による影響などについての解析も可能である。

2. リガンド結合

被験化合物が転写因子のリガンドになるかどうかを調べる方法として，①ゲル濾過法，②ビーズ法，③時間分解蛍光共鳴エネルギー転移（time-resolved fluorescence resonance energy transfer：TR-FRET）法がある[14]。①と②は，核内レセプターなどの転写因子に対する標識リガンドの結合と被験化合物による競合作用を調べる方法である。大腸菌で発現・精製した転写因子を用い，ゲル濾過カラムあるいは転写因子を結合させたビーズを用いて，リガンド結合量の変化をシンチレーションカウンターで測定する。③は，核内レセプターがリガンドと結合するとコアクチベーターをリクルートするという現象を利用した方法である（図❹）。

おわりに

本稿では薬物代謝酵素と薬物トランスポーターの転写活性化による誘導機構について代表例を概説した。誘導現象は，実験動物とヒトのどちらでも起こりうる現象である。しかし，リファンピシンがヒトPXRを活性化するのに対し，ラットPXRとマウスPXRに対しては活性化せず，pregnenolone 16α-carbonitrile（PCN）はその逆である。同様に，TCPOBOPはマウスCARを活性化するのに対し，ヒトCARとラットCARに対しては活性化しない。核内レセプターのリガンド認識における種差については，不明な点が残されており，転写因子を介した誘導の予測においては種差の存在を十分に考慮する必要がある。

用語解説

1. **核内レセプター**：Znフィンガー型の転写因子。50種以上の遺伝子からなるスーパーファミリーを形成しており，ステロイドホルモンやビタミンをリガンドとする受容体と内因性リガンド未知のオーファン受容体に分類される。薬物代謝酵素および薬物トランスポーターの誘導に関与する核内レセプターには，retinoid X recepor，vitamin D receptorに加え，オーファン受容体であるpregnane X receptor，constitutive androgen receptor，hepatocyte nuclear factor 4 alphaなどがある。
2. **転写活性化**：一般に，RNAポリメラーゼが転写開始点近傍で基本転写因子群と複合体を形成することにより基本転写が開始する。基本転写に必要な遺伝子領域をプロモーター，転写を活性化する領域をエンハンサーと呼ぶ。プロモーターやエンハンサーに転写因子が結合し転写が活性化する。
3. **コリプレッサー**：転写因子に結合することにより転写を制御するコレギュレーターのうち，負の作用を示すもの。NCoR（nuclear receptor corepressor），SMRT（silencing mediator of repressed transcription）が含まれる。リガンド非存在下では，コリプレッサーが核内レセプターと結合することにより転写が抑制されている。リガンド結合による核内レセプターの構造変化に伴ってコリプレッサーが解離し，コアクチベーターがリクルートされる。
4. **核内レセプター結合モチーフ**：核内レセプターが結合するゲノムDNA上の配列。コンセンサス配列（AGGTCA）が1つから8つの塩基をはさんで繰り返されている。繰り返し配列には，同方向の繰り返し（direct repeat：DR），内向きで相補的な繰り返し（inverted repeat：IR），外向きで相補的な繰り返し（everted repeat：ER）があり，n個の塩基をはさんだモチーフはそれぞれDRn, IRn, ERnと標記される。
5. **コアクチベーター**：転写因子に結合することにより転写を制御するコレギュレーターのうち，正の作用を示すもの。SRC-1（steroid receptor coactivator-1），SRC-2（GRIP-1 / TIF2とも呼ぶ），SRC-3（pCIP, RAC3, TRAM1, ACTRとも呼ぶ）がある。誘導性のコアクチベーターとしてPGC-1（peroxisome proliferators activated receptor gamma coactivator-1）も存在する。

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現在の研究テーマは，核内レセプターを介した薬物代謝酵素の発現制御に関する研究。

Severe Toxicities After Irinotecan-Based Chemotherapy in a Patient With Lung Cancer: A Homozygote for the *SLCO1B1*15* Allele

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Abstract: Irinotecan is used widely in the treatment of several malignancies, but unpredictable severe toxicities such as myelosuppression and delayed-type diarrhea are sometimes experienced. Polymorphism of the *UGT1A1* gene is one of the likely reasons for interindividual differences in irinotecan pharmacokinetics and severe toxicity. Also, polymorphic organic anion-transporting polypeptide 1B1 (OATP1B1, *SLCO1B1*) is reported to be involved in the hepatocellular uptake of SN-38. A 61-year-old man with lung cancer developed severe toxicities, including grade 3 diarrhea, grade 4 leukopenia, and grade 4 neutropenia, after the first cycle of irinotecan (60 mg/m^2) plus cisplatin chemotherapy. The irinotecan and SN-38 areas under the concentration–time curve from time zero to infinity in this patient were 43% and 87% higher than the corresponding mean values for 10 other patients with lung cancer treated with irinotecan (60–100 mg/m^2) normalized for the dose of irinotecan. Analysis of genetic variants in genes encoding the drug-metabolizing enzyme (*UGT1A1*) and transporter (*SLCO1B1*) involving irinotecan disposition revealed that this patient was homozygous for the *SLCO1B1*15* allele, which may result in severe toxicities attributable to the extensive accumulation of SN-38. Screening of *SLCO1B1*15* is suggested to be useful in irinotecan chemotherapy to avoid unpredicted severe toxicity, although the homozygous genotype is rare among the Japanese.

Key Words: pharmacogenetics, *SLCO1B1*, irinotecan, pharmacokinetics, toxicity

(*Ther Drug Monit* 2007;29:666–668)

INTRODUCTION

Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin [CPT-11]) has displayed promising results in several malignancies such as lung and colorectal cancers. Irinotecan is a camptothecin analog that is mainly converted by carboxylesterase to an active metabolite, SN-38 (7-ethyl-10-hydroxycamptothecin), a potent topoisomerase I inhibitor.[1] Subsequently, SN-38 is conjugated to an inactive glucuronic acid conjugate (SN-38G) by UDP-glucuronosyltransferase 1A1 (UGT1A1).[2] Large interindividual variability in the pharmacokinetics of active metabolite SN-38 is likely important in the clinical outcome and toxicity (including myelosuppression and diarrhea) of irinotecan-based chemotherapy.[3] In particular, interindividual differences in the glucuronidation activity of UGT1A1 have been involved in development of severe toxicities and are explained in part by genetic variation.[4–6] Of the known genetic variants in the *UGT1A1* gene, *UGT1A1*28*, characterized by an extra seventh dinucleotide (TA) insertion in the $(TA)_6$TAA-box in the promoter region, is the most common, leading to decreased converting activity of SN-38 to SN-38G and resulting in increased plasma SN-38 level and severe irinotecan toxicity.[4–6] In addition, *UGT1A1*6* (211G>A)[5] and **60* (−3279T>G)[6] variants have been correlated with a reduction in SN-38 glucuronide formation.

Recently, an in vitro study indicated that SN-38 is a very good substrate for organic anion-transporting polypeptide 1B1 (OATP1B1, *SLCO1B1*), which is expressed on the basolateral membrane in the hepatocytes responsible for the hepatocellular uptake of several compounds from systemic circulation.[7] Previously, we found that the *SLCO1B1*15* (388A>G and 521T>C) variant was associated with a higher plasma concentration of pravastatin, a substrate of OATP1B1.[8] Therefore, to evaluate the contribution of genetic variants in the *UGT1A1* and *SLCO1B1* genes to the variability in irinotecan pharmacokinetics, we performed pharmacokinetic studies at the first administration (cycle) of irinotecan in 11 patients with lung cancer. This study was approved by the Ethics Review Board of Tottori University, and informed consent was obtained from all individuals. We report the case, a patient homozygous for the *SLCO1B1*15* allele, who showed an extensive accumulation of SN-38 after irinotecan administration resulting in severe toxicities.

Received for publication January 22, 2007; accepted April 25, 2007.

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This study was supported by Health and Labor Sciences Research Grants from the Ministry of Health, Labor and Welfare, Tokyo, Japan.

