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AUTOINDUCTION OF MKC-963 [(*R*)-1-(1-CYCLOHEXYLETHYLAMINO)-4-PHENYLPHTHALAZINE] METABOLISM IN HEALTHY VOLUNTEERS AND ITS RETROSPECTIVE EVALUATION USING PRIMARY HUMAN HEPATOCYTES AND CDNA-EXPRESSED ENZYMES

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ABSTRACT:

MKC-963, (*R*)-1-(1-cyclohexylethylamino)-4-phenylphthalazine, a potent inhibitor of platelet aggregation, was synthesized and used in clinical trials in the 1990s. In the process of clinical study, it was found that urinary excretion ratios for 6β-hydroxycortisol and free cortisol increased significantly in parallel with decreases in the plasma concentrations of MKC-963 after repeated oral administration of the compound to healthy volunteers. These findings suggested that MKC-963 caused autoinduction (defined as the ability of a drug to induce enzymes that enhance its own metabolism, resulting in dispositional tolerance) in humans, and clinical studies using the compound were stopped. This experience prompted us to reevaluate the effects of this compound on CYP3A4 using primary human hepatocytes and cDNA-expressed human cytochrome P450 (P450) enzymes to determine whether the autoinduction of MKC-963 metabolism in humans could have been predicted if these in vitro systems had been used for the evaluation of MKC-963 in the preclinical study. The results of in vitro study showed that MKC-963 increased CYP3A4 mRNA expression level and activity of testosterone 6β-hydroxylation to extents similar to those observed with rifampicin in primary human hepatocytes. In addition, approximately 90% of the MKC-963 metabolism in human liver microsomes was estimated to be attributable to CYP3A4. These in vitro findings are in good agreement with the results of clinical study, suggesting that studies using human hepatocytes and cDNA-expressed human P450s are useful for assessing the autoinductive nature of compounds under development before starting clinical studies.

MKC-963, (*R*)-1-(1-cyclohexylethylamino)-4-phenylphthalazine (Fig. 1), a potent inhibitor of platelet aggregation, was synthesized and used in clinical trials by Mitsubishi Chemical Corp. (Tokyo, Japan) in the 1990s. In the process of clinical trials, the urinary excretion of 6β-hydroxycortisol (6β-OHF) and free cortisol (F) was studied after repeated oral administration of MKC-963 in human volunteers to determine whether this compound induces CYP3A4 or not. This was because the compound would be used for treatment of circulatory disorders together with drugs such as antihypertensives, antihyperlipidemics or antidiabetes. Many of these drugs are metabolized by CYP3A4 (Li et al., 1995; Lehmann et al., 1998; Prueksaritanont et al., 2003; Jerling et al., 2005), a predominant P450 enzyme found in the adult human liver that catalyzes the oxidation of a wide variety of exogenous compounds (Guengerich et al., 1986). In addition, CYP3A4 had been reported to be induced by several drugs, including rifampicin, phenytoin, and phenobarbital, that caused clinical drug-drug interactions (Holtbecker et al., 1996; Anderson 1998; Ridtitid et al., 2002). Moreover, measurement of the urinary ratio of 6β-OHF and F (6β-OHF/F) had been regarded as a safe and simple method for evaluating induction of CYP3A4 because it is noninvasive and does not require administration of a probe drug to volunteers (Galteau and Shamsa, 2003).

In this clinical study on MKC-963, we found that 6β-OHF/F increased significantly in parallel with decreases in the plasma concentrations of MKC-963 after repeated oral administration of the compound to healthy volunteers. This finding suggested that CYP3A4 is induced by MKC-963 and that the compound itself is an autoinducer in humans. Because autoinduction[1] was thought to reduce the therapeutic response of MKC-963 and might cause clinical problems,

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[1] Autoinduction is defined as the ability of a drug to induce enzymes that enhance its own metabolism, resulting in dispositional tolerance.

ABBREVIATIONS: MKC-963, (*R*)-1-(1-cyclohexylethylamino)-4-phenylphthalazine; d5MKC-963, MKC-963 with 5 hydrogen substituted by deuterium in the phenyl ring; MGB, minor groove binder; AUC, area under the plasma concentration-time curve; C_{max}, maximum plasma concentration; t_{max}, time to reach C_{max}; $t_{1/2}$, terminal half-life; F, cortisol; 6β-OHF, 6β-hydroxycortisol; P450, cytochrome P450; CL, in vitro clearance.

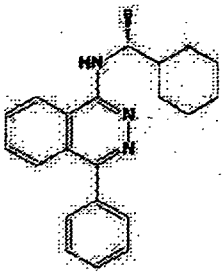

FIG. 1. Chemical structure of MKC-963.

the clinical study on MKC-963 was abandoned at that time. Thus, we have recently decided to reevaluate the effects of this compound on CYP3A4 using primary human hepatocytes and cDNA-expressing human P450 enzymes to determine whether we could have predicted the autoinduction of MKC-963 metabolism if we had used these in vitro systems for the preclinical evaluation of MKC-963.

In this paper, we describe the results of the clinical study on the pharmacokinetics of MKC-963 and its effects on the urinary excretion ratio of 6β-OHF and F after repeated oral administration of the compound to healthy volunteers, and the results of in vitro studies on the effects of MKC-963 on the expression and activities of CYP3A4 and identification of the P450 enzyme(s) responsible for the metabolism of MKC-963 using primary human hepatocytes and cDNA-expressed human P450 enzymes, respectively. The results suggest that these in vitro systems would have been useful for the prediction of the autoinductive nature of MKC-963 in the preclinical study.

Materials and Methods

Materials. MKC-963 was provided by Mitsubishi Chemical Corp. (Tokyo, Japan), and its chemical purity was 99.8%. Rifampicin, testosterone and 6β-hydroxytestosterone were purchased from Sigma-Aldrich (St. Louis, MO), Tokyo Kasei Kogyo Co. (Tokyo, Japan), and Sumika Chemical Analysis Service, Ltd. (Osaka, Japan), respectively. All other chemicals were of analytical reagent grade.

In Vivo Study. *Subjects.* Six healthy male volunteers aged between 20 and 35 years were recruited for the study. They were within ±20% of their ideal body weight and in good general health according to routine medical history and laboratory data. They did not use any medications for at least 2 weeks before and were not using any concurrent medications during the study. All of them agreed to refrain from consumption of alcohol and grapefruit or grapefruit juice during the study. Subjects who had clinically significant abnormalities on preliminary examination, those who had a history of drug or food allergies or a history of drug or alcohol abuse, and those who had donated blood or received an investigational drug within 4 months before the start of this study were excluded from this study.

Study Protocol. The subjects received a single oral dose of MKC-963 on day 1 and on day 14, and two oral doses per day with a 12-h interval for 12 days (from day 2 to day 13). Each dose was 120 mg, and the drug was supplied as tablets (40 mg). The oral doses were administered with 100 ml of water at 9:00 AM after breakfast or at 9:00 PM after dinner. Breakfast and dinner were standardized for all the subjects. Blood samples (each 4 ml) were collected by venipuncture at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h after the first administration of MKC-963 on days 1 and 14 and at 0, 1, and 2 h on days 2, 5, 8, and 11. The blood samples collected were centrifuged at 1500*g* for 10 min at 4°C, and plasma samples were separated and stored at −20°C until analyses. Urine samples were pooled over a period of 24 h and collected at the end of designated days: the day before and 1, 2, 5, 8, 11, and 14 days after starting drug administration. The urine was kept cool during collection, and then the total volume was recorded and a 10-ml aliquot was stored at −20°C until analyses.

The study was conducted at Hohsen Clinic, Research Center for Clinical Pharmacology, The Kitasato Institute (Tokyo, Japan), and the protocol was approved by the institutional review board. The study was conducted in accordance with the guidelines on good clinical practice and the ethical standards for human experimentation established by the Declaration of Helsinki. Each subject gave written informed consent.

Determination of MKC-963 Concentrations in Plasma. Plasma concentrations of MKC-963 were determined by liquid chromatography-tandem mass spectrometry. d5MKC-963 was used as an internal standard. The plasma (0.5 ml) was mixed with 0.4 ml of Titrisol buffer (pH 9) and applied on a solid-phase extraction column (Extrelut-1; Merck KGaA, Darmstadt, Germany). The MKC-963 and internal standard were isolated from the column with 5 ml of diethyl ether. The organic extract was dried under nitrogen and reconstituted in 1 ml of acetonitrile. The sample was separated by a Waters HPLC system (Waters, Milford, MA) equipped with a Capcell Pak CN column (5 μm, 35 × 4.6 mm in internal diameter; Shiseido, Tokyo, Japan). The mobile phase consisted of acetonitrile/water/acetic acid (90:10:1, v/v/v) and the flow rate was maintained at 0.2 ml/min. MKC-963 and the internal standard were detected by tandem mass spectrometry using a Finnigan TSQ7000 mass spectrometer (Thermo Electron Corp., Waltham, MA). For mass spectral detection, the following precursors to product ion reactions were monitored: m/z 332.1 > m/z 222.1 for MKC-963 and m/z 337.0 > m/z 227.1 for d5MKC-963. The standard curves were linear from 0.1 ng/ml to 50 ng/ml. The interassay precision (% CV) assessed from the blank plasma to which known concentrations of the analytes was added (final concentrations of 0.1 ng/ml to 50 ng/ml) ranged from 2.0% to 7.7%. The limit of sensitivity of the assay was 0.01 ng/ml.

Pharmacokinetic Parameters. The pharmacokinetic parameters of MKC-963 were estimated by noncompartmental methods with the use of WinNonlin V4.1 (Pharsight Corporation, Mountain View, CA). The values of C_{max} and t_{max} were determined directly from the plasma concentration-time profiles. The area under the plasma concentration-time curve (AUC) from 0 to 24 h was determined by the linear trapezoidal rule from the beginning of drug administration to the last quantifiable data point. The value of $t_{1/2}$ was calculated by linear regression analysis of the last elimination phase after log transformation of the data.

Determination of Urinary 6β-OHF and F. Determination of 6β-OHF and F in urine samples was performed by using enzyme immunoassay kits for urinary 6β-OHF (Stabiligen, Villers-Les-Nancy, France) and F (Biométreux, Marcy l'Etoile, France), respectively, according to the manufacturer's instructions. The cross-reactivity of these kits for urinary F and 6β-OHF were 4.4 and 1.1%, respectively.

In Vitro Study. *Human Primary Hepatocytes and Treatment with MKC-963.* Cryopreserved human hepatocytes (lot 100, white female, 74 years old) were obtained from In Vitro Technologies, Inc. (Baltimore, MD). Hepatocytes were suspended in Hepatocyte Culture Medium (Cambrex, Walkersville, MD), centrifuged at 50*g* for 3 min, and resuspended in the same medium. The cells were plated onto Matrigel-coated 24-well plates at a density of 1.5×10^5 cells/well and were maintained in an atmosphere of 95% air and 5% CO_2 at 37°C. The cell viability was more than 80% assessed by a trypan blue exclusion test. Stock solutions of MKC-963 and rifampicin were prepared in dimethyl sulfoxide and were diluted before each use. Treatments of hepatocytes with chemicals were begun on the fourth day after seeding and continued for 4 days. The hepatocytes were treated with dimethyl sulfoxide (final concentration of 0.2%), rifampicin (10 μM), a positive control, or MKC-963 (0.25 μM). The concentration of MKC-963 used in the present study was determined considering that C_{max} of MKC-963 was 0.29 μM when 120 mg of MKC-963 was administered orally to human volunteers (Fig. 2). The concentration of rifampicin used in the present study also corresponded nearly to C_{max} of rifampicin after an oral administration of 450 to 600 mg in patients with tuberculosis (Smith, 2000).

