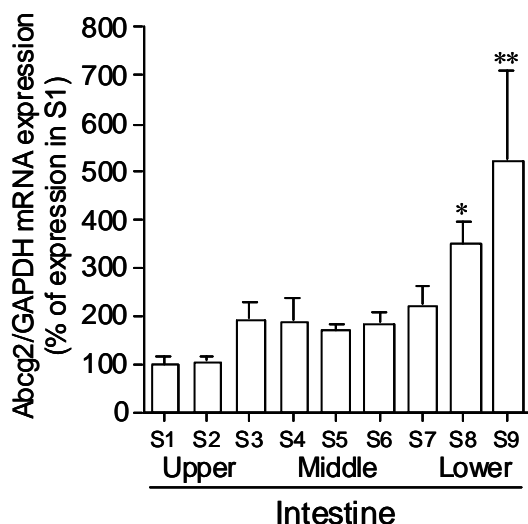


Figure 2: Regional differences in Abcg2 mRNA expression in the intestine of male Wistar rats determined by RT-PCR. Rats were sacrificed, and their tissues were quickly collected into RNA later^{TM} . Total RNA was extracted from the tissue using a GenElute $^{\text{TM}}$ Mammalian Total RNA kit. PCR products were separated on a 3% agarose gel stained with 100 ng/mL ethidium bromide, to visualize the DNA bands. Data were obtained after a densitometric analysis of three electrophoretograms derived from three rats. Results are expressed as percentages of the ratio of Abcg2 mRNA to GAPDH mRNA in the S1 region. Each bar represents the mean \pm S.E. for three rats. * and ** $p < 0.05$ and 0.01 significantly different from S1, respectively.

Next, the distribution of Abcc2 mRNA was examined in male Wistar rats. Abcc2 mRNA was expressed weakly in the intestine but strongly in the liver (Figure 1B), unlike Abcg2 mRNA (Figure 1A). In addition, Abcc2 mRNA levels in the intestine increased slightly from the upper to middle region, and subsequently decreased in the lower region (Figure 3). These results were supported by the findings on the distribution of Abcc2 in the intestine of Sprague-Dawley rats (Gotoh

et al., 2000; Mottino et al., 2000). Therefore, the distribution of Abcc2 was suggested to be different from that of Abcb1 or Abcg2. However, enzymes which contribute to phase II detoxifying reactions, e.g., UDP-glucuronosyltransferase, glutathione S-transferases, and sulfotransferases, are known to be located in enterocytes. These activities were reported to be the greatest in the proximal portion with decreases observed further down the intestine (Clifton and Kaplowitz, 1977; Koster et al., 1985; Pinkus et al., 1977). The distribution of Abcc2 in the intestine was suggested to be similar to that of phase II detoxifying enzymes including glutathione S-transferase and UDP-glucuronosyltransferase. Abcc2 is known to be responsible for the intestinal excretion of glutathione, glucuronide, or sulfate conjugated, and some non-conjugated, organic anions (Adachi et al., 2005; Chu et

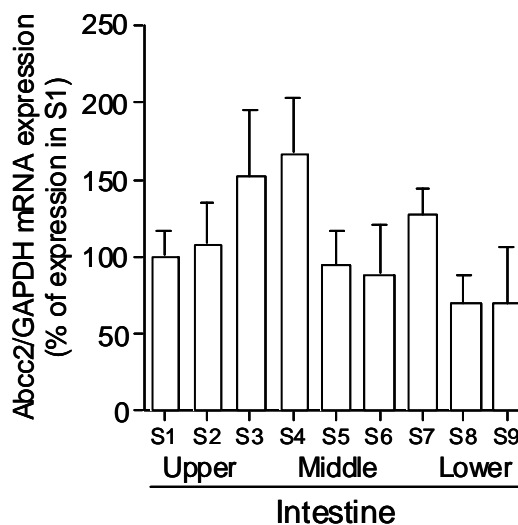


Figure 3: Regional differences in Abcc2 mRNA expression in the intestine of male Wistar rats determined by RT-PCR. Rats were sacrificed, and their tissues were quickly collected into RNA later^{TM} . Total RNA was extracted from the tissue using a GenElute $^{\text{TM}}$ Mammalian Total RNA kit. PCR products were separated on a 3% agarose gel stained with 100 ng/mL ethidium bromide, to visualize the DNA bands. Data were obtained after a densitometric analysis of three electrophoretograms derived from three rats. Results are expressed as percentages of the ratio of Abcc2 mRNA to GAPDH mRNA in the S1 region. Each bar represents the mean \pm S.E. for three rats.

al., 2004; Gotoh et al., 2000). Consequently, Abcc2 and conjugating enzymes may also act together to metabolize and secrete xenobiotics into the intestinal lumen, although Abcc2 was predominantly distributed in the liver.

Detailed and systematic studies have demonstrated marked differences among species in the bioavailability of many drugs (Cao et al., 2006; Chiou and Buehler, 2002; Takahashi et al., 2008). Understanding the relationship between the oral absorption and bioavailability of drugs in humans and experimental animals is important in drug discovery and development. In addition, drug transporters including Abcb1, Abcc2, and Abcg2 have been reported to contribute to the bioavailability of some drugs (Dietrich et al., 2003; Greiner et al., 1999; Marchetti et al., 2008). Therefore, the present findings should prove useful for drug development.

In conclusion, Abcg2 was expressed predominantly in the intestine rather than liver, whereas Abcc2 predominated in the liver, in male Wistar rats. In addition, regional differences in the expression of Abcg2 but not Abcc2 were observed in the intestine.

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Conflict of interest: The authors declare that no conflict of interest exists.

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