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TABLE 1. Patient Characteristics

	No.	Sex	Age (Years)	PS	Diagnosis	Dose, mg/m² (On Days) Irinotecan	Cisplatin	*SLCO1B1* *15	*UGT1A1* *60	*28	*6
This case	1	M	61	0	SCLC, ED	60 (1, 8, 15)	60 (1)	+/+	−/−	−/−	−/−
Other patients	2	F	72	0	SCLC, ED	60 (1, 8, 15)	60 (1)	−/−	−/−	−/−	−/−
(nos. 2–11)	3	M	78	0	SCLC, ED	60 (1)	–	−/−	−/−	−/−	−/−
	4	F	53	0	NSCLC, Sq	80 (1)	–	−/+	−/−	−/−	−/−
	5	M	80	2	NSCLC, Sq	100 (1)	–	−/+	−/−	−/−	−/−
	6	M	64	0	SCLC, ED	60 (1, 8, 15)	60 (1)	−/−	−/+	−/+	−/−
	7	M	50	0	SCLC, LD	60 (1, 8, 15)	60 (1)	−/+	−/+	−/+	−/−
	8	M	40	0	NSCLC, Ad	60 (1, 8, 15)	80 (1)	−/−	+/+	−/+	−/−
	9	M	67	0	NSCLC, Ad	80 (1)	–	−/−	+/+	−/−	−/−
	10	M	53	0	NSCLC, Ad	60 (1, 8, 15)	60 (1)	−/+	−/−	−/−	−/+
	11	M	58	2	SCLC, ED	100 (1)	–	−/+	−/−	−/−	−/+

M, male; F, female; PS, performance status; SCLC, small-cell lung cancer; NSCLC, nonsmall-cell lung cancer; ED, extensive disease; LD, limited disease; Ad, adenocarcinoma; Sq, squamous cell carcinoma.

SCLC is commonly staged using the VA Lung Cancer Group staging system.[9] This system classifies patients into LD or ED. LD is defined as disease confined to one hemithorax, in the absence of a malignant effusion, with disease that can be encompassed in one radiation port. Disease that does not meet this criteria is defined as ED.

CASE REPORT

A 61-year-old man (Eastern Cooperative Oncology Group performance status 0) was admitted to our hospital and diagnosed with small-cell lung cancer (extensive disease[9]) (Table 1). He was treated with irinotecan (60 mg/m² on days 1, 8, and 15) in combination with cisplatin (60 mg/m² on day 1) of a 28-day cycle as second-line treatment. Irinotecan was administered in the presence of oral alkalization (sodium bicarbonate, magnesium oxide, metoclopramide, and ursodeoxycholic acid) to reduce irinotecan-induced delayed diarrhea. He had also received lansoprazole, rebamipide, allopurinol, and mosapride. Toxicity was graded according to the Common Terminology Criteria for Adverse Events, version 3.0. After the first cycle of chemotherapy, he developed severe side effects, including grade 3 diarrhea (day 15), grade 4 leukopenia (day 14), and grade 4 neutropenia (day 14), and then required the continuous administration of granulocyte-colony stimulating factor and antibiotics; therefore, chemotherapeutic treatment on day 15 was discontinued. In the control group (ie, patient nos. 2–11, Table 1), patient no. 7 (grade 4 neutropenia and grade 3 diarrhea), and patient no. 8 (grade 4 neutropenia) were experiencing serious adverse effects during the first cycle of chemotherapy, but all were manageable.

Blood samples (each of 2 mL) for all enrolled patients with lung cancer were obtained at 0.5, 1, 1.5, 2, 4, 8, 12, and 24 hours after the start of irinotecan infusion (90-minute intravenous infusion) on day 1 of the first cycle. Serum concentrations of total irinotecan, SN-38, and its glucuronide (SN-38G) at the first administration of irinotecan were measured by high-performance liquid chromatography according to previously described methods.[10,11]

The areas under the concentration–time curve from time zero to infinity (AUCs) of irinotecan, SN-38, and SN-38G in this patient were 4553.2, 260.7, and 864.4 ng × hr/mL, respectively. The pharmacokinetic parameters of irinotecan were compared with 10 other patients with lung cancer receiving irinotecan (Table 2). The irinotecan and SN-38 AUCs (ng × hr/mL) normalized by the dose (mg/m²) of irinotecan in this patient (75.9 and 4.3) were 43% and 87%

TABLE 2. Pharmacokinetics of Irinotecan and Its Metabolites in the Case and 10 Other Patients

	*SLCO1B1*15*			
	*15/*15		−/− or −/*15	
Pharmacokinetic Parameters	Case	Patient Nos. 2–5	Patient Nos. 6–11	Patient Nos. 2–11
CLcr (mL/min)	71.8	64.5 ± 18.0	87.3 ± 12.8	78.2 ± 18.1
Total bilirubin (mg/mL)	0.5	0.4 ± 0.1	0.4 ± 0.2	0.4 ± 0.2
AST (IU/L)	28.0	24.0 ± 11.5	26.8 ± 11.3	25.7 ± 10.8
ALT (IU/L)	31.0	18.5 ± 8.3	24.7 ± 11.8	22.2 ± 10.5
$CL_{irinotecan}$ (L/hr/m²)	13.2	16.4 ± 1.1	19.7 ± 2.8	18.4 ± 2.8
$AUC_{irinotecan}$/dose	75.9	58.5 ± 5.0	49.5 ± 6.2	53.1 ± 7.2
AUC_{SN-38}/dose	4.3	1.6 ± 0.4	2.8 ± 0.6*	2.3 ± 0.8
AUC_{SN-38G}/dose	14.4	9.8 ± 3.7	7.4 ± 2.2	8.4 ± 2.9
REC ($AUC_{SN-38}/AUC_{irinotecan}$)	0.057	0.028 ± 0.009	0.057 ± 0.014*	0.045 ± 0.019
REG (AUC_{SN-38G}/AUC_{SN-38})	3.3	6.2 ± 1.3	2.4 ± 0.7*	3.9 ± 2.1

Genetic characteristics of the *UGT1A1* gene are summarized in Table 1.

Each value is expressed as the mean ± standard deviation.

CLcr, creatinine clearance; CL, total clearance; AUC/dose, area under the concentration–time curve from time zero to infinity (ng × hr/mL) normalized by dose (mg/m²); REC, relative extent of conversion of irinotecan into SN-38; REG, relative extent of glucuronidation to SN-38 into SN-38G.

*$P < 0.05$ when compared with the group including patient nos. 2–5 was analyzed with Mann-Whitney U test.

higher than the corresponding mean values for the other 10 patients (53.1 and 2.3) (Table 2). Plasma concentrations of irinotecan and SN-38 in this case were remarkably higher than those in other patients.

Genotyping of *UGT1A1*6* was performed by polymerase chain reaction–restriction fragment length polymorphism methods as previously reported.[4] Also, *UGT1A1*28* and **60* were determined with polymerase chain reaction–single-strand conformation polymorphisms or direct sequencing using gene-specific primers (5′-AAGTGAACTCCCTGCTACCTT-3′ [forward primer] and 5′-CC ACTGGGATCAACAGTATCT-3′ [reverse] for *UGT1A1*28* and 5′-GTCATAGTAAGCTGGCCAAGGGTAGAG-3′ [forward] and 5′-CA TCGGCTGCCCACCTGAATAAA-3′ [reverse] for *UGT1A1*60*). This patient did not harbor any of these variants in the promoter and the coding regions of the *UGT1A1* gene (Table 1). Haplotyping of *SLCO1B1*15* was identified according to previously described methods.[8] In the *SLCO1B1* gene, this patient was a homozygous carrier of the *SLCO1B1*15* allele (Table 1).

SN-38 is metabolized to SN-38 glucuronide by hepatic UGT1A1 and excreted into feces (8.24% of dose) and urine (3.02%).[12] Severe hematologic and gastric toxicities are sometimes observed in patients homozygous for the *UGT1A1*28* allele,[4,6] but this genotyping pattern was not found in our patients. The AUC ratio of SN-38G to SN-38 (relative extent of glucuronidation to SN-38 into SN-38G, 3.3) in this case was lower than that in four other patients (patient nos. 2–5, group 1) with a reference allele for the *UGT1A1* gene (6.2 ± 1.3, mean ± standard deviation) as homozygosity and comparable with six other patients (patient nos. 6–11, group 2) harboring at least one variant allele of the *UGT1A1* gene (2.4 ± 0.7) (Table 2). These results suggest that glucuronidation capability was not the major determinant of the severe toxicities observed in this patient.