RNA Extraction and Real-Time PCR. Total RNA was extracted using TRIzol reagent (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's instructions. All samples were stored at −80°C until used for cDNA preparation. One microgram of total RNA was reverse-transcribed into cDNA with random hexamers using a SuperScript II Transcription system (Invitrogen Corp) according to the manufacturer's instructions. The expression levels of specific mRNAs were determined by using a quantitative real-time PCR method. The primer and TaqMan minor groove binder (MGB) probe sets were designed by using Primer Express software (Applied Biosystems, Foster City, CA). The sequences (5′ to 3′) for the primers and probes are as follows: CYP3A4, forward primer (GCAGGAGGAAATTGATGCAGTT), TaqMan MGB probe [FAM (Applied Biosystems)-ATAAGGCACCACCCACCTA-MGB], and reverse primer (CTGAGCGTTTCATTCACCACC); β-actin, forward primer (CCTGGCACCCAGCACAAT), fluorogenic probe [VIC

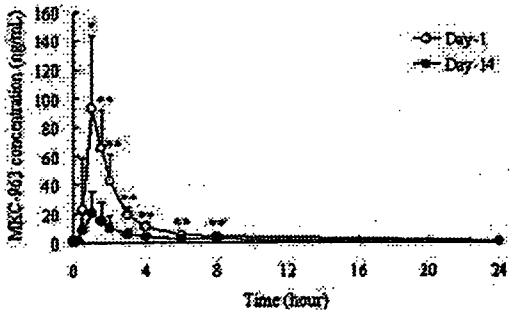

FIG. 2. Plasma concentration-time profiles of MKC-963 on day 1 (open circles) and day 14 (closed circles) after oral administration of 120 mg to six healthy subjects. Data are expressed as means ± S.D. *, $p < 0.05$; **, $p < 0.01$.

(Applied Biosystems)-ATCATTGCTCCTCCTGAG-MGB], and reverse primer (CCGATCCACACGGAGTACTTG). The sequence of fluorogenic probe for CYP3A4 was one base different from that of CYP3A5, which is recognized by MGB-probe according to the supplier's manuals. Cycling conditions of the PRISM 7900 Sequence Detection system (Applied Biosystems) were 50°C for 2 min and 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. CYP3A4 mRNA levels in cultured human hepatocytes were expressed as ratio against β-actin mRNA levels.

Determination of CYP3A4 Activities in Human Hepatocyte Culture. CYP3A4 activities were determined by the measurement of 6β-hydroxylation activities for testosterone in intact hepatocytes cultured on 24-well plates (Donato et al., 1995). After treatment with chemicals, monolayers were incubated with testosterone (250 μM) for 30 min. Quantification of 6β-hydroxytestosterone was performed by high-performance liquid chromatography (Donato et al., 1993).

Identification of P450 Enzyme(s) Contributing to the Metabolism of MKC-963. Recombinant P450 enzymes expressed in insect cells infected with baculovirus containing human P450 and human NADPH-P450 reductase cDNA inserts were obtained from BD Gentest (Woburn, MA). Incubation mixtures contained cDNA-expressed P450s (50 pmol/ml) in potassium phosphate buffer (pH 7.4), an NADPH-generating system, and MKC-963. Substrate (2 μM MKC-963) was incubated at 37°C for 0, 5, 15, and 30 min with microsomes expressing CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP3A4, and determined by liquid chromatography/mass spectrometry. The remaining percentage of MKC-963 was calculated using the $t = 0$ value as 100%. Then, in vitro clearance of each P450 enzyme (CL) was estimated from the following equation: CL (μl/min/pmol P450) = −slope (1/min)/P450 concentration (pmol P450/ml) × 1000, where slope was determined from linear regression analysis between log percentage of MKC-963 and incubation time (Obach, 1999), and P450 concentration was the concentration of recombinant P450 enzyme in the incubation mixture. CL was corrected with the P450 contents in native human liver microsomes (Rodrigues, 1999) as follows: Corrected CL = CL × enzyme content of each P450. Therefore, the contribution of each P450 enzyme to overall clearance was estimated from the following equation: Contribution of each P450 enzyme (%) = corrected CL for each P450 enzyme/sum of corrected CL × 100.

Statistics. Statistical analysis was performed with SAS software (version 8.2; SAS Institute, Cary, NC). A P value of <0.05 was considered statistically significant.

Results

In Vivo Study. *Pharmacokinetics of MKC-963.* Plasma concentration-time profiles of MKC-963 showed a dramatic change after repeated oral administration of the compound (120 mg) to healthy subjects. As shown in Fig. 2, the mean (±S.D.) plasma concentrations of MKC-963 on day 14 at 1 to 8 h after administration were significantly lower than those on day 1. As a result, C_{max} and AUC values on day 14 had decreased by 77% and 69%, respectively, compared with the values on day 1 (Table 1). There were no notable differences between t_{max} and $t_{1/2}$ values on day 1 and those on day 14 (Table 1).

Figure 3 shows the changes in mean plasma concentrations of MKC-963 at 1 and 2 h after administration from day 1 to day 14. As shown in this figure, the plasma concentrations of MKC-963 at 1 h decreased significantly ($p < 0.05$) from day 2 to day 14, and those at 2 h also showed significant ($p < 0.05$) decreases from day 5 to day 14.

TABLE 1

Pharmacokinetic parameters of MKC-963 on day 1 and day 14 after repeated oral administration of 120 mg to six healthy subjects

Data are expressed as means ± S.D. except for t_{max} data, which are given as median with range.

	Day 1	Day 14
C_{max} (ng/ml)	96.2 ± 46.7	22.6 ± 14.8**
t_{max} (h)	1 (1–1.5)	1 (0.5–1)
AUC (h · ng/ml)	206.0 ± 76.5	64.8 ± 31.8**
$t_{1/2}$ (h)	7.2 ± 2.1	10.9 ± 5.0

** $p < 0.01$.

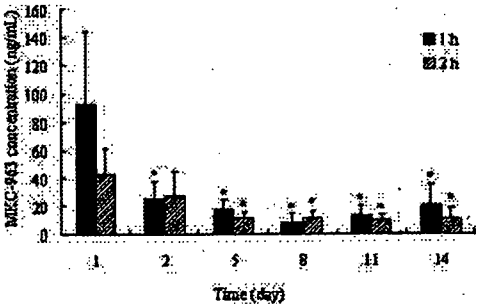

FIG. 3. Plasma concentrations of MKC-963 at 1 h and 2 h after oral administration of the compound (120 mg) to six healthy subjects on days 1, 2, 5, 8, 11, and 14. Data are expressed as means ± S.D. *, $p < 0.05$.

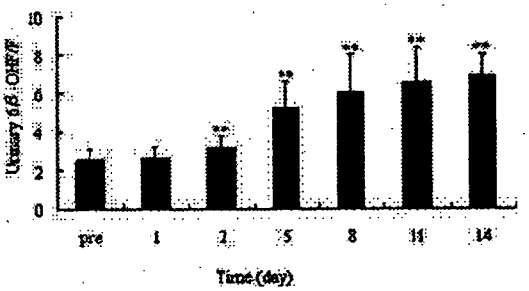

FIG. 4. Twenty-four-hour urinary excretion ratios of 6β-hydroxycortisol and free cortisol in six healthy subjects on the day before the start of administration and on days 1, 2, 5, 8, 11, and 14. Results are expressed as means ± S.D. **, $p < 0.01$.

Urinary 6β-OHF/F. Figure 4 shows the mean 24-h urinary excretion ratios of 6β-OHF and F on the day before the start of administration and from day 1 to day 14. The mean value of 6β-OHF/F increased significantly ($p < 0.05$) from day 2 to day 14 compared with the value on the day before the start of administration, and all subjects showed increases in the urinary excretion ratios of 6β-OHF from day 2 to day 14.

In Vitro Study. *Primary Human Hepatocytes.* The effects of MKC-963 (0.25 μM) on the expression of CYP3A4 mRNA and on the activity for testosterone 6β-hydroxylation were investigated using human primary hepatocytes. The effect of rifampicin (10 μM) was also investigated as a positive control. As shown in Fig. 5A, MKC-963 increased the expression level of CYP3A4 mRNA by 6-fold, comparable to the effect of rifampicin (increase of approximately 11-fold). Testosterone 6β-hydroxylation activity was also increased by 9-fold in the presence of MKC-963, which is also comparable to the effect of rifampicin (14-fold increase, Fig. 5B).

Identification of P450 Enzyme(s) Involved in the Metabolism of MKC-963. cDNA-expressed human P450s were used to estimate the

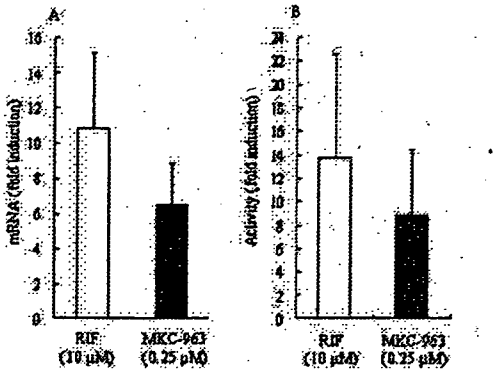

FIG. 5. Effects of rifampicin (10 μM) and MKC-963 (0.25 μM) on CYP3A4 mRNA (A) and activity of testosterone 6β-hydroxylation in primary human hepatocyte cultures (B). Total RNA was extracted, and CYP3A4 and β-actin mRNA levels were measured by real-time PCR methods as described under *Materials and Methods*; then, CYP3A4 mRNA was normalized to β-actin and compared with that of a vehicle control. The mean absolute ratio for the control hepatocytes was 0.051 ± 0.02 (A). For measurement of CYP3A4 activity, testosterone was incubated with intact hepatocytes and metabolite was analyzed as described under *Materials and Methods*; then, CYP3A4 activities were compared with those of a vehicle control. The mean activity of control hepatocytes was 2.3 ± 0.7 pmol/min/10^5 cells (B). Results are expressed as means ± S.D. of three experiments.

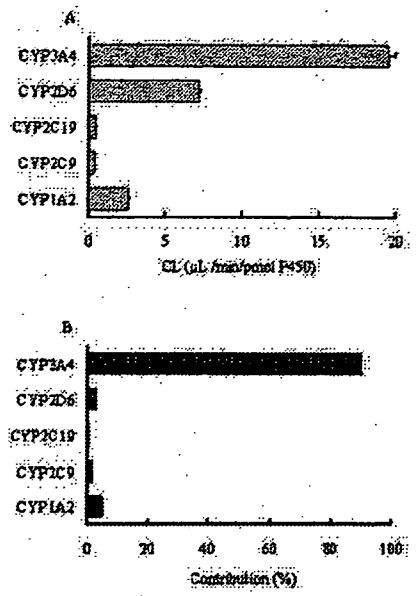

FIG. 6. Metabolic clearance of MKC-963 in microsomes from insect cells expressing P450 enzymes (50 pmol/ml) (A) and the contributions (percentage) of each P450 enzyme to the total clearance of MKC-963 in five P450 enzymes corrected by the P450 contents in native human liver microsomes as described under *Materials and Methods* (B). MKC-963 (2 μM) was incubated in the presence of CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP3A4 for 0, 5, 15, and 30 min, at 37°C. Each value is the mean ± S.D. of triplicate assays.

enzyme(s) of P450 mainly responsible for the metabolism of MKC-963 in humans. As shown in Fig. 6A, MKC-963 was metabolized by CYP3A4 extensively, by 2D6 moderately, and by CYP1A2 to some extent. However, when the contribution of individual P450 enzymes to the overall metabolic clearance of MKC-963 was estimated by P450 contents in human liver microsomes, approximately 90% of the MKC-963 metabolism in human liver microsomes was estimated to be attributable to CYP3A4 (Fig. 6B).

Discussion

The results of the in vitro study showed that MKC-963 increased CYP3A4 mRNA expression level and activity of testosterone 6β-hydroxylation to extents similar to those observed with rifampicin in primary human hepatocytes (Fig. 5). In addition, approximately 90% of the MKC-963 metabolism in human liver microsomes was estimated to be attributable to CYP3A4 (Fig. 6B). These findings suggest that MKC-963 is a potent inducer of CYP3A4, which catalyzes the metabolism of MKC-963 itself. These in vitro findings are in good agreement with the results of the clinical study showing that the urinary excretion ratio of 6β-OHF and F, a marker of CYP3A4 induction, increased significantly (Fig. 4) and that AUC and C_{max} of MKC-963 decreased dramatically after repeated oral administration of the compound to healthy volunteers (Table 1). Therefore, these findings suggest that MKC-963 is a relatively potent autoinducer of CYP3A4 in humans. Moreover, the findings also suggest that in vitro studies using primary human hepatocytes coupled with cDNA-expressed P450 enzymes would have been useful to predict or assess the autoinductive character of MKC-963 in the preclinical study.

In the present study, we used 0.25 μM MKC-963 since C_{max} of MKC-963 was 0.29 μM when 120 mg of MKC-963 was administered orally to human volunteers (Fig. 2). The concentration of rifampicin was also set at 10 μM because it is almost the same as C_{max} of rifampicin after oral administration of 450 to 600 mg in patients with tuberculosis (Smith, 2000). At that dose, interaction of rifampicin with a number of drugs has been reported (Holtbecker et al., 1996; Villikka et al., 1999; Ridtitid et al., 2002). Under these conditions, the effect of MKC-963 on CYP3A4 in the primary culture of human hepatocytes was comparable to the effect of rifampicin, suggesting that MKC-963 is a potent inducer of CYP3A4, similar to rifampicin even in vivo. This assumption is supported by the in vivo observation that MKC-963 increased urinary excretion ratios of 6β-OHF and F to an extent similar to that reported previously for rifampicin (Ohnhaus et al., 1989; Kovacs et al., 1998; Tran et al., 1999). Therefore, the concentration of the test compound used in the study on human hepatocytes appears to be an important factor for assessing the potential to induce CYP3A4 in vivo (Smith, 2000). In fact, it has been reported that thiazolidinediones, including troglitazone, pioglitazone, and rosiglitazone, showed the potential to induce CYP3A4 in human hepatocytes but that only troglitazone showed drug-drug interactions due to the induction of CYP3A4 in vivo (Sahi et al., 2003). Although the mechanism remains unknown, the authors speculated that the concentrations of pioglitazone and rosiglitazone do not reach concentrations sufficient to induce CYP3A4 in in vivo situations (Sahi et al., 2003). In a preclinical study, however, the actual concentrations of test compounds in plasma or other human organs are generally unknown. Thus, prediction of concentrations of the test compound in plasma or other human organs by animal scale-up (Mitsuhashi et al., 1990; Izumi et al., 1996) or by extrapolation of in vitro clearance obtained from human liver microsomes, human hepatocytes, or cDNA-expressed P450s to in vivo clearance in humans (Iwatsubo et al., 1997; Ito et al., 1998) appears to be essential to predict the ability of the test compound to induce P450 in in vivo situations.

Although the results of the present study clearly showed the induction of CYP3A4 after repeated oral administration of MKC-963, there were some differences in the time courses of changes in the indicators of induction. There was some delay in changes in the urinary ratio of 6β-OHF and F (Fig. 4) compared with those of the plasma concentrations of MKC-963 at 1 h and 2 h after administration (Fig. 3). As shown in Fig. 3, the plasma concentrations of MKC-963 decreased dramatically on the second day after starting repeated oral administration of MKC-963, whereas the urinary ratios of 6β-OHF and F did not show a remarkable change on day 2 but showed a considerable change on day 5 (Fig. 4). The reason for this difference in the time

courses of these indicators is unknown, but it may be due to the difference in the involvement of intestinal CYP3A4 in the metabolism of MKC-963. CYP3A4 has been reported to be expressed in small intestinal epithelial cells (Watkins et al., 1987; Kolars et al., 1992) as well as in hepatic parenchymal cells (Guengerich et al., 1986; Shimada et al., 1994). Intestinal CYP3A4 has recently been suggested to be a major factor in determining the extent of first-pass metabolism and, hence, oral bioavailability of drugs (Hall et al., 1999). Because MKC-963 was administered orally in the present study, plasma concentration of MKC-963 should be affected by the induction of CYP3A4 in the small intestine. Although we do not know whether MKC-963 induces CYP3A4 in the small intestine 1 day after oral administration, Kolars et al. (1992) reported that rifampicin induces small intestinal CYP3A4 mRNA within 24 h. Therefore, it is conceivable that induction of intestinal CYP3A4 by MKC-963 occurred very rapidly, and reduced the bioavailability of MKC-963 and decreased its plasma concentration within 2 days after starting drug administration. In contrast, it has been reported that the formation of 6β-OHF is primarily mediated by the liver and that intestinal metabolism plays a minor role (Galteau and Shamsa, 2003). Therefore, it is possible that the induction of CYP3A4 in the liver is slower than that in the small intestine and that change in the urinary excretion ratio of 6β-OHF and F was therefore delayed compared with that of plasma concentration of MKC-963 after repeated oral administration. In support of this speculation, half-maximal changes in 6β-OHF/F have been reported to be achieved 2 to 3 days after rifampicin administration (Ohnhaus et al., 1989).

It should be noted that hepatocytes used in this study were derived from one donor; therefore, CYP3A4 induction may have been less remarkable if other livers with low levels of CYP3A4 were used in the present study. In this case, it is possible that metabolism by CYP2D6 and CYP1A2 could be a predominant route, and autoinduction of MKC-963 metabolism may not be as remarkable as that observed in the present study.

Finally, CYP3A5 may also be involved in the autoinduction of MKC-963 metabolism. This is because substrate specificities of CYP3A5 and CYP3A4 are similar and overlapped (Wrighton et al., 1990). In addition, CYP3A5 has been reported to be induced by rifampicin in primary human hepatocytes (Zhuo et al., 2004). However, both the catalytic activity and specific content of CYP3A5 in the human liver or small intestine are much lower than those of CYP3A4 (Wrighton et al., 1990; Rodrigues, 1999). Furthermore, the extent of induction of CYP3A5 by rifampicin in human hepatocytes is less than that of CYP3A4 (Zhuo et al., 2004). In addition, primer sets of CYP3A4 used in the present study were specific for CYP3A4 and do not recognize CYP3A5. Therefore, it seems that the contribution of CYP3A5 to the autoinductive nature of MKC-963, if any, is much smaller than that of CYP3A4.

In summary, in vivo findings suggesting autoinduction of MKC-963 metabolism prompted us to reevaluate the autoinductive nature of the compound using primary human hepatocytes and cDNA-expressed human P450 enzymes. The results showed that MKC-963 increased CYP3A4 mRNA expression level and activity of testosterone 6β-hydroxylation to extents similar to those observed with rifampicin in primary human hepatocytes. In addition, approximately 90% of the MKC-963 metabolism in human liver microsomes was estimated to be attributable to CYP3A4. These findings are in good agreement with the clinical study. Therefore, in vitro studies using human hepatocytes coupled with cDNA-expressed human P450s appear to be useful for assessing the autoinductive nature of compounds under development before starting clinical studies.

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Expert Opinion

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Genetic polymorphisms of drug transporters: pharmacokinetic and pharmacodynamic consequences in pharmacotherapy

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There has been increasing appreciation of the role of drug transporters in pharmacokinetic and pharmacodynamic consequences in pharmacotherapy. The clinical relevance of drug transporters depends on the localisation in human tissues (i.e., vectorial movement), the therapeutic index of the substrates and inherent interindividual variability. With regard to variability, polymorphisms of drug transporter genes have recently been reported to be associated with alterations in the pharmacokinetics and pharmacodynamics of clinically useful drugs. A growing number of preclinical and clinical studies have demonstrated that the application of genetic information may be useful in individualised pharmacotherapy for numerous diseases. However, the reported effects of variants in certain drug transporter genes have been inconsistent and, in some cases, conflicting among studies. Furthermore, the incidence of almost all known variants in transporter genes tends to be racially dependent. These observations suggest the necessity of considering interethnic variability before extrapolating pharmacokinetic data obtained in one ethic group to another, especially in the early phase of drug development. This review focuses on the impact of genetic variations in the function of drug transporters (ABC, organic anion and cation transporters) and the implications of these variations for pharmacotherapy from pharmacokinetic and pharmacodynamic viewpoints.

Keywords: drug transporter, genetic polymorphism, pharmacodynamics, pharmacokinetics

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1. Introduction

Many types of drug transporters are expressed in various human tissues, such as the intestine, liver, kidney, skin and the brain, and play roles in drug absorption, distribution and excretion. Accordingly, it is reasonable to hypothesise that factors influencing transport capability could lead to important consequences for interindividual differences in disposition kinetics and interaction profiles of clinically useful drugs, susceptibility to side effects, and treatment efficacy. Among these factors, genetic polymorphism is highly important. The identification of allelic variations and their functional confirmations (i.e., genotype–phenotype relationship) is a necessary step towards the use of genetic information for individualised pharmacotherapy. These backgrounds have led to the study of single nucleotide polymorphisms (SNPs), which has progressed rapidly and generated remarkable findings, and some SNPs have been shown to alter both the expression and function of their gene products. This review highlights recent studies by the groups of Ieiri and others on the role of drug transporter gene polymorphisms in pharmacokinetic and pharmacodynamic consequences in pharmacotherapy. The scope of this review is strictly limited to

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Table 1. General features of drug transporters (localisation in human tissues, substrates and inhibitors)