Irinotecan is mainly converted by carboxylesterase 2 (CE-2) to an active metabolite, SN-38. Functional genetic polymorphisms in the *CE-2* gene are extremely rare (0.3%) in the Japanese.[13] The AUC ratio of SN-38 to irinotecan in this patient (relative extent of conversion of irinotecan into SN-38, 0.057) was higher (0.028) than and comparable (0.057) with the mean values in patients in group 1 and group 2, respectively (Table 2). In addition to CE-2, irinotecan is known to be metabolized by CYP3A4 to form inactive metabolites.[14] Coadministration of the CYP3A4 inhibitor may lead to the increasing formation of SN-38[15]; however, none of our patients was receiving medications known to interact with irinotecan. Furthermore, no patients with both renal and liver dysfunctions were included in this study (Table 2). These findings suggest that the higher plasma concentrations of irinotecan and SN-38 in this case could not be explained by the functional deficiency of either metabolizing enzyme.

Nozawa et al[7] reported that OATP1B1 transports SN-38, but not irinotecan and SN-38G in HEK293 cells, and demonstrated that the *SLCO1B1*15* allele exhibits decreased transport activities for SN-38 in *Xenopus* oocytes. In contrast to these in vitro findings, this patient, who was homozygous for the *SLCO1B1*15* allele, showed higher plasma concentrations of SN-38 and irinotecan than the mean values of 10 other patients, including five heterozygous carriers of the *SLCO1B1*15* allele. These results were consistent with the findings of Xiang et al.[16] However, in the present study, we did not observe significant differences in irinotecan (noncarriers, 51.4 ± 7.4; heterozygosity, 54.7 ± 7.3), SN-38 (noncarriers, 2.3 ± 0.7; heterozygosity, 2.3 ± 1.0), and SN-38G (noncarriers, 8.4 ± 4.2; heterozygosity, 8.4 ± 1.4) AUCs normalized by the dose of irinotecan between noncarriers and heterozygous carriers of the *SLCO1B1*15* allele, possibly attributable to the small sample size of the study and mismatched genotypes of the *UGT1A1* polymorphisms. Although the reasons for the discrepancy between previous in vitro and our in vivo findings are not clear, this case suggests that the low transport activity of OATP1B1 leads to increased not only SN-38, but also irinotecan exposure in humans.

This is the first report suggesting that homozygosity of the *SLCO1B1*15* allele is important for the variability in irinotecan and SN-38 dispositions and is implicated in the unpredicted accumulation of plasma SN-38, resulting in irinotecan-related severe toxicities. In the present study, contribution of comedicated cisplatin to the observed toxicities could not be excluded; however, low transport activity of OATP1B1 attributable to the *SLCO1B1*15* allele may lead to increased systemic exposure of SN-38 by reduced hepatocellular uptake of SN-38 from the systemic circulation. Although the frequency of homozygous carriers of *SLCO1B1*15* is low (0.8% in the Japanese),[8] genotyping of this variant may be useful to avoid severe toxicities after irinotecan treatment with a standard body surface area-based dose.

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SLCO1B1 (OATP1B1, an Uptake Transporter) and *ABCG2* (BCRP, an Efflux Transporter) Variant Alleles and Pharmacokinetics of Pitavastatin in Healthy Volunteers

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To investigate the contribution of genetic polymorphisms of *SLCO1B1* and *ABCG2* to the pharmacokinetics of a dual substrate, pitavastatin, 2 mg of pitavastatin was administered to 38 healthy volunteers and pharmacokinetic parameters were compared among the following groups: 421C/C*1b/*1b (group 1), 421C/C*1b/*15 (group 2), 421C/C*15/*15 and 421C/A*15/*15 (group 3), 421C/A*1b/*1b (group 4), 421A/A*1b/*1b (group 5), and 421C/A*1b/*15 (group 6). In *SLCO1B1*, pitavastatin area under plasma concentration-time curve from 0 to 24 h (AUC_{0-24}) for groups 1, 2, and 3 was 81.1 ± 18.1, 144 ± 32, and 250 ± 57 ng h/ml, respectively, with significant differences among all three groups. In contrast to *SLCO1B1*, AUC_{0-24} in groups 1, 4, and 5 was 81.1 ± 18.1, 96.7 ± 35.4, and 78.2 ± 8.2 ng h/ml, respectively. Although the *SLCO1B1* polymorphism was found to have a significant effect on the pharmacokinetics of pitavastatin, a nonsynonymous *ABCG2* variant, 421C > A, did not appear to be associated with the altered pharmacokinetics of pitavastatin.

Pitavastatin is a highly potent inhibitor of 3-hydroxymethylglutaryl coenzyme A reductase and is used for the treatment of hypercholesterolemia.[1] In humans, pitavastatin is scarcely metabolized by the cytochrome P450 2C9,[2,3] and lactonization is another known metabolic pathway.[4,5] The lactone form can be reversibly converted to the parent drug.[4] Cumulative evidence has indicated that various active transport mechanisms are involved in its distribution and disposition kinetics. Pitavastatin is taken up efficiently from the circulation into hepatocytes by an organic anion-transporting polypeptide (OATP) 1B1 (formally known as OATP-C or OATP2, gene *SLCO1B1*), a sodium-independent bile-acid transporter expressed at the sinusoidal membrane of human hepatocytes responsible for the hepatocellular uptake of a variety of endogenous and foreign chemicals.[6–8] In addition to the uptake process, a recent study demonstrated that breast cancer resistance protein (BCRP, gene *ABCG2*) is involved in the biliary excretion of pitavastatin.[9] BCRP is expressed at the apical membrane in the placenta (trophoblast cells), liver (bile canalicular membrane of hepatocytes), kidney, and intestine (enterocytes).[10–13] The biliary excretion clearance of pitavastatin in Bcrp1 (−/−) mice was 10 times lower than that in control mice;[9] thus, at least two drug transporters, OATP1B1 and BCRP, contribute to hepatic uptake and efflux of pitavastatin in humans.

A number of single nucleotide polymorphisms have been identified in *SLCO1B1* and some of these single nucleotide polymorphisms are associated with a significant change in the transporter activity of OATP1B1. Two commonly occurring single nucleotide polymorphisms, 388A > G (130Asn > Asp) and 521T > C (174Val > Ala), are found to cause a remarkable change in the disposition of OATP1B1 substrates such as statins (pravastatin[14–17] and pitavastatin[18]), fexofenadine,[19] and repaglinide.[20] Interestingly, most human studies have demonstrated that subjects with haplotypes *SLCO1B1*5*, *15, or *17, all haplotypes harboring the 174Val > Ala variant, showed increased plasma levels of substrates as compared with subjects having the *SLCO1B1*1a* (130Asn174Val) or *1b (130Asp174Val) allele as homozygosity. Furthermore, recent studies reported that the

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Received 19 October 2006; accepted 9 February 2007; published online 25 April 2007. doi:10.1038/sj.clpt.6100190

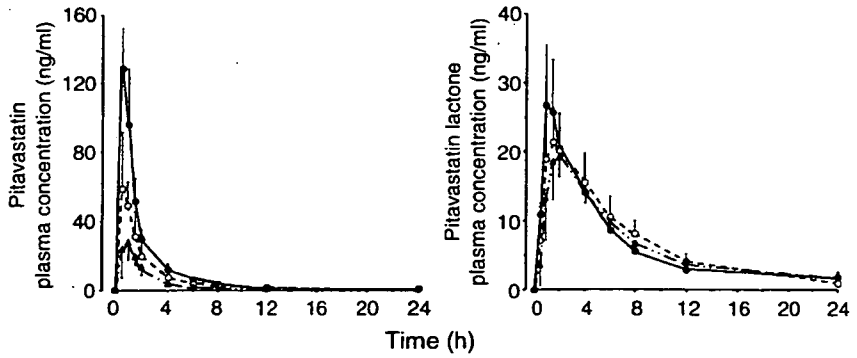

Figure 1 Effect of *SLCO1B1* haplotype on pharmacokinetics of pitavastatin. Plasma concentration–time profiles of pitavastatin and pitavastatin lactone after oral administration of 2 mg pitavastatin in 421C/C*1b/*1b subjects (closed triangles, $n = 11$), 421C/C*1b/*15 subjects (open circles, $n = 8$), and 421C/C*15/*15 and 421C/A*15/*15 subjects (closed circles, $n = 3$).