Name (gene nomenclature)	Chromosome localisation	Main localisation (tissue or subcellular)	Substrates (clinically useful drugs)	Inhibitors (clinically useful drugs)
MDR1 or P-gp (*ABCB1*)	7q21.1	Canalicular membrane (hepatocytes) Brush-border membrane of proximal tubular cells (kidney) Brush-border membrane (enterocytes) Capillary endothelial cells (brain and testis) Placental trophoblast	Anticancers (docetaxel, etoposide, paclitaxel, topotecan, vinblastine) Antihypertensives (diltiazem, losartan) Antiarrhythmics (digoxin, verapamil) Antivirals (indinavir, nelfinavir) Antibiotics (erythromycin, sparfloxacin) Immunosuppressants (ciclosporin, tacrolimus) Others (cimetidine, fexofenadine, loperamide, phenytoin, morphine, ondansetron)	Amiodarone, amitriptyline, diltiazem, dipyridamole, phenothiazines, propafenone, propranolol, quinidine, spironolactone, tamoxifen
MRP2 (*ABCC2*)	10q24	Canalicular membrane (hepatocytes) Brush-border membrane of proximal tubular cells (kidney)	Bilirubin, diglucuronide, sulfates, glutathione conjugates, benzbromarone, indomethacin, vinblastine, telmisartan	Ciclosporin, glibenclamide
BCRP (*ABCG2*)	4q22	Canalicular membrane (hepatocytes) Apical membrane of syncytiotrophoblast cells (placenta, membrane facing maternal blood) Luminal membranes of villous epithelial cells (small intestine and colon)	Epirubicin, topotecan, doxorubicin, daunorubicin, etoposide, SN-38, reserpine	
OATP1A2 or OATP-A (*SLCO1A2*)	12p12	Cerebral endothelial cells luminal membrane (intestinal enterocytes)	Thyroid hormones (T4 and T3), prostaglandin E2, fexofenadine, quinidine	Dexamethasone, erythromycin, quinidine, verapamil
OATP1B1 or OATP-C (*SLCO1B1*)		Basolateral (sinusoidal) Plasma membrane (hepatocytes)	Thyroid hormones (T4 and T3), methotrexate, pravastatin, rifampicin, prostaglandin E2	
OATP1B3 or OATP8 (*SLCO1B3*)		Basolateral (sinusoidal) Plasma membrane (hepatocytes)	Thyroid hormones (T4 and T3), leukotriene C4, digoxin, methothrexate, rifampicin	
OATP2B1 or OATP-B (*SLCO2B1*)	11q13	Basolateral (sinusoidal) Plasma membrane (hepatocytes) Apical membrane (enterocytes)	Narrow substrate specificity (pH dependent?)	
OCT1 (*SLC22A1*)	6q26	Basolateral (sinusoidal) Plasma membrane (hepatocytes)	Acylclovir, ganciclovir, metformin	Acebutolol, amantadine, cimetidine, disopyramide, midazolam, prazosin, quinidine, verapamil
OCT2 (*SLC22A2*)		Basolateral membrane of proximal tubular cells (kidney) Apical side of the distal tubule (kidney)?	Amantadine, metformin, neurotransmitters, monoamine	Desipramine, procainamide
OCT3 (*SLC22A3*)	6q26 – 27	Placenta	Cimetidine, tyramine, neurotransmitters, monoamine	Clonidine, desipramine, imipramine, prazosin, procainamide

BCRP: Breast cancer-resistance protein; OAT: Organic anion transporter; OATP: Organic anion-transporting polypeptide; OCT: Organic cation transporter; MDR: Multi-drug resistance; MRP: Multi-drug resistance-associated protein; P-gp: P-glycoprotein.

Table 1. General features of drug transporters (localisation in human tissues, substrates and inhibitors) (continued)

Name (gene nomenclature)	Chromosome localisation	Main localisation (tissue or subcellular)	Substrates (clinically useful drugs)	Inhibitors (clinically useful drugs)
OAT1 (*SLC22A6*)	11q12.3	Basolateral membrane of proximal tubular cells (kidney)	Methotrexate	β-Lactam antibiotics, diuretics, NSAIDs, probenecid
OAT2 (*SLC22A7*)	6q21.1 – 2	Basolateral (sinusoidal) Plasma membrane (hepatocytes)	Methotrexate, prostaglandin E2	
OAT3 (*SLC22A8*)	11q12.3	Basolateral membrane of proximal tubular cells (kidney) Brush-border membrane of choroid plexus cells and in capillary endothelial cells (brain)	Cimetidine, methotrexate, salicylate, prostaglandin E2	

BCRP: Breast cancer-resistance protein; OAT: Organic anion transporter; OATP: Organic anion-transporting polypeptide; OCT: Organic cation transporter; MDR: Multi-drug resistance; MRP: Multi-drug resistance-associated protein; P-gp: P-glycoprotein.

observations from human (healthy volunteers and patients) studies. This review focuses on the following transporters: ABC transporters (P-glycoprotein [P-gp]/multi-drug resistance 1 [MDR1/ABCB1], multi-drug resistance-associated protein 2 [MRP2/ABCC2] and breast cancer-resistance protein [BCRP/ABCG2]), organic anion-transporting polypeptide family (OATP1A2 [OATP-A]/SLCO1A2, OATP1B1 [OATP-C]/SLCO1B1, OATP1B3 [OATP8]/SLCO1B3 and OATP2B1 [OATP-B]/SLCO2B1), organic anion transporter family (OAT1/SLC22A6, OAT2/SLC22A7 and OAT3/SLC22A8) and organic cation transporter family (OCT1/SLC22A1 and OCT2/SLC22A2).

2. General features

2.1 Localisation in human tissues and basic function

P-gp/MDR1 (ABCB1) is expressed in the small and large intestines, adrenal gland, placental trophoblasts, kidney, liver, pancreas (pancreatic ductile cell) and capillary endothelial cells of the brain and testes (Table 1) [1-4]. Evidence including findings in knockout mice support that P-gp excretes substrate drugs via the canalicular membrane of hepatocytes into the bile, via the brush-border membrane of enterocytes into the gut lumen and via the brush-border membrane of proximal tubules into the urine [5,6]. P-gp in trophoblasts and endothelial cells of the blood–brain barrier (BBB) contribute to the function of blocking the transfer of xenobiotics across the human placenta and preventing the entry of substrates into the CNS [7-9].

Although at least 13 structurally and functionally related family members have been identified in MRPs (ABCC proteins), their localisation, expression levels and substrate specificity are different [10,11]. MRP2 (ABCC2 protein) is expressed at the apical membrane in liver hepatocytes, renal proximal tubule cells and enterocytes of the intestine [12-15], and plays roles in the biliary excretion, intestinal excretion and urinary excretion of the substrates [10,11].

Similar to P-gp and MRP2, BCRP (ABCG2 protein) is expressed at the apical membrane in the placenta (trophoblast cells), liver (bile canalicular membrane of hepatocytes), kidney and intestine (enterocytes) [16-19]. The tissue distribution of BCRP suggests that its major physiological role may be the regulation of intestinal absorption and biliary secretion of substrates, and protection of the fetus and brain from toxic xenobiotics. Unlike most other ABC transporters (e.g., P-gp and MRPs), which are characterised by 2 nucleotide-binding domains (NBD) and 12 transmembrane domains (TMD), BCRP has a single NBD at the amino terminus followed by 6 TMDs. Thus, BCRP is a so-called half-transporter and may form a homodimer, although heterodimeric forms are possible [20-24].

OATP1A2 (OATP-A) was first isolated from human liver; however, subsequent studies have identified its expression in the brain, lung, kidney and testes [25,26]. Recently, OATP1A2 has been reported to be expressed on the luminal membrane of human intestinal enterocytes, and to play a possible role in fexofenadine absorption from the intestine [26,27].

Both OATP1B1 (OATP-C) and -3 (OATP8; 80% amino acid identity to OATP-C) have liver-specific tissue distribution [28-31]. Because the uptake of substrates from the blood into hepatocytes, mediated by uptake transporters in the basolateral membrane, is the first step in the hepatocellular elimination process in the human body, the role of these transporters in the liver is of special interest. So far, the functional characterisation of OATP1B1 in the human body has been elucidated progressively among the OATP family due to its liver-specific expression.

Similar to OATP1B1 and -3, OATP2B1 (OATP-B) is predominantly found in the liver, but is also expressed in various tissues, including the brain, lung, kidney, placenta, heart, intestine and testis [32,33]. OATP2B1 is found on the basolateral membrane of hepatocytes, suggesting that this transporter functions in an uptake capacity to remove substrates from the portal circulation [33].

OAT1 and -3 are substantially expressed in the kidney, and localised on the basolateral membrane of the proximal tubules [34,35]. They uptake substrates from the blood side into the proximal tubule cell [36]. Because of key molecules in

renal excretion, OAT1 and -3 have been reported to be responsible for antibiotic- or antiviral-related nephrotoxicity [37-40]. In general, OAT family members are mainly expressed in the kidney; however, OAT2 is abundantly expressed on the basolateral membrane of the liver and, to a lesser extent, in the kidney [41,42]. In the brain, OAT3 is localised on the brush-border membrane of choroids plexus cells, suggesting it functions as the blood–cerebrospinal fluid barrier [43,44].

OCT1 is primarily expressed in the basolateral membrane of hepatocytes and is thought to play a fundamental role in the uptake of substrates into the liver [45-48]. In contrast, OCT2 is detected predominantly in the kidney and is likely to be the major transporter for the uptake of many cations from the blood sides into renal epithelial cells [48]. OCT3 has much more widespread tissue distribution at the mRNA level: aorta, skeletal muscle, prostate, salivary gland, adrenal gland and placenta [49]. Among these tissues, the placental expression level is relatively high.

2.2 Substrate drugs

P-gp accepts a broad spectrum of structurally and functionally unrelated drugs (Table 1). P-gp substrates, inducers and inhibitors are listed in detail elsewhere [50-52]. Interestingly, there is a strong overlap in substrate specificity and tissue distribution between P-gp and CYP3A4/5 [53,54].

MRP2 also has broad substrate specificity covering anticancer drugs [55,56] and organic anions derived from phase I and II metabolism of xenobiotics [57-59].

BCRP recognises various compounds such as negative or positive charge, organic anions and sulfate conjugates [60,61]; however, there is considerable, but not complete, overlap in substrates, especially for anticancer drugs among P-gp, MRP2 and BCRP [62,63].

In general, the substrate specificity of most OATPs is extremely broad and shows substantial overlap between different members of the superfamily. Substrates of OATP1A2 include various endogenous compounds such as bile acids, steroid hormones and thyroid hormones [25,64-66]. In contrast, information on the substrate specificity of OATP2B1 is limited at present [33]. OATP1B1 is involved in the hepatic uptake of a broad array of endogenous compounds such as leukotriene C4, prostaglandin E2, bilirubin and its glucuronides conjugates [29,67]. Furthermore, a variety of drugs, including 3-hydroxy-3-methylgluatryl coenzyme A reductase (HMG-CoA-reductase) inhibitors (e.g., pravastatin and pitavastatin), have been identified as OATP1B1 substrates [30,68,69]. Although OATP1B3 shares substrates with OATP1B1, OATP1B3 is the only OATP member known to transport digoxin [31,33,70].

Substrates of OAT1 and -3 include relatively small and hydrophilic organic anions, such as methotrexate, antiviral agents, β-lactam antibiotics and NSAIDs [40,71,72]. OAT2 also transports small and hydrophilic organic anions including salicylate and indometacin [73].

OCT1, -2 and -3 all transport a broad range of structurally diverse organic cations with extensively overlapping substrate specificities [45]. Clinically useful drugs for which transport has been demonstrated include antiparkinsonians (amantadine), antidiabetics (biguanide metformin) and the H_2-receptor agonist cimetidine [45].

3. Sites of polymorphisms and allelic frequency in different ethnic populations

ABCB1, the MDR1 gene, is located on chromosome 7 at q21, with 28 exons encoding a protein of 1280 amino acids [74]. Recently, Bodor *et al.* [75] used several different human cell lines as well as lymphocytes and liver samples to investigate eventual differences between tissues and/or subjects regarding the *ABCB1* gene locus, and confirmed the length of the *ABCB1* gene is most likely 209 kb, as indicated in the database (accession number NT007933). The first evidence of the presence of naturally occurring polymorphisms in human *ABCB1* was reported by Mickley *et al.* [76] who found two SNPs in exon 21 (2677G→T) and 24 (2995G→A) (Figures 1 and 2). Subsequently, screening of the entire *ABCB1* gene has been undertaken by various laboratories and, so far, numerous SNPs have been identified [77-82]. Some SNPs are nonsynonymous; for example, G→T (2677G→T) and G→A (2677G→A) transversions at position 2677 in exon 21, located on the intracellular side of P-gp after transmembrane region 10, result in an amino acid change from Ala at codon 893 to Ser and Thr, respectively. In contrast, 1236C→T (exon 12) and 3435C→T (exon 26) are synonymous. Interestingly, some SNPs, such as 1236C→T, 2677G→T/A and 3435C→T are closely linked; thus, haplotype-oriented assignment has been taken into consideration in recent genotype–phenotype studies [78,83-86].

The allelic frequency distributions of SNPs in *ABCB1* have been reported in various racial populations (Table 2). The incidence of the most known SNPs, but also haplotypes, is highly racially dependent. The above-mentioned three variants are found at 45 – 55% frequency in Caucasians and 35 – 50% in Japanese, but only at 5 – 10% frequency in African-Americans. Interethnic differences in the distribution of the variants are a possible cause of interethnic differences in the pharmacokinetics of P-gp substrate drugs. Differences in the oral bioavailability of ciclosporin and tacrolimus and the incidence of resistance and more aggressive tumours are illustrated as samples [87-90].

ABCC2 (MRP2 gene) is composed of 32 exons encoded by an ~ 45-kb gene located on chromosome 10q24 [91,92]. Similar to the *ABCB1* gene, numerous variations have been identified in the *ABCC2* gene. Genetic analysis of *ABCC2* is well documented in patients with Dubin–Johnson syndrome (DJS), an autosomal recessive disorder characterised by conjugated hyperbilirubinaemia. At present, at least 16 variants have been identified in DJS patients, and a wide variety of genetic mechanisms, including missense mutation, nonsense mutation, splice site mutation and deletion mutation, are responsible for DJS [93]. In healthy Japanese volunteers

Figure 1. Schematic representation of secondary structures in drug transporters, with some nucleotide substitutions.
BCRP: Breast cancer-resistance protein; Exc: Extracellular; Inc: Intracellular; Mnb: Membrane; MRP: Multi-drug resistance-associated protein; NBD: Nucleotide-binding domain; P-gp: P-glycoprotein.

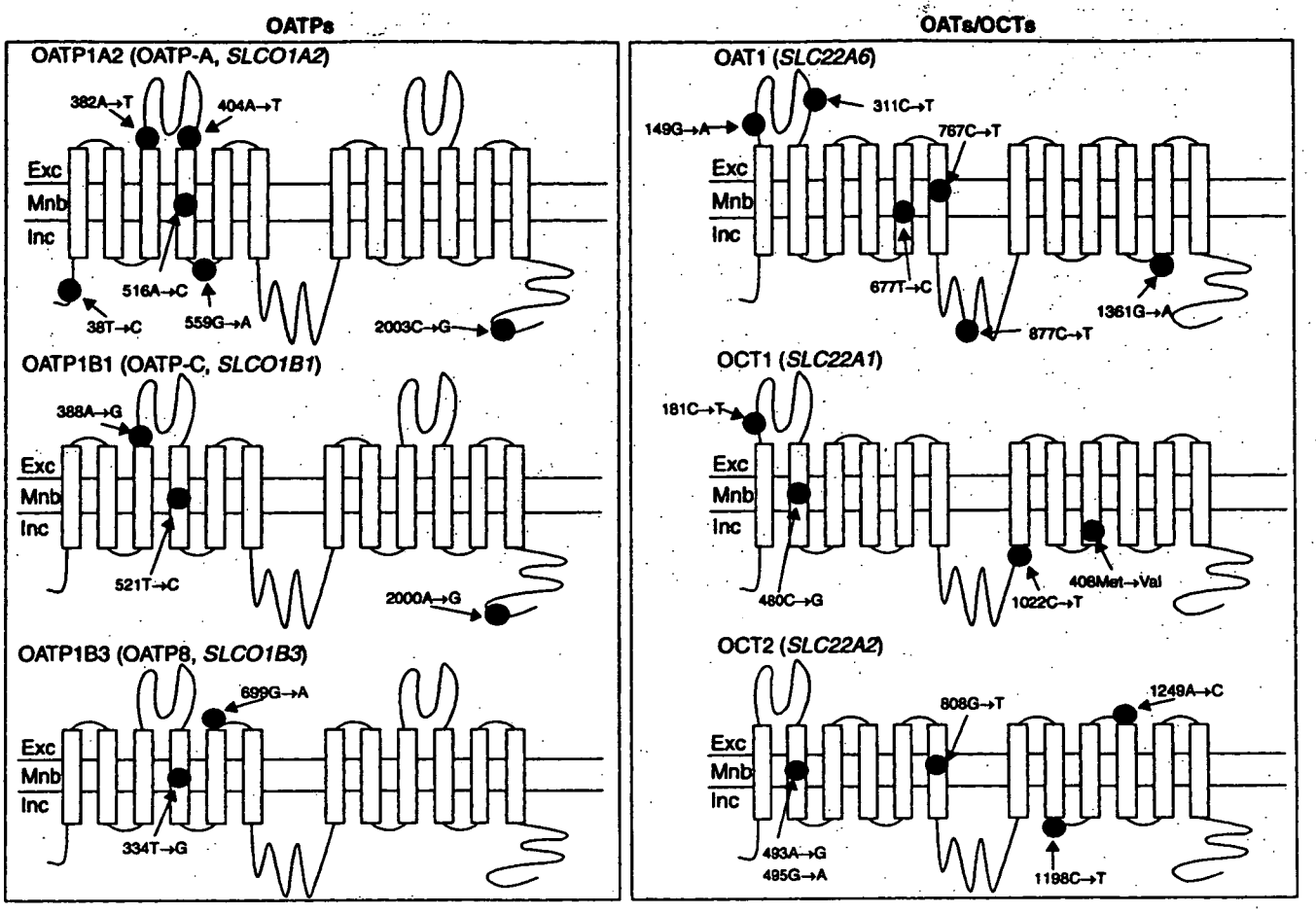

Figure 2. Schematic representation of secondary structures in drug transporters, with some nucleotide substitutions.
Exc: Extracellular; Inc: Intracellular; Mnb: Membrane; OAT: Organic anion transporter; OATP: Organic anion-transporting polypeptide; OCT: Organic cation transporter.

Table 2. Summary of racial genetic data of naturally occurring variations of human drug transporters.

Gene	Mutation	Location	Effect	Allelic frequency (n)		
				Japanese (48 – 220)	Caucasians (37 – 461)	African-Americans (23 – 200)
ABCB1 (MDR1)	-129T→C	Exon 1b	Noncoding	0.94/0.06	0.94 – 0.97/ 0.03 – 0.06	
	1236C→T	Exon 12	Synonymous	0.35/0.65	0.54 – 0.66/ 0.34 – 0.46	0.79 – 0.85/ 0.15 – 0.21
	2677G→(T/A)	Exon 21	893Ala→(Ser/Thr)	0.36 – 0.44/ 0.36 – 0.42/ 0.20 – 0.22	0.50 – 0.56/ 0.38 – 0.46/ 0.02 – 0.10	0.85 – 0.89/ 0.10 – 0.15/ 0.00 – 0.01
	3435C→T	Exon 26	Synonymous	0.51 – 0.62/ 0.38 – 0.49	0.43 – 0.54/ 0.46 – 0.57	0.74 – 0.84/ 0.16 – 0.26
ABCC2 (MRP2)	-24C→T	Promoter	Noncoding	0.81/0.19		
	1249G→A	Exon 10	417Val→Ile	0.88/0.13		
	2302C→T	Exon 18	768Arg→Trp	0.99/0.01		
	2366C→T	Exon 18	789Ser→Phe	0.99/0.01		
	4348G→A	Exon 31	1450Ala→Thr	0.99/0.01		
ABCG2 (BCRP)	34G→A	Exon 2	12Val→Met	0.81 – 0.83/ 0.17 – 0.19	0.90 – 0.96/ 0.04 – 0.10	0.94/0.06
	376C→T	Exon 4	126Gln→ (stop codon)	0.98 – 0.99/ 0.01 – 0.02	1.00/0.00	1.00/0.00
	421C>→A	Exon 5	141Gln→Lys	0.67 – 0.73/ 0.27 – 0.33	0.86 – 0.89/ 0.11 – 0.14	0.95 – 0.97/ 0.02 – 0.05
	1515C (deletion)	Exon 13	509Met→ (stop codon)	0.995/0.005		
SLCO1A2 (OATP-A)	38T→C	Exon 1	13Ile→Thr		0.89 – 0.94/ 0.06 – 0.11	0.98/0.02
	382A→T	Exon 4	128Asn→Tyr		1.00/0.00	0.99/0.01
	516A→C	Exon 5	172Glu→Asp		0.95 – 0.98/ 0.02 – 0.05	0.98/0.02
	559G→A	Exon 5	187Ala→Thr		0.99/0.01	1.00/0.00
	2003C→G	Exon 14	668Thr→Ser		0.99/0.01	0.96/0.04
SLCO1B1 (OATP-C)	-11187G→A	Promoter	Noncoding		0.93/0.07	
	388A→G	Exon 4	130Asn→Asp	0.37/0.63	0.53 – 0.69/ 0.31 – 0.47	0.25/0.75
	521T→C	Exon 5	174Val→Ala	0.84 – 0.89/ 0.11 – 0.16	0.82 – 0.88/ 0.12 – 0.18	0.98/0.02
SLCO1B3 (OATP8)	334T→G	Exon 3	112Ser→Ala	0.70/0.30	0.76/0.24	0.49/0.51
	699G→A	Exon 6	233Met→Ile	0.70/0.30	0.76/0.24	0.49/0.51
SLCO2B1 (OATP-B)	9-bp deletion	Exon 2	Frame shift	0.93/0.07		
	1457C→T	Exon 10	486Ser→Phe	0.69/0.31	0.86/0.14	0.63/0.37
SLC22A6 (OAT1)	149G→A	Exon 1	50Arg→His		1.00/0.00	0.97/0.03
	311C→T	Exon 1	104Pro→Leu		1.00/0.00	0.99/0.01

BCRP: Breast cancer-resistance protein; MDR: Multi-drug resistance; MRP: Multi-drug resistance-associated protein; OAT: Organic anion transporter; OATP: Organic anion-transporting polypeptide; OCT: Organic cation transporter.

Table 2. Summary of racial genetic data of naturally occurring variations of human drug transporters (continued)

Gene	Mutation	Location	Effect	Allelic frequency (n)		
				Japanese (48 – 220)	Caucasians (37 – 461)	African-Americans (23 – 200)
	677T→C	Exon 4	226Ile→Thr		0.99/0.01	1.00/0.00
	767C→T	Exon 4	256Ala→Val		1.00/0.00	0.99/0.01
	877C→T	Exon 5	293Arg→Trp		1.00/0.00	0.98/0.02
	1361G→A	Exon 8	454Arg→Gln		1.00/0.01	0.99/0.01
SLC22A8 (OAT3)	1166C→T	Exon 8	389Ala→Val	0.99/0.01		
SLC22A1 (OCT1)	181C→T	Exon 1	61Arg→Cys	1.00/0.00	0.91 – 0.93/ 0.07 – 0.09	1.00/0.00
	262T→C	Exon 1	88Cys→Arg	1.00/0.00	0.99/0.01	1.00/0.00
	480C→G	Exon 2	160Phe→Leu	0.89/0.11	0.78 – 0.93/ 0.07 – 0.22	0.99/0.01
	1022C→T	Exon 6	341Pro→Leu	0.84/0.16	1.00/0.00	0.92/0.08
	17857G→A	Exon 7	401Gly→Ser	1.00/0.00	0.97 – 0.99/ 0.01 – 0.03	0.99/0.01
	17878A→G	Exon 7	408Met→Val	0.17/0.83	0.40/0.60	0.26/0.74
	17914(ATG) deletion	Exon 7	420Met deletion	1.00/0.00	0.81 – 0.84/ 0.16 – 0.19	0.97/0.03
	32870G→A	Exon 9	465Gly→Arg	1.00/0.00	0.96 – 0.99/ 0.01 – 0.04	1.00/0.00
SLC22A2 (OCT2)	495G→A	Exon 2	165Met→Ile	1.00/0.00	1.00/0.00	0.99/0.01
	601C→T	Exon 3	200Thr→Met	0.99/0.01	1.00/0.00	1.00/0.00
	808G→T	Exon 4	270Ala→Ser	0.83 – 0.87/ 0.13 – 0.17	0.84/0.16	0.89/0.11
	1198C→T	Exon 7	400Arg→Cys	1.00/0.00	1.00/0.00	0.98/0.02
	1294A→C	Exon 8	432Lys→Gln	1.00/0.00	1.00/0.00	0.99/0.01
SLC22A3 (OCT3)	1270A→T	Exon 7	424Thr→Ser	0.99/0.01		

BCRP: Breast cancer-resistance protein; MDR: Multi-drug resistance; MRP: Multi-drug resistance-associated protein; OAT: Organic anion transporter; OATP: Organic anion-transporting polypeptide; OCT: Organic cation transporter.