*SLCO1B1*1b* allele showed more enhanced transport activity than the *1a allele.[17,21]

Systematic mutation analysis of the *ABCG2* gene has been performed in various ethnic populations and more than 40 single nucleotide polymorphisms have been identified.[22–25] The two most frequent nonsynonymous mutations identified in humans are 34G>A (12Val>Met in exon 2) and 421C>A (141Gln>Lys in exon 5). After intravenous administration, plasma levels of diflomotecan were significantly higher in patients with the 421C/A than the 421C/C genotype.[26] These results were supported by *in vitro* experiments showing that BCRP expression of the 421C>A variant was reduced compared with the wild-type,[22,27,28] suggesting that carriers of the 421C>A variant may have decreased clearance (increased plasma levels) and/or increased bioavailability.

In view of the pharmacokinetics, at least two genes (*i.e.*, *SLCO1B1* and *ABCG2*) are of interest as candidates that may lead to large interindividual variability in the pharmacokinetics and clinical outcome of pitavastatin therapy. Recently, Chung *et al.*[18] evaluated the contribution of *SLCO1B1* haplotypes to pitavastatin pharmacokinetics and demonstrated that subjects with the *15 allele showed significantly higher dose-normalized pitavastatin plasma levels. Although these observations are similar trends to pravastatin, no homozygotes for the *15 allele participated in their study. Very recently, Zhang *et al.*[29] studied the role of *ABCG2* 421C>A variant in rosuvastatin pharmacokinetics in 14 healthy volunteers and indicated that the AUC of rosuvastatin was lower in the 421C/C group than in the (421C/A and 421A/A) group. Although all statins share a common action mechanism, they differ in terms of their chemical structures, pharmacokinetics, and pharmacodynamics.[30]

With this background in mind, we designed this study to confirm the role of *SLCO1B1* and *ABCG2* polymorphisms in the pharmacokinetics of pitavastatin in healthy volunteers. In this study, we selected volunteers from our panels based on their genotypes of *SLCO1B1* (*1b and *15) and *ABCG2* (421C>A). In addition, we investigated the importance of intestinal BCRP in the pharmacokinetics of pitavastatin using Bcrp1 (−/−) mice.

RESULTS

No clinically undesirable signs and symptoms possibly attributed to the administration of pitavastatin were recognizable throughout the study. All subjects completed the study successfully according to the protocol.

Pitavastatin pharmacokinetics in relation to *SLCO1B1* and *ABCG2* genotypic status

After oral administration, the mean plasma concentrations of pitavastatin were significantly higher ($P<0.01$) in group 3 subjects ($n=3$, homozygotes for the *SLCO1B1*15* allele, 421C/C*15/*15 ($n=2$) and 421C/A*15/*15 ($n=1$)) compared with group 1 subjects ($n=11$, homozygotes for the *SLCO1B1*1b* allele, 421C/C*1b/*1b), and group 2 subjects, heterozygotes for the *SLCO1B1*15* allele ($n=8$, 421C/C*1b/*15), had values between those in group 1 (*i.e.*, *1b/*1b) and group 3 (*15/*15) subjects at all observation points (**Figure 1**). The mean ($\pm$SD) AUC_{0-24} of pitavastatin in groups 1 (*1b/*1b), 2 (421C/C*1b/*15), 3 (*15/*15), and 6 (421C/A*1b/*15) was 81.1 ± 18.1, 144 ± 32, 250 ± 57, and 121 ± 25 ng h/ml, respectively. The mean apparent oral clearance (CLt) of pitavastatin in groups 1 (*1b/*1b), 2 (421C/C*1b/*15), 3 (*15/*15), and 6 (421C/A*1b/*15) was 0.43 ± 0.13, 0.24 ± 0.04, 0.15 ± 0.03, and 0.29 ± 0.07 l/h/kg, respectively. The group 3 (*15/*15) subjects had the highest AUC value and the lowest CLt value among all study volunteers. A similar trend was observed in peak concentration (C_{max}) values, but not in elimination rate constant (K_e) values. Although the difference did not reach the level of significance, volume of distribution/bioavailability (V_d/F) values tended to be lower in subjects with the *15 allele; the mean V_d/F in group 3 (*15/*15) was 30% of that in group 1 (*1b/*1b). In contrast to pitavastatin, no significant intergenotypic differences were observed in any mean pharmacokinetic parameters of pitavastatin lactone in this experiment (**Table 1** and **Figure 1**).

The mean plasma concentration–time curves of pitavastatin and pitavastatin lactone in relation to *ABCG2* genotypic status are shown in **Figure 2**. The pharmacokinetic parameters are also summarized in **Table 1**. There were no significant differences in any of the pharmacokinetic

Table 1 Pharmacokinetic parameters of pitavastatin and its lactone form in each genotyping group

Genotype			Pitavastatin					Pitavastatin lactone		
		n	AUC_{0-24}	CLt	C_{max}	K_e	Vd/*F*	AUC_{0-24}	C_{max}	K_e
SLCO1B1	*ABCG2*		(ng h/ml)	(l/h/kg)	(ng/ml)	(per hour)	(l/kg)	(ng h/ml)	(ng/ml)	(per hour)
*1b/*1b	421C/C	11	81.1±18.1	0.43±0.13	31.2±11.4	0.06±0.03	0.58±0.27	154±27	20.4±4.4	0.07±0.01
*1b/*15	421C/C	8	144±32[a]	0.24±0.04[a]	70.7±18.1[a]	0.06±0.02	0.27±0.09	169±38	22.3±5.4	0.08±0.02
*15/*15	421C/C 421C/A	3	250±57[a,b]	0.15±0.03[a]	129±24[a]	0.06±0.01	0.16±0.07	153±31	27.2±8.8	0.07±0.01
*1b/*1b	421C/A	7	96.7±35.4	0.37±0.13	41.7±12.4	0.06±0.03	0.46±0.25	145±38	18.9±3.1	0.06±0.02
*1b/*1b	421A/A	3	78.2±8.2	0.42±0.01	42.1±6.3	0.05±0.02	0.48±0.21	140±47	22.1±4.9	0.06±0.01
*1b/*15	421C/A	6	121±25	0.29±0.07	57.7±7.6	0.05±0.01	0.26±0.05	125±18	18.8±2.6	0.07±0.01

AUC_{0-24}, area under plasma concentration–time curve from 0 to 24 h; CL_t, total clearance; C_{max}, peak concentration; V_d/F, volume of distribution/bioavailability. Data are presented as the mean±SD. [a]Significantly different from values in *SLCO1B1*1b/*1b421C/C* subjects as determined by analysis of variance with Fisher's least significant difference test ($P<0.01$). [b]Significantly different from values in *SLCO1B1*1b/*15421C/C* subjects as determined by analysis of variance with Fisher's least significant difference test ($P<0.01$).