(n = 48), Ito *et al.* [79] analysed the entire *ABCC2* gene and found six SNPs. Among them, 1249G→A in exon 10, a non-synonymous mutation (417Val→Ile) was frequently observed with an allelic frequency of 12.5%. Only one heterozygote (allelic frequency is 1%) was observed out of 48 volunteers for 2302C→T (768Arg→Trp in exon 18), 2366C→T (789Ser→Phe in exon 18) and 4348G→A (1450Ala→Thr in exon 31).

The *ABCG2* gene is located at 4q22 and encodes a 72-kDa membrane protein composed of 655 amino acids [22,94]. So far, systematic mutation analysis of the *ABCG2* gene has been performed in various ethnic populations, and > 40 SNPs have been identified [95-98]. The two most frequent non-synonymous mutations identified in humans are 34G→A (12Val→Met in exon 2) and 421C→A (141Gln→Lys in exon 5). When comparing the frequencies of the three major variants (i.e., 34G→A, 376C→T and 421C →A) among three ethnic populations (Japanese, Caucasian and African-American), Japanese subjects had significantly higher frequencies of 34G→A and 421C→A than the other two ethnic groups. Interestingly, these three variants occurred simultaneously, and the following four haplotypes were identified: G-C-C, G-C-A, A-C-C and G-T-C with their corresponding allelic frequencies of 46, 35, 18 and 1%, respectively [97]. Thus, similar to other transporter genes, the genetic frequency of *ABCG2* appears to be dependent on ethnicity.

Two recent studies have been conducted to identify SNPs in the *SLCO1A2* gene using genomic DNA samples from various ethnic populations [99,100]. Iida *et al.* [99] screened 27-kb wide for *SLCO1A2* in a Japanese population (n = 48). They did not detect SNPs in the exonic regions, but identified several variations in the 5'-flanking region. Among them, three variations (-916G→A, -526T→C and -189A/ins) are of interest because they are located within important transcriptional regulatory

regions (e.g., hepatic nuclear factor 1α). In contrast, Lee *et al.* [100] screened all 14 exons of *SLCO1A2* and identified 6 nonsynonymous SNPs with an allelic frequency in the range of 1.0 – 11.1%. They also demonstrated that allelic frequencies of six identified SNPs are dependent on ethnicity using ethnically defined DNA (European, African, Chinese and Hispanic-Americans).

So far, at least 15 nonsynonymous *SLCO1B1* SNPs have been identified in various ethnic populations. Among them, two commonly occurring nonsynonymous SNPs, 388A→G (130Asn→Asp in exon 4) and 521T→C (174Val→Ala in exon 5), are of special interest, due to not only their marked consequences in transport capability, but also interethnicity in allelic frequency. In addition to SNP-based analysis, haplotype-oriented assessment has also been well documented [101-104]. At least 17 haplotypes have been recognised so far. Major haplotypes in humans are as follows: *SLCO1B1*1a* (130Asn174Val), *-*1b* (130Asp174Val), *-*5* (130Asn174Ala), *-*15* (130Asp174Ala) and *-*17* (-11187G→A130Asp174Ala). Although the frequency of *SLCO1B1*5* is extremely low in Asian and black populations, the frequency in Caucasians is ~ 15%. In contrast, *SLCO1B1*15* is more common in Asian populations [69,101,103]. Interestingly, although the allelic frequency of 521T→C is similar between Asians and Caucasians (~ 15%), their haplotype patterns are different. In Asian populations, the 521T→C polymorphism is combined with the 388A→G variant. In recent Japanese data, the -11187G→A variant is also tightly linked (~ 100%) to the *SLCO1B1*15* allele [105].

A recent report has described the identification of SNPs in *SLCO1B3* in a population of Japanese individuals [99]. Based on this study and the authors' unpublished data, at least two nonsynonymous SNPs, 334T→G (112Ser→Ala in exon 3) and 699G→A (233Met→Ile in exon 6), exist with an allelic frequency ranging 0.24 – 0.51. Because the frequencies of these two SNPs were identical in all ethnic populations studied, these SNPs may occur simultaneously (being haplotyped).

Interestingly, collective evidence indicates that the frequency of nonsynonymous SNPs in OAT family genes (*SLC22A6*, *SLC22A7* and *SLC22A8*) appears extremely lower (< 1%) [99,103,106,107], suggesting these genes are relatively intolerant of nonsynonymous changes. Fujita *et al.* [106] focused on *SLC22A6* (OAT1 gene) and identified 6 nonsynonymous SNPs using 267 DNA samples from an ethnically diverse population. Only two SNPs, 149G→A (50Arg→His in exon 1) and 877C→T (293Arg→Trp in exon 5), were present at ≥ 1% in at least one ethnic population. They also identified 17 distinct haplotypes. Xu *et al.* [107] resequenced the coding regions of four OAT member genes from an ethnically diverse, healthy population (n = 192), and identified two nonsynonymous SNPs in *SLC22A6*, three in *SLC22A7*, one in *SLC22A8* and eight in *SLC22A9*, with an allelic frequency in the range of 0.01 – 0.03.

Some groups have screened for genetic variants of *SLC22A1* (encoding OCT1) in various ethnic populations [108-110]. Kerb *et al.* [110] identified 4 nonsynonymous SNPs (61Arg→Cys, 88Cy→Arg, 160Phe→Leu, 401Gly→Ser) and 1 deletion (420Met→del) in 57 Caucasian samples, with respective allelic frequencies of 9.1, 0.6, 22.0, 3.2 and 16.0%. Subsequently, Shu *et al.* [109] also detected numerous variations from five different ethnic groups. Some known SNPs (e.g., 41Phe→Leu and 117Pro→Leu) were observed in at least one ethnic population, suggesting ethnic diversity in *SLC22A1* polymorphism.

Similar to *SLC22A1*, several genetic variants in the coding region of *SLC22A2* (OCT2 gene) have been identified [111,112]. *SLC22A2* polymorphism was recently investigated comprehensively by screening all 11 exons as well as intronic sequence using 247 ethnically diverse DNA samples [112]. Among eight nonsynonymous SNPs, four (165Met→Ile, 270Ala→Ser, 400Arg→Cys and 432Lys→Gln) were polymorphic, with ethnic-specific allelic frequencies ≥ 1%. Novel variations, including SNPs and deletion, have also been reported in recent Japanese studies [111,113].

4. Impact of polymorphisms on pharmacotherapy

4.1 Pharmacokinetic consequences

In the pharmacogenomics of the *ABCB1* gene, Hoffmeyer *et al.* [77] first reported that a synonymous SNP, 3435C→T, was associated with significantly reduced intestinal P-gp content in subjects with the T/T genotype in comparison with subjects homozygous for the C allele (C/C), leading to higher steady-state plasma concentrations after the oral administration of digoxin in T/T subjects. After this report, a remarkably large number of clinical studies have been conducted around the world on the association of the *ABCB1* genotype and pharmacokinetic phenotypes. Most studies have focused on SNPs in the following two exons, 21 (2677G→T/A) and 26 (3435C→T); however, as summarised in recent reviews [50,82,114-116] and Table 3, the published observations conflict even when using the same probe drug and even among the same racial group. For example, Sakaeda *et al.* [117] conducted an ABCB1 genotype–phenotype study using digoxin as a probe and found that the AUC of digoxin in the absorption phase was significantly lower in subjects with 3435T/T genotype than in 3435C/C subjects. These observations are in line with a finding by Kim *et al.* [81], but are in contrast to the findings of Hoffmeyer *et al.* [77] and Kurata *et al.* [118]. In order to overcome these disagreements, some researchers have incorporated haplotype-oriented analysis into the genotype–phenotype study [83-86,119]. Recent studies have demonstrated that haplotype assessment represents more precise prediction of the pharmacokinetics of certain drugs such as digoxin [119] and ciclosporin [83].

Wang *et al.* [120] introduced new approach for the evaluation of the 3435C→T variant. The level of mRNA expression can be regulated in a *cis* or *trans* fashion, and the *cis*-acting polymorphism changes the expression of the gene transcript

Table 3. Impact of *ABCB1* gene variants on PK of drug substrates

Polymorphism	Population	Drug	Functional effect of the variant allele	Ref.
3435C→T	Caucasian HV	Digoxin	Increased AUC after single dose for T/T	[77]
	Caucasian HV	Digoxin	Higher AUC and C_{max} under steady state for T/T*	[119]
	Japanese HV	Digoxin	Higher BA after single dose for T/T*	[118]
	Caucasian and African HV	Digoxin	Higher AUC after single dose for T/T	[191]
	Caucasian HV	Digoxin	No difference in PK data after single dose	[192]
	Japanese HV	Digoxin	Decreased AUC after single dose for T/T	[117]
	Korean HV	Fexofenadine	Higher AUC and C_{max} after single dose for T/T*	[193]
	Caucasian HV	Fexofenadine	No difference in PK data after single dose	[194]
	Caucasian and African HV	Fexofenadine	Decreased AUC after single dose for T/T	[81]
	Asian HT patients	Ciclosporin	Higher AUC under steady state for T/T*	[84]
	Caucasian RT patients	Ciclosporin	No difference in C_{min} under steady state	[195]
	Caucasian and African HV	Ciclosporin	No difference in AUC after single dose	[196]
	Caucasian RT patients	Ciclosporin	Decreased AUC under steady state for C/T and T/T	[197]
	LT patients	Ciclosporin	Higher plasma (or serum) level/dose ratio under steady state for T/T	[198]
	RT patients	Tacrolimus	Higher C_{min} under steady state for T/T	[199]
	HT paediatric patients	Tacrolimus	Higher C_{min} under steady state for C/T and T/T	[200]
	RT patients	Tacrolimus	No difference in C_{min} under steady state	[201]
	Caucasian HV	Talinolol	No difference in AUC*	[202]
	Chinese HV	Talinolol	No difference in AUC after single dose*	[203]
	Caucasian HIV-1 patients	Nelfinavir, efavirenz	Lower C_{min} under steady state for T/T	[143]
	HIV patients	Atazanavir	Lower drug level under steady state for T/T	[204]
	Caucasian HV	Loperamide	No difference in PK data after single dose	[205]
	HV	Dicloxacillin	No difference in C_{max} after single dose	[206]
	Turkish HV	Phenytoin	Higher drug level under steady state for T/T	[207]
	Japanese schizophrenic patients	Risperidone	No difference in C_{min} under steady state	[208]
	ALL paediatric patients	Vincristine	No difference in PK data*	[160]
2677G→(T/A)	Japanese HV	Digoxin	Higher BA after single dose for T/T*	[118]
	Caucasian and African HV	Digoxin	Higher AUC after single dose for T/T	[191]
	Caucasian HV	Digoxin	No difference in PK data after single dose	[192]
	Japanese HV	Digoxin	Lower AUC after single dose for T/T	[209]
	Caucasian and African HV	Fexofenadine	Decreased AUC after single dose for T/T	[81]
	Korean HV	Fexofenadine	Decreased AUC after single dose for A/A*	[193]
	Asian HT patients	Ciclosporin	Higher AUC under steady state for T/T*	[84]
	HT paediatric patients	Tacrolimus	Higher C_{min} under steady state for G/T and T/T	[200]

*Including haplotype assessments.
ALL: Acute lymphoblastic leukaemia; BA: Bioavailability; HT: Heart transplant; HV: Healthy volunteers; LT: Liver transplant; PK: Pharmacokinetics; RT: Renal transplant.