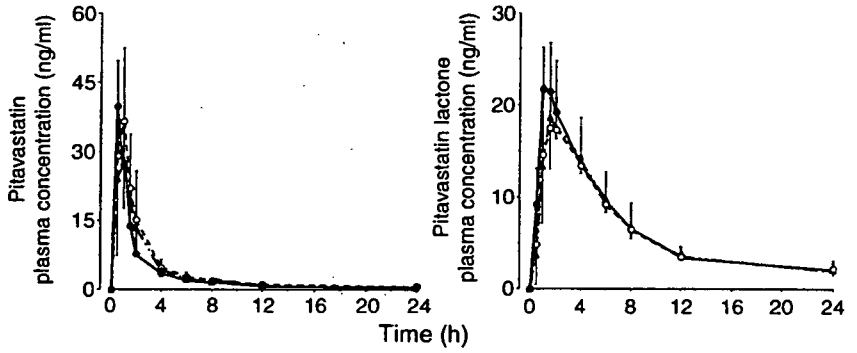

Figure 2 Effect of *ABCG2* genotype on pharmacokinetics of pitavastatin. Plasma concentration–time profiles of pitavastatin and pitavastatin lactone after oral administration of 2 mg pitavastatin in 421C/C*1b/*1b subjects (closed triangles, $n=11$), 421C/A*1b/*1b subjects (open circles, $n=7$), and 421A/A*1b/*1b subjects (closed circles, $n=3$).

parameters for either pitavastatin or pitavastatin lactone among the three *SLCO1B1* matched (*i.e.*, homozygotes for the *1b allele) *ABCG2* groups: group 1 ($n=11$, 421C/C*1b/*1b), group 4 ($n=7$, 421C/A*1b/*1b), and group 5 ($n=3$, 421A/A*1b/*1b). The mean AUC_{0-24} of pitavastatin in groups 1 (*i.e.*, 421C/C), 4 (421C/A*1b/*1b), 5 (421A/A), and 6 (421C/A*1b/*15) was 81.1±18.1, 96.7±35.4, 78.2±8.2, and 121±25 ng h/ml, respectively.

Role of BCRP in the intestinal absorption of pitavastatin in mice *in vivo*

To investigate the involvement of Bcrp1 in the intestinal absorption of pitavastatin, we evaluated its pharmacokinetics using control and Bcrp1 (−/−) mice. The mean AUC up to 4 h after administration was 213±13 and 221±17 ng h/ml (mean±SE, $n=4$) in control and Bcrp1 (−/−) mice, respectively ($P>0.05$). The time profiles of the plasma concentration of pitavastatin did not show any significant difference between control and Bcrp1 (−/−) mice (**Figure 3**).

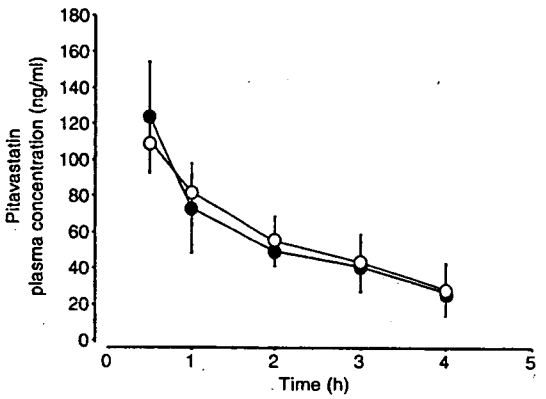

Figure 3 Time profiles of plasma concentration of pitavastatin after its oral administration (10 mg/kg) to control and Bcrp1 (−/−) mice. Closed and open circles represent the time profile of plasma concentration of pitavastatin in control and Bcrp1 (−/−) mice, respectively.

DISCUSSION

The primary objective of this study was to evaluate whether the polymorphism of two drug transporter genes contribute to large interindividual variability in the pharmacokinetics of pitavastatin, a dual substrate of OATP1B1 and BCRP. The important findings were that (1) significant differences in AUC_{0-24} and C_{max} of pitavastatin, but not in those of the lactone form, were observed among subjects with different *SLCO1B1* genotypes; and (2) in contrast to *SLCO1B1*, no significant differences in any pharmacokinetic parameters

were observed among genotyping groups of *ABCG2*. To ensure the quality of the study, we selected *SLCO1B1* and *ABCG2* genotyping-matched volunteers from our panels. However, unfortunately, as the frequency of the *SLCO1B1*15/*15* is low in Japanese populations,[15] we recruited one miss-matched 421C/A subject in group 3 (*i.e.*, *15/*15), which could have consequences for the interpretation of the results.

In this study, the mean AUC_{0-24} and C_{max} values in homozygotes for the *SLCO1B1*15* allele were 3.1- and 4.1-fold higher, respectively, than those in *SLCO1B1*1b/*1b* subjects, and heterozygotes had values between the two homozygous groups. These findings were consistent with a recent study conducted by Chung *et al.*[18] Although no homozygotes for the *15 allele were included in their study, they found dose-normalized AUC and C_{max} of pitavastatin to be 1.4- and 1.8-fold higher, respectively, in subjects heterozygous for the *15 allele versus subjects without this allele. Several transporters are known to be involved in the hepatic uptake of clinically important drugs in humans. Among them, a recent *in vitro* study indicated that the uptake clearance of pitavastatin in human hepatocytes could be almost completely accounted for by OATP1B1 and OATP1B3 (OATP8), but approximately 90% of the total hepatic clearance could be accounted for by OATP1B1.[6] Thus, similar to pravastatin, OATP1B1 is suggested to play an important role in the hepatic uptake of pitavastatin in humans.

The 174Val > Ala variant has been consistently associated with reduced transport activity of OATP1B1 both *in vitro*[14,31] and *in vivo*.[15–18] As selective distribution to the liver may also be the first step for the pharmacological action of pitavastatin, subjects with this variant (*i.e.*, *5, *15, and *17 alleles) are expected to exhibit a reduced cholesterol-lowering effect owing to the lower pitavastatin concentration in hepatocytes, despite high plasma concentrations and AUC of pitavastatin. To date, some groups have reported the implication of the *SLCO1B1* polymorphism in the lipid-lowering efficacy of statins under multiple-dose conditions[32] and chronic treatment.[33–35] Igel *et al.*[32] conducted a healthy volunteer study ($n = 16$) and demonstrated no significant difference in the lipid-lowering efficacy of pravastatin between the variant allele (*15 and *17 alleles) and control groups after treatment with 40 mg pravastatin daily for 3 weeks, despite considerably higher plasma pravastatin concentration in the variant group. Similarly, in a study of 33 patients with hypercholesterolemia treated with pravastatin (mean dose of 9.4 mg/day), the genotype-dependent difference in the lipid-lowering effect in the initial phase of treatment (8 weeks) disappeared after 1 year of treatment.[33] In addition to experimental designs, such as the male/female ratio[36] and racial background, numerous genetic factors may have an impact on the response to statins.[32,37] Furthermore, the 174Val > Ala variant is associated with reduced transport activity, but does not lead to loss of activity,[14,31] making it conceivable that there is no significant impact of the genotype on the clinical efficacy of statins during long-term treatment; however, a high plasma concentration, on the other hand, is known as a risk factor for the myotoxic effects of statins.[38] The effect of the polymorphism of the *SLCO1B1* gene on the clinical efficacy and adverse events of pitavastatin will be the subject of further investigation.

In this study, the AUC_{0-24} of the lactone form was comparable with that of the acid form, and the pharmacokinetics of lactone was not affected by the *SLCO1B1* polymorphism. The profile of the hepatic uptake of lactone has not yet been elucidated; however, our study indicated that OATP1B1 can be ruled out as a candidate transporter in humans. As the lactone form can be reversibly converted to the acid form in the body, it can be asked whether a comparably high serum concentration of lactone contributes to the clinical efficacy of pitavastatin. In the acid/lactone interconversion of pitavastatin, the following pathways have been proposed: the first step is the glucuronidation of pitavastatin to form UM-2 as an intermediate to the lactone form by uridine 5′-diphosphate glucuronosyl transferases (UGT1A1, 1A3, and 2B7). The glucuronic acid moiety is subsequently converted nonenzymatically to its lactone form. After conversion, some of the resulting lactone form changes to the acid form by hydrolysis. Cytochrome p450 3A4-mediated metabolism of the lactone form was also observed in human hepatic microsomes.[3,4] Furthermore, in addition to the liver, lactone may form in extrahepatic tissues such as the kidney and intestine.[4] Although the metabolism of pitavastatin is complex, lactonization is the major metabolic pathway in humans.[4] These findings suggest that a certain amount of pitavastatin acid produced from the lactone form by hepatic interconversion has a clinical impact on the lipid-lowering effect. Further study with regard to the *SLCO1B1* polymorphism, in which the pitavastatin lactone form is used as a test drug, is required.

In addition to AUC_{0-24}, the mean C_{max} was higher in subjects with the *15 allele than in subjects without this allele. To discuss this point, we estimated the pharmacokinetic data using WinNonlin. Although the difference did not reach the level of significance, V_d/F values tended to be lower in subjects with the *15 allele (**Table 1**). The V_d of pitavastatin in the liver ($V_{d_{liver}}$) can be estimated by the following equation: $K_{P_{liver}} \times V_{liver}$, where $K_{P_{liver}}$ is the $C_{P_{liver}}/C_{P_{plasma}}$ ratio (approximately 23.0 in rats),[9] and V_{liver} is the liver volume (approximately 78.4 ml/kg).[39] Estimated $V_{d_{liver}}$ (1.8 l/kg) and V_d/F (1.0 l/kg)[9] are comparable, suggesting that the liver is the major organ for the distribution of pitavastatin in rats. In addition, total clearance of pitavastatin (0.15–0.43 l/h/kg in this study) is relatively lower than that of other statins in humans (800–1000 l/h for simvastatin,[40] 0.8–2.7 l/h/kg for pravastatin,[15] 80–200 l/h for rosuvastatin,[41] and 150–1200 l/h for atorvastatin[42]). Taking these *in vivo* findings into consideration, a decreased V_d of pitavastatin owing to low transport activity is one of the possible reasons for high C_{max} values in subjects with the *15 allele. Nevertheless, changes in F cannot be denied in such subjects because there is no

intravenous data for pitavastatin to determine absolute value of bioavailability.

In this study, no change in K_e values was observed. Although a firm conclusion cannot be reached regarding possible changes in V_d versus F (again, because of no intravenous data), *OATP1B1*15* allele may be associated with decreased CL_t and V_d values simultaneously, which may cancel out the change in K_e values.

It was somewhat unexpected that there were no significant differences in any pharmacokinetic parameters among *ABCG2* (421C>A) genotypic groups because some *in vivo* studies demonstrated that the 421C>A allele was associated with changes in the pharmacokinetics of certain clinically important substrate drugs, such as diflomotecan[26] and topotecan.[43] In addition, Zhang *et al.*[29] recently studied rosuvastatin pharmacokinetics in relation to the *ABCG2* 421C>A polymorphism in 14 healthy volunteers. Although they used an insufficient number of subjects, they demonstrated that the AUC of rosuvastatin was lower in subjects with the 421C/C genotype than in subjects with 421A variant(s). Available data indicate that the 421C>A variant was associated with remarkably decreased BCRP expression compared with wild-type cells and human placental samples,[22,27] which may lead to the following changes based on its localization; increased absorption at the intestinal epithelium, and/or decreased biliary excretion of substrates, thereby resulting in elevated plasma concentrations in subjects with 421A variant(s). Although both pitavastatin and rosuvastatin seem to be good substrates for BCRP,[9,44] our present findings were clearly in contrast to the findings reported by Zhang *et al.*[29] This discrepancy may be explained as follows: a recent *in vitro* experiment using double-transfected Madin-Darby cainine kidney (MDCK) II monolayers expressing OATP1B1 and human canalicular efflux transporters indicated that the significant transport of pitavastatin was observed in OATP1B1/MDR1 and OATP1B1/MRP2 as well as OATP1B1/BCRP double transfectants.[9] These results suggest that multiple organic anion transporters across the canalicular membrane in the liver are involved in the biliary excretion of pitavastatin.

Hirano *et al.*[9] have previously shown that the biliary excretion of pitavastatin in Bcrp1 (−/−) mice was drastically reduced compared with that in control mice after constant infusion, although the steady-state plasma concentration in Bcrp1 (−/−) mice is not different from that in control mice owing to the extensive metabolism of pitavastatin in mice. As BCRP is also expressed on the brush-border membrane of enterocytes, reduced function because of modulation of the expression level or recognition/affinity capability may lead to the increasing intestinal absorption of substrate drugs; therefore, we compared the pharmacokinetics of pitavastatin after oral administration in Bcrp1 (−/−) and control mice. Similar to the constant infusion, the time profiles of the plasma concentration of pitavastatin were not different between Bcrp1 (−/−) and control mice (**Figure 3**). These results suggest that, in contrast to the biliary excretion, Bcrp1 is not important as a determinant of intestinal absorption. In our animal study, the dose of pitavastatin was considerably higher and also the plasma concentrations appeared to be somewhat higher in mice than those observed in our subjects. In addition to these experimental designs, the metabolic profile of pitavastatin is reported to be different between mice and men;[9] however, our present findings suggest that the contribution of BCRP to the pharmacokinetics of pitavastatin is not significant as our expectations in humans.

Although all statins share a common action mechanism, they differ in terms of their chemical structures, pharmacokinetics, and pharmacodynamics. In particular, transporters involved in biliary excretion are different among statins; for example, ABCC2 (MRP2) is reported to be responsible for pravastatin both *in vitro* and *in vivo*.[9,45,46] In addition to efflux, a recent study indicated that multiple transporters are involved in the hepatic uptake of statins.[47] These differences make the understanding of the transport mechanism of statins difficult. Differences in the pharmacokinetic profiles between acid and lactone forms of pitavastatin observed in this study can be partially attributed to the contribution of multiple transporters.[48] However, hepatic uptake of pravastatin and pitavastatin, and probably rosuvastatin,[41] by OATP1B1 seems to be the major determinant of their overall pharmacokinetic profiles (*i.e.*, elimination and tissue distribution) in humans. Indeed, numerous OATP1B1-mediated drug–drug interactions have been reported to date, suggesting that transporter-mediated hepatic uptake is the main determinant of plasma clearance, even for drugs undergoing extensive metabolism.[49]

METHODS

Subjects and genotyping of *SLCO1B1* and *ABCG2*. After approval by the Ethics Review Board of Kyushu University and Kyushu Clinical Pharmacology Research Clinic, 38 healthy male volunteers (age, 20–32 years; weight, 52.4–72.4 kg) gave written informed consent to participate in the study. None had taken any drugs for at least 1 week before the study. Each subject was physically normal and had no antecedent history of significant medical illness or hypersensitivity to any drugs, and each had a body mass index between 17.6 and 26.4 kg/m^2. Their health status was judged to be normal on the basis of a physical examination with screening of blood chemistry, a complete blood count and urinalysis, and an electrocardiogram just before the study.

The genotyping methods of *SLCO1B1* and *ABCG2* have been described previously.[15,22] Single-strand conformation polymorphism and polymerase chain reaction–restriction fragment length polymorphism methods were used for genotyping. In this study, *1b and *15 alleles and 421C>A variant were identified for *SLCO1B1* and *ABCG2* genes, respectively, and all subjects were divided into the following groups: 421C/C*1b/*1b (group 1, $n=11$), 421C/C*1b/*15 (group 2, $n=8$), 421C/C*15/*15 ($n=2$) and 421C/A*15/*15 ($n=1$) (group 3), 421C/A*1b/*1b (group 4, $n=7$), 421A/A*1b/*1b (group 5, $n=3$), and 421C/A*1b/*15 (group 6, $n=6$).

Study protocol. The participants came to the clinic after an overnight fast. They were required to abstain from alcohol for 2 days before drug administration and during the period of hospitalization and were served standard meals on the study day. Each volunteer received a single oral dose of 2 mg of pitavastatin (Livalo, Kowa,

Nagoya, Japan) with 150 ml of water. Venous blood samples (7 ml each) to determine pitavastatin and pitavastatin lactone concentrations were obtained just before and 0.5, 1, 1.5, 2, 4, 6, 8, 12, and 24 h after dosing. Plasma samples were immediately separated after centrifugation and stored at −70°C until analyzed.