Table 3. Impact of *ABCB1* gene variants on PK of drug substrates (continued)

Polymorphism	Population	Drug	Functional effect of the variant allele	Ref.
	RT patients	Tacrolimus	Higher drug level under steady state for T/T*	[210]
	Caucasian HV	Talinolol	Slightly higher in AUC for T/A and T/T*	[202]
	Chinese HV	Talinolol	No difference in AUC after single dose*	[203]
	ALL paediatric patients	Vincristine	No difference in PK data*	[160]

*Including haplotype assessments.

ALL: Acute lymphoblastic leukaemia; BA: Bioavailability; HT: Heart transplant; HV: Healthy volunteers; LT: Liver transplant; PK: Pharmacokinetics; RT: Renal transplant.

from the allele carrying the polymorphism, leading to the allelic expression imbalance. In order to test for the presence of *cis*-acting polymorphisms in human *ABCB1* that might be responsible for altered mRNA expression of the 3435T allele, they measured differences in allelic mRNA expression between the 3435T and 3435C allele using liver samples from heterozygous individuals carrying the 3435C→T SNP. They indicated that mRNA expression of the 3435C allele was significantly higher than that of the 3435T allele (3435C/3435T ratios in the range of 1.06 – 1.16). Based on the experiments including *in vitro* transfection of mixtures of *ABCB1* variants carrying all possible combinations of 1236C→T, 2677G→T and 3435C→T, they concluded that 3435C→T is the main functional polymorphism affecting mRNA levels, by altering mRNA stability. Interestingly, allelic expression imbalance has been observed in other pharmacokinetic genes such as *ABCG2*, *CYP3A5* and *CYP3A4* [97,121,122].

The question arises as to why the contribution of SNPs to the pharmacokinetics of some probes (e.g., digoxin and fexofenadine) differs among reports. The reasons for this discrepancy remain to be addressed; however, multiple tissue expression of P-gp with various vectorial movements and no suitable specific probe drug for P-gp function may contribute. Recently, Brunner *et al.* [123] measured the brain distribution of a model P-gp substrate, the calcium-channel inhibitor verapamil [124], using positron emission tomography in two groups of healthy volunteers. To these authors' knowledge, this is the first evaluation of P-pg function, as a 'gatekeeper' (i.e., regulating drug uptake to highly sensitive tissue brain), in the BBB directly. They indicate no difference in the brain distribution of [^{11}C]verapamil between the TTT haplotype (1236T, 2677T and 3435T) and the wild-type CGC haplotype (1236C, 2677G and 3435C). Because positron emission tomography has sensitivity in the lower picomolar range for tissue concentrations of drug molecules to be measured, and because P-gp-triggered active efflux may be an unyielding barrier in the brain penetration of substrate drugs, their findings that failed to show an effect of *ABCB1* gene polymorphisms on P-gp functions in the BBB are notable.

Sparreboom *et al.* [125] first studied the effects of naturally occurring, common variant *ABCG2* 421C→A on the pharmacokinetics of diflomotecan, a synthetic derivative of camptothecin, in 22 adult white patients with cancer. They found that plasma levels of diflomotecan after intravenous administration were significantly higher (~ 300%) in patients with 421C/A genotype than in 421C/C patients. However, despite expectations of significant genotype-dependent regulation in intestinal absorption due to its enriched localisation, the pharmacokinetics of diflomotecan did not differ between the two genotype groups after oral administration. Although further investigation is required to resolve this issue, these observations partially agree with some *in vitro* studies, indicating that the *ABCG2* 421C→A allele is associated with low BCRP expression levels [97,126,127]. These *in vitro* observations suggest that carriers of the 421C→A allele may have decreased clearance (increased plasma levels) and/or increased bioavailability. In a preliminary fashion, Sparreboom *et al.* [128] also reported that the heterozygous 421C/A allele observed in 2 patients was associated with a 1.34-fold increased oral bioavailability of topotecan compared with that in 10 patients with the 421C/C genotype. In contrast, de Jong *et al.* [129] reported no difference in the pharmacokinetic parameters of irinotecan and SN-38 between patients with and without the *ABCG2* 421C→A allele. They noted that other processes involved in irinotecan metabolism and elimination that exhibit great interindividual variation might be overshadowing any effect of this *ABCG2* polymorphism.

As described previously, because MRP2 (ABCC2 protein) is responsible for the export of conjugated drug metabolites from hepatocytes to bile, and because many *ABCC2* variants are known to be associated with DJS, naturally occurring *ABCC2* variants are expected to be involved in large interindividual differences in pharmacokinetic and pharmacodynamic consequences of substrate drugs; however, no sufficient human data have been reported so far. To the authors' knowledge, at least two studies have been carried out to assess possible associations of genetic variants in *ABCC2* with phenotypes (i.e., cellular exposure of nelfinavir [130] and pharmacokinetics of pravastatin [102]). However, there were no significant associations between phenotype indices and SNPs and/or haplotypes at *ABCC2*.

Facilitative hepatic uptake from the portal circulation by OATP1B1 is thought to contribute to tissue selectivity and therapeutic response to HMG-CoA reductase inhibitors (statins). Nishizato *et al.* [103] screened genetic polymorphism in the *SLCO1B1* using DNA from 120 Japanese healthy

volunteers and conducted a clinical study to examine whether variants alter transport activity with pravastatin as a selective probe drug. Subjects with the *SLCO1B1*15* allele (130Asp174Ala) had reduced total and nonrenal clearance, as compared with those with the *SLCO1B1*1b* allele (130Asp174Val), and the difference between **1b/*1b* and **1b/*15* subjects was significant. In their study, only one subject harboured the **15/*15* genotype, with nonrenal clearance about a tenth of that in the **1b/*1b* genotype. They first demonstrated that commonly occurring SNPs in the *SLCO1B1* gene are likely to be associated with altered pharmacokinetics of substrate drugs in humans. Niemi *et al.* [102] also evaluated the relationship between *SLCO1B1* variants and the pharmacokinetics of pravastatin. In heterozygous carriers of **15B* (130Asp174Ala), the mean pravastatin AUC_{0-12} was 93% higher compared with noncarriers and, in heterozygous carriers of **17* (-11187G→A and 130Asp174Ala), it was 130% higher compared with noncarriers. They also reported no significant associations between *SLCO2B1*, *ABCC2* or *ABCB1* polymorphisms and the pharmacokinetics of pravastatin. Based on the healthy volunteers study, Mwinyi *et al.* [104] found that **5* allele (130Asn174Ala) delayed the hepatocellular uptake of pravastatin, whereas **1b* allele seemed to accelerate OATP1B1-dependent uptake of the drug. Chung *et al.* [69] characterised the effects of *SLCO1B1* alleles, **1a*, **1b* and **15* on the pharmacokinetics of pitavastatin. Despite small sample sise, the dose-normalised AUC and C_{max} of pitavastatin were 1.4- and 1.8-fold higher, respectively, in subjects heterozygous for the **15* allele versus subjects not varying this allele. Similar to pravastatin, the **15* allele is suggested to be associated with decreased pitavastatin uptake from blood into hepatocytes. Systematic exposure to rosuvastatin had been observed to be ~ 2-fold higher in Japanese subjects living in Japan compared with white subjects in Western Europe or the US [131-133]. Because OATP1B1 contributes to the hepatic uptake of rosuvastatin [134], in order to determine whether polymorphisms in the *SLCO1B1* gene contribute to any pharmacokinetic differences, Lee *et al.* [135] conducted a pharmacokinetic study including four racial populations. They found that *SLCO1B1* 521T→C did not account for the clear population differences in rosuvastatin exposure among white subjects and Asian groups. Although no 521C/C homozygote in Asian subjects seems to be the most likely reason for failed to show up the differences, they concluded that the pharmacogenetics of other rosuvastatin disposition pathways may better explain the ethnic differences in pharmacokinetics [136].

The H_1-receptor antagonist fexofenadine is a P-gp substrate [65]; however, association between the pharmacokinetics of fexofenadine and polymorphism of the *SLCO1B1* gene has recently been reported [137]. The mean total AUC of fexofenadine in the -11187G/G521C/C subjects was 76% higher in subjects with the 521T/C genotype and 127% higher in subjects with the 521T/T genotype. These results suggest that OATP1B1 is involved in fexofenadine exposure, and may partly explain the conflicting observations between fexofenadine pharmacokinetics and *ABCB1* polymorphism.

Niemi *et al.* [138] investigated possible associations between the pharmacokinetics of repaglinide, a meglitinide analogue antidiabetic drug, and SNPs in genes encoding for OATP1B1, P-gp, CYP2C8 and CYP3A5 in 56 healthy subjects. Multiple regression analysis indicated that the *SLCO1B1* 521T→C and *CYP2C8*3* allele were independent predictors of the $AUC_{0-\infty}$ and C_{max} of repaglinide; the $AUC_{0-\infty}$ in the subjects with 521C/C genotype was 107 and 188% higher, respectively, than in subjects with the 521C/T or 521T/T genotype; however, surprisingly, only *SLCO1B1* -11187G→A was significantly associated with an enhanced effect of repaglinide on blood glucose, even though SNPs at positions -11187 and 521 are haplotyped.

Very recently, the authors studied the effects of polymorphism of *SLCO1B1*, particularly the **1b* allele, on the pharmacokinetics of three anionic drugs, pravastatin, valsartan and temocapril in a three-way crossover manner in 23 healthy Japanese volunteers [139]. The authors found that AUC of pravastatin in **1b/*1b* carriers was 65% of that in **1a/*1a* carriers, and AUC of valsartan and temocapril in each subject was significantly correlated with that of pravastatin. These results suggest that: i) *SLCO1B1*1b* allele enhances the hepatic uptake activity of pravastatin; and ii) OATP1B1 is one of the determinant factors governing interindividual variability in the pharmacokinetics of these three drugs. Reduction of pravastatin AUC in the **1b* subjects was well consistent with the above-mentioned study conducted by Mwinyi *et al.* [104].

Two studies have examined the effects of *SLCO1B1*5* and **15* on the functional properties of OATP1B1 using cDNA transfected cells. Kameyama *et al.* [140] evaluated transport capability by transient expression system of HEK293 and HeLa cells using endogenous conjugates, estradiol-17β-D-glucuronide and estrone-3-sulfate, and statins as substrates. Kinetic analysis of pravastatin and atorvastatin showed that K_m values were not altered, but V_{max} values decreased significantly in cells expressing the variants. Immunocytochemical study showed that the variant-typed proteins were localised not only at the plasma membrane, but also in the intracellular space. In contrast, Iwai *et al.* [141] indicated that all SNP variants expressed in HEK293 cells were predominantly located on the cell surface without changes in K_m values for the transport of 17β-estradiol 17β-D-glucuronide. However, the normalised V_{max} value (by the protein expression level estimated from western blotting) for *SLCO1B1*15* was drastically decreased to < 30% compared with **1a*. Although the observation of lower V_{max} values in the *SLCO1B1*15* cells was similar between the studies, the expression manner was clearly controversial. There is no good reason for this discrepancy; however, we need to be careful in expecting *in vivo* cellular localisation from the results of *in vitro* expression system.