Quantification of pitavastatin and pitavastatin lactone in plasma. The concentrations of pitavastatin and its lactone in plasma were measured by high-performance liquid chromatography (HPLC) according to the methods of Kojima *et al.*[50] with a minor modification. The plasma sample (1.0 ml) was mixed with 0.2 ml internal standard (I-1938, 125 ng/ml; supplied by Nissan Chemical Industries (Saitama, Japan)), 0.2 ml water, 10 μl acetonitrile, and 0.5 ml 1.0 M potassium dihydrogenphosphate in a colored tube. The sample mixture was extracted with 8 ml of methyl *tert*-butyl ether by shaking for 10 min on a horizontal shaker at 200 r.p.m. and by centrifuging for 10 min at approximately 1,720 × *g* (at 4°C). The organic layer was transferred to another colored tube and subsequently diazomethane-diethyl ether solution (0.5 ml) was added. The reaction mixture was kept at room temperature for 30 min. To degrade excessive diazomethane, 1.0 M potassium dihydrogenphosphate (2 ml) was added to the mixture. After centrifuging for 10 min at 1,720 × *g*, the organic layer was evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was reconstituted in 150 μl of mobile phase for pre-separation and an aliquot of 80 μl was injected into the HPLC system. Column-switching HPLC (using a six-port switching valve) was performed with two Cosmosil-C18-MS-II columns (150 × 4.6 mm internal diameter; Nacalai Tesque, Kyoto, Japan) for pre-separation and analytical separation. Two mobile phases, 0.2 M ammonium acetate buffer (pH 4.0)–acetonitrile (5:5, v/v) for pre-separation and 0.2 M acetic acid–acetonitrile (5:5, v/v) for analytical separation, were maintained at a flow rate of 1.0 ml/min. Detection was carried out at 250 nm with a UV detector. Column temperature was maintained at 40°C. Calibration curves for both analytes ranged from 0.5 to 200 ng/ml. This HPLC method was validated only for the measurement of serum concentrations of pitavastatin and lactone in the human study. The intraday coefficient of variation values were less than 13.0% and intraday accuracies were between −14.0 and 6.0%, within the concentration range of the calibration curves for both analytes. Interday coefficient of variation values were less than 5.0% and interday accuracies were between −2.4 and 4.7%. The limits of quantification for both analytes were set to 0.5 ng/ml.[50]

***In vivo* study in mice and quantification of pitavastatin by liquid chromatography/mass spectrometry.** Male FVB control and Bcrp1 (−/−) mice weighing approximately 28–33 g were used throughout these experiments. Pitavastatin was orally administered to both mice at a dose of 10 mg/kg. Blood samples were collected from the tail vein at 0.5, 1, 2, 3, and 4 h after oral administration of pitavastatin. The plasma sample (10 μl) was mixed with 100 μl methanol containing the internal standard (atorvastatin, 80 ng/ml), followed by centrifugation (10,000 × *g*) at 4°C for 10 min. Atorvastatin was synthesized by Kowa (Tokyo, Japan). The supernatant (80 μl) was mixed with 50 μl water and subjected to HPLC (Waters 2695; Waters, Milford, MA). Liquid chromatography/mass spectrometry analysis of pitavastatin was performed with an Inertsil ODS-3 column (50 × 2.1 mm internal diameter, particle size 5 μm) (GL Sciences, Tokyo, Japan). The mobile phase consisted of methanol–ammonium formate buffer (pH 4.0, 7:3, v/v) and the flow rate was 0.5 ml/min. The mass spectrometry instrument used for this work was a ZQ micro-mass (Waters) equipped with a Z-spray source and operated in the positive-ion electrospray ionization mode. The Z-spray desolvation temperature, capillary voltage, and cone voltage were 350°C, 3400 V and 40 V, respectively. The *m/z* monitored for pitavastatin and atorvastatin was 422.3 and 559.0, respectively. No chromatographic interference was found for pitavastatin and atorvastatin in extracts from blank plasma. The retention times of pitavastatin and atorvastatin were 1.5 and 1.4 min, respectively. The calibration curves for pitavastatin ranged from 5 to 1000 ng/ml. This liquid chromatography/mass spectrometry method was validated only for the measurement of serum concentration of pitavastatin in the animal study. For pitavastatin, quality control samples covering the whole concentration range showed high intra- and interday accuracy and reproducibility with a coefficient of variation and bias below 10%.

Pharmacokinetics and statistical analysis. C_{max} was obtained directly from the data. AUC_{0-24} was calculated by the linear trapezoidal rule. We calculated the CL_t of pitavastatin as follows: $CL_t = Dose/AUC_{0-24}$. The K_e was estimated using least-squares regression analysis from the terminal postdistribution phase of the concentration–time curve. To assess differences in the V_d/F of pitavastatin in relation to the *SLCO1B1* polymorphism, we also estimated model-dependent parameters (one-compartment open model with first-order elimination and no lag time) using the WinNonlin 5.0.1 program (Pharsight, Mountain View, CA). Statistical differences among the data for each group were determined by analysis of variance, followed by Fisher's least significant difference test. $P<0.05$ was considered statistically significant.

ACKNOWLEDGMENTS

This study was supported by a Health and Labour Sciences Research Grant from the Ministry of Health, Labour and Welfare for Research on Advanced Medical Technology.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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J Hum Genet (2007) 52:117–122
DOI 10.1007/s10038-006-0087-0

ORIGINAL ARTICLE

Human organic cation transporter (*OCT1* and *OCT2*) gene polymorphisms and therapeutic effects of metformin

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Received: 25 August 2006 / Accepted: 27 October 2006 / Published online: 17 November 2006

Abstract Organic cation transporters (OCTs) are responsible for the hepatic and renal transport of metformin. In this study we analyzed variants of *OCT1* and *OCT2* genes in 33 patients (24 responders and nine non-responders) based on the hypothesis that polymorphisms in both genes contribute to large inter-patient variability in the clinical efficacy of metformin. The sequences of the 5′-flanking and coding regions of the two genes of interest were screened by single-strand conformation polymorphism (SSCP) analysis. To compare the causative factors between responders and non-responders, we performed stepwise discriminant functional analysis. Age, body mass index (BMI) and treatment with lipid-lowering agents were demonstrated as positive predictors, and two mutations in the *OCT1* gene, –43T > G in intron 1 and 408Met > Val (1222A > G) in exon 7, were negative and positive predictors, respectively, for the efficacy of metformin; the predictive accuracy was 55.5% ($P < 0.05$). Subsequent study indicated that OCT1 mRNA levels tended to be lower in human livers with the 408Met (1222A) variant, though the differences did not reach the level of significance. In this study it is suggested that *OCT1* and *OCT2* gene polymorphisms have little contribution to the clinical efficacy of metformin.

Keywords Metformin · OCT1 · OCT2 · Polymorphisms · Pharmacokinetics · Pharmacodynamics

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Introduction

Metformin is one of the most commonly used drugs for the treatment of type 2 diabetes, but we sometimes encounter patients who do not respond sufficiently, even under approved dosage conditions (e.g., 500–750 mg/day in Japan). Although the effects of metformin on glycemic control and lipids have been reported to be dose dependent, recent pharmacogenomic studies indicate that genetic polymorphisms of drug-metabolizing enzymes and transporters should be taken into consideration when large inter-patient variability in the intensity and duration of both drug effects and side effects is observed. Among various pharmacokinetic-related genes, since renal secretion, not hepatic metabolism, is the major route of elimination of metformin, the contribution of genetic variations in drug transporters is of interest.

Human organic cation transporters (OCTs; OCT1–3) are poly-specific transporters of small and hydrophilic

organic cations, including toxic substances, endogenous compounds (e.g., dopamine and serotonin), and clinically used drugs (e.g., procainamide and amantadine) (Jonker and Schinkel 2004). Among the OCT family, OCT1 is expressed predominantly in the basolateral membrane of hepatocytes, and mouse Oct1, which is homologically and functionally similar to OCT1, is responsible for the hepatic uptake of metformin (Wang et al. 2002, 2003). Although the precise mechanism of the action of metformin remains unclear, it is believed that hepatic uptake is an essential step in reducing hepatic glucose production as well as the occurrence of life-threatening side effects such as lactic acidosis (Hundal et al. 2000; Stumvoll et al. 1995; Wang et al. 2002). Recently, a number of single nucleotide polymorphisms (SNPs) has been identified in the *OCT1* gene. Some of these SNPs have been found to be associated with altered in vitro transport activity (Hundal et al. 2000; Sakata et al. 2003; Shu et al. 2003; Takeuchi et al. 2003).

In the kidney, OCT2, another subfamily of the OCT family, is expressed on the basolateral membrane of the proximal tubule epithelium and is involved in the uptake of many xenobiotics from the bloodstream into renal epithelial cells (Jonker and Schinkel 2004). Kimura et al. (2005) demonstrated that metformin is a good substrate for OCT2, using HEK293 cells expressing OCT2. Similar to those in the *OCT1* gene, functionally different variants have been identified in the *OCT2* gene (Leabman et al. 2002).

We hypothesized that large inter-patient variability in the clinical efficacy of metformin may occur as a result of variations in *OCT1* and/or *OCT2*. In this report we evaluated the functional significance of genetic polymorphisms of *OCT1* and *OCT2* genes with regard to the efficacy of metformin in patients with type 2 diabetes. To date, no study has addressed the genotype–phenotype relationship in light of *OCT* in humans.

Materials and methods

Study subjects

Thirty-three patients (nine men and 24 women; mean age 60 years, range 29–73 years) treated with metformin for at least 1 month were enrolled. We excluded patients who discontinued metformin because of adverse effects (e.g., diarrhea and headache). There are no generally accepted criteria in the clinical cut-off point to divide patients into responders and non-responders. Thus, we selected the criteria empirically, based on our clinical experiences and a previous report (Takei et al. 2001) as follows: (1) responders [n = 24; mean age 62 years, range 29–73 years; mean body mass index (BMI) 25.4 kg/m^2, range 20.4–34.5 kg/m^2], i.e., those whose HbA_{1c} levels had decreased by more than 0.5% from the baseline within 3 months of metformin therapy and had remained low for more than 3 months; and (2) non-responders (n = 9; mean age 56 years, range 34–69 years; mean BMI 25.1 kg/m^2, range 17.8–30.6 kg/m^2), i.e., those for whom either metformin therapy had been discontinued within 3 months and/or after another hypoglycemic drug (e.g., sulfonylurea) had been added to the therapy because of insufficient improvement in HbA_{1c} levels. Eighteen of the responders and six of the non-responders were treated with the maximum approved daily dose in Japan (i.e., 750 mg/day). Eight of the responders and four of the non-responders received metformin monotherapy, and others were co-medicated with sulfonylurea, α-glycosidase inhibitor or insulin. This study was approved by the Ethics Review Board of the Faculty of Medicine, Tottori University, and all subjects gave informed consent before participating.

Identification of variants in *OCT1* and *OCT2* genes

Genomic DNA was extracted from peripheral blood. The primer design was based on the sequence of the 5'-flanking region and the intron/exon junction of *OCT1* and *OCT2* genes (GenBank accession number AL353625 for *OCT1*, AL162582 for *OCT2*). Primers were designed to divide all 11 exons of each gene into fragments of approximately 350 bp so that mutations could be screened by subsequent single-strand conformation polymorphism (SSCP) analysis. Polymerase chain reaction (PCR) products were sequenced either directly or after subcloning on an ABI 3100 automatic sequencer (Applied Biosystems, Foster City, VA, USA).

Quantitative real-time PCR

Total RNA was extracted with an RNAeasy kit (Qiagen, Hilden, Germany) from 58 human liver samples (33 Caucasian and 25 Japanese non-diabetic donors), and reverse transcribed into cDNA using oligo dT primers and reverse transcriptase. OCT1 mRNA was quantified by real-time PCR using an ABI PRISM 7700 sequence detector (Applied Biosystems) with SYBR-green detection of reaction products. Primers for OCT1 mRNA were directed at a sequence that spans the junction of exons 9 and 10, corresponding to open reading frame 1437–1509; 5'-CAC

CCCCTTCATAGTCTTCAG-3′ (forward) and 5′-GCC CAACACCGCAAACAAAAT-3′ (reverse). The copy number of the transcript was measured against the copy-number standard curve of cloned target templates consisting of serial tenfold dilution points. β_2-microglobin mRNA was used as the reference gene for OCT1 mRNA.

Statistical analysis

The significance of differences in allelic frequency was calculated by χ^2 analysis using 2×2 contingency tables. Statistical differences among the data for each group were determined by analysis of variance (ANOVA), followed by the Fisher least significant difference test. To compare the causative factors between responders and non-responders, we performed stepwise discriminant functional analysis. At each step, improvement in the χ^2 and the P values was used to check whether the variable entered at that step significantly improved the discrimination. The independent variables were as follows: polymorphisms, gender, age, duration of disease, types and numbers of co-medicated anti-hyperglycemic drugs, daily dose of metformin, BMI, aspartate aminotransferase, alanine aminotransferase, total cholesterol, high-density lipoprotein (HDL) and treatment with lipid-lowering agents (statins and fibrates). Data are shown as means ± SDs. A P value <0.05 was considered to be significant.

Results

Although the time course of change in the mean daily dose of metformin (milligrams per kilogram per day) and the initial level of HbA_{1c} did not differ between the two groups, the mean HbA_{1c} level was significantly lower in the responder group than in the non-responder group during metformin therapy (Fig. 1).

To identify polymorphisms, we performed PCR–SSCP analysis of all 11 exons of the two genes of interest (*OCT1* and *OCT2*), using DNA obtained from all patients, and the allelic frequency was compared between the responder and non-responder groups. In the *OCT1* gene, 11 polymorphisms were detected by SSCP analysis and identified by subsequent sequencing; none were novel polymorphisms (Table 1). Of these, five SNPs resulted in the following amino acid substitutions: 123C > G (41Phe > Leu), 350C > T (117Pro > Leu), 480C > G (160Phe > Leu), 1022C > T (341Pro > Leu), and 1222A > G (408Met > Val). Although 480C > G, 1022C > T, and 1222A > G variants had a relatively

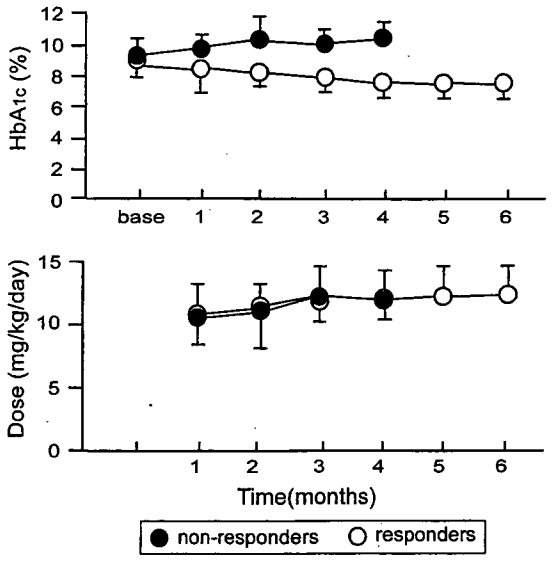

Fig. 1 Time course of changes in HbA_{1c} and metformin daily dose during the observation period in responders and non-responders

high incidence, 123C > G and 350C > T were observed in one patient as heterozygosity. In the *OCT2* gene, two non-synonymous variants were observed: 602C > T (201Thr > Met) and 808G > T (270Ala > Ser). Altogether, there were no remarkable differences in the prevalence of any mutation between responders and non-responders.

The result of discriminant functional analysis is shown in Table 2. Variables selected by the discriminant process were age, BMI, treatment with lipid-lowering agents and two mutations in the *OCT1* gene (–43T > G and 1222A > G). Other variables, such as duration of disease, daily dose of metformin, and types of co-medicated anti-hyperglycemic drugs, had no significant effect on the discrimination. Although age, BMI and treatment with lipid-lowering agents were demonstrated as positive predictors, –43T > G and 1222A > G (408Met > Val) were negative and positive predictors, respectively, for the efficacy of metformin. Total predictive accuracy using these factors was 55.5% ($\chi^2 = 5.59$, $P < 0.05$).

As shown in Table 1, since the frequency of the 408Met allele tended to be higher in non-responders than in responders (0.28 vs 0.19), and since the non-synonymous 408Met > Val variant was selected as a positive predictor, we next examined the association of the 408Met > Val (1222A > G) variant with the expression of OCT1 mRNA in the human liver samples (Fig. 2). Of