Although many SNPs have been identified in the OCT2 gene, no pharmacogenomic human study has yet been

Table 4. Impact of the *ABCB1* (MDR1) genetic variant on PD of drug substrates and their consequences on disease states

Population (disease)	Polymorphism	Drug	Outcome marker	Effect	Ref.
Caucasian RT patients	3435C→T	Ciclosporin	Acute rejection	No significant difference	[195]
Caucasian patients (depression)	3435C→T	Nortriptyline	Nortriptyline-induced postural hypotension	Higher in T/T	[211]
Caucasian patients (HIV infection)	3435C→T	Nelfinavir, efavirenz	CD4 recovery with treatment	Higher in T/T	[143]
HIV patients	3435C→T		Virological suppression	Higher in T/T	[212]
Caucasian patients (HIV infection)	3435C→T		CD4 recovery with treatment	No significant difference	[145]
HIV patients	3435C→T	Efavirenz	Drug-induced HDL-cholesterol level	Higher in C/C	[213]
Paediatric HT patients	3435C→T 2677G→T	Corticosteroids	Steroid weaning 1 year after HT	Higher in T/T	[214]
AML patients	1236C→T 2677G→(T/A) 3435C→T	Menu of SHG-AML-96	OS and PR	Higher OS and low PR in T/T	[155]
AML patients	2677G→T		OS and PR	No significant difference	[156]
ALL patients	3435C→T		OS	No significant difference	[157]
Colon cancer patients	3435C→T		Susceptibility to colon cancer	Higher in T/T	[179]
Japanese LT patients	2677G→(T/A)	Tacrolimus	Tacrolimus-induced neurotoxicity	Higher in T/T	[161]
RT patients	3435C→T	Ciclosporin	Ciclosporin-induced tremor	No significant difference	[215]
RT patients	2677G→(T/A) 3435C→T	Ciclosporin	Ciclosporin-induced nephrotoxicity	Higher in T/T (donor side)	[162]
Lung transplant patients	2677G→T 3435C→T	Tacrolimus + predonisolone + azathiopurine	Acute persistent rejection	Higher in patients with the C allele	[216]
RT patients	3435C→T	Ciclosporin	Gingival overgrowth	No significant difference	[163]
Caucasian patients (renal epithelial cell cancer)	3435C→T		Susceptibility to renal tumour	Higher in T/T	[181]
Caucasian patients (ulcerative colitis)	3435C→T		Susceptibility to ulcerative colitis	Higher in T/T	[168]
Caucasian patients (Crohn's disease)	3435C→T		Susceptibility to Crohn's disease	No significant difference	[168]
White Spanish patients	2677G→(T/A) 3435C→T		Susceptibility to Crohn's disease	Higher in 2677T/3435C	[175]
White Scottish patients	2677G→T 3435C→T		Susceptibility to ulcerative colitis	Higher in 3435T/T	[174]
Caucasian patients (Parkinson's disease)	3435C→T		Susceptibility to Parkinson's disease	Early onset in T/T (trend)	[164]

5-HT: 5-Hydroxytryptamine; ALL: Acute lymphoblastic leukaemia; AML: Acute myeloid leukaemia; HDL: High-density lipoprotein; HT: Heart transplant; LT: Liver transplant; OS: Overall survival; PD: Pharmacodynamics; PR: Probability of relapse; RT: Renal transplant.

Table 4. Impact of the *ABCB1* (MDR1) genetic variant on PD of drug substrates and their consequences on disease states (continued)

Population (disease)	Polymorphism	Drug	Outcome marker	Effect	Ref.
Parkinson's disease patients	3435C→T		Susceptibility to pesticide-induced Parkinson's disease	Higher in C/T	[165]
Breast cancer patients	3435C→T		Response to preoperative chemotherapy	Decreased resistance in T/T	[217]
Epileptic patients	3435C→T		Response to antiepileptics	Higher in T/T	[152]
Epileptic patients	3435C→T		Response to antiepileptics	No significant difference	[154]
Cancer patients	3435C→T	5-HT_3 receptor antagonists	Antiemetic response (granisetron)	Higher in T/T (first 24 h)	[218]
Japanese patients (schizophrenia)	2677G→(T/A) 3435C→T	Bromperidol	Response to bromperidol		[219]

5-HT: 5-Hydroxytryptamine; ALL: Acute lymphoblastic leukaemia; AML: Acute myeloid leukaemia; HDL: High-density lipoprotein; HT: Heart transplant; LT: Liver transplant; OS: Overall survival; PD: Pharmacodynamics; PR: Probability of relapse; RT: Renal transplant.

reported. A recent study of monozygotic twin pairs showed that genetic factors contribute substantially to the renal clearance of metformin [142]. Genetic variation in the OCT2 is expected to explain the large interindividual variability in the pharmacokinetics of metformin.

Fujita *et al.* [106] conducted a small family based clinical study to determine the renal elimination of a model OAT1 substrate, adefovir, with regard to a nonfunctional variant, OAT1 1361G→A. They did not observe significant differences in renal clearance or renal secretory clearance in family members heterozygous for 1361G→A allele, in comparison with family members who did not carry this variant.

In OAT3 pharmacogenomics, one study reported no remarkable differences in both the mean renal and renal secretory clearances of pravastatin among the genotypic groups evaluated (reference, 723T→A and 1166C→T) [103].

4.2 Pharmacodynamic consequences

An association of *ABCB1* gene polymorphisms with pharmacodynamic consequences was reported for HIV therapy, antiepileptic pharmacotherapy, chemotherapy, adverse effects of P-gp substrates such as immunosuppressants and tricyclic antidepressants, and others (Table 4).

Fellay *et al.* [143] have studied the association between the response to antiretroviral treatment and the *ABCB1* genotype in 123 HIV-1-infected patients treated with efavirenz or nelfinavir. They found that patients with 3435T allele have a better response to the drugs after treatment for 6 months, as determined by an increased $CD4^+$ count. Haas *et al.* [144] also found that the 3435T/T genotype was associated with a decreased likelihood of virological failure and decreased emergence of efavirenz-resistant virus, but not with plasma efavirenz exposure after long-term follow up lasting up to 3 years. In contrast, Nasi *et al.* [145] and Winzer *et al.* [146] failed to find an association between the *ABCB1* genotype and virological and immunological responses to antiretroviral therapy.

P-gp can transport antiepileptic drugs [147], and the association of the multiple-drug resistance phenotype in epilepsy with increased lesional P-gp expression levels in resected brain tissues has been speculated [148-151]. Based on these backgrounds, Siddiqui *et al.* [152] genotyped 3435C→T in 315 patients with epilepsy, and demonstrated that patients with drug-resistant epilepsy were more likely to have the C/C genotype than T/T, when compared with patients with drug-responsive epilepsy. However, similar to antiretroviral therapy, controversial observations have been reported by at least two independent laboratories [153,154].

Multi-drug resistance is one of the most serious problems in the failure of chemotherapy, and some clinical studies with regard to *ABCB1* polymorphism and clinical outcomes have been conducted; however, as shown in Table 4, contribution of the *ABCB1* gene variants on outcome markers (e.g., overall survival and probability of relapse) in acute lymphoblastic leukaemia or acute myeloid leukaemia patients is controversial among the studies [155-160].

Numerous adverse reactions including neurotoxicity, nephrotoxicity and gingival hyperplasia are sometimes observed in patients treated with immunosuppressants. Yamauchi *et al.* [161] studied the correlation of the *ABCB1* polymorphism with tacrolimus-induced neurotoxicity (e.g., convulsion and tremor) in patients after living-related donor liver transplantation, and found that the 2677T allele might be a positive predictor of toxicity. Similarly, although daily dose, trough levels, and the concentration per dose ratio were not different between case and control groups, the donor's *ABCB1* 3435T/T genotype was reported to be a predictor of ciclosporin-induced nephrotoxicity [162]. Drug transporters in salivary glands have not yet been characterised; however,

Table 5. Impact of gene variants of the drug transporters PK and PD of substrates and their effect on disease states.

Gene	Polymorphism	Population	Drug/substrate	PK	PD	Functional effect of the variant allele	Ref.
ABCG2 (BCRP)	421C→T (141Gly→Lys)	White cancer patients (n = 22)	Diflomotecan	Yes		Higher AUC for C/T	[125]
	421C→T (141Gly→Lys)	European Caucasian cancer patients (n = 84)	Irinotecan (SN-38 and SN-38G)	Yes		No significant change in PK	[129]
	421C→T (141Gly→Lys)	2 cancer patients	Topotecan	Yes		Higher BA for C/T	[128]
SLCO1B1 (OATP1B1)	**15*	Japanese HV	Pravastatin	Yes		Higher AUC for **15/*15*	[103]
	521T→C	Japanese hyperlipidaemia (n = 66)	HMG-CoA reductase inhibitors		Response to cholesterol-lowering effect	Lower in C/T	[188]
	**15*	Japanese patients	Pravastatin, atorvastatin		Susceptibility to statin-induced myopathy	Higher in **15* patients	[220]
	521 T→C -11187G→A	Caucasian HV (n = 20)	Fexofenadine	Yes		Higher AUC in C/C	[137]
	**17*	Caucasian HV (n = 41)	Pravastatin	Yes	Response to cholesterol synthesis inhibition	Higher AUC and smaller response in **17* subjects	[189]
	521 T→C -11187G→A	HV (n = 56)	Repaglinide	Yes	Response to glucose-lowering effect	Higher AUC in C/C Increased response in G/A	[138]
	388A→G 521T→C	4 racial populations (HV)	Rosuvastatin	Yes		Higher AUC for C/T and C/C	[135]
	**1a, *1b, *15*	Korean HV (n = 24)	Pitavastatin	Yes		Higher AUC for **15* subjects	[69]
	**1a, *1b, *5*	Caucasian HV (n = 30)	Pravastatin	Yes		Higher AUC for **5* subjects Lower AUC for **1b* subjects	[104]
	**15B, *17*	Caucasian HV (n = 41)	Pravastatin	Yes		Higher AUC for **15B* and **17* subjects	[102]
SLC22A6 (OAT1)	1361G→A (454Arg→Gln)	1 African-American family	Adefovir	Yes		No change in renal clearance	[106]
SLC22A8 (OAT3)	1166C→T (389Ala→Val)	1 heterozygote (Japanese HV)	Pravastatin	Yes		No change in renal clearance	[103]

BA: Bioavailability; BCRP: Breast cancer-resistance protein; HMG-CoA: 3-Hydroxy-3-methylgluatryl coenzyme A; HV: Healthy volunteers; OAT: Organic anion transporter; OATP: Organic anion-transporting polypeptide; PD: Pharmacodynamics; PK: Pharmacokinetics.

Drozdzik *et al.* [163] reported no association between *ABCB1* polymorphism and gingival overgrowth in kidney transplant patients with ciclosporin treatment.

Although the physiological role of P-gp is not fully elucidated, it is conceivable that P-gp acts as a cellular barrier at numerous levels in the human body. Therefore, genotype-dependent P-gp function may contribute to disease susceptibility. So far, a number of studies have been reported on the association of *ABCB1* polymorphism with the following diseases: Parkinson's disease [164-167], inflammatory bowel diseases (ulcerative colitis and Crohn's disease) [168-175], cancers (leukaemia [155,176,177], colon cancers [178-180], renal epithelial tumours [181] and glioma [182]), primary biliary cirrhosis [183], rheumatoid arthritis [184] and hypertension [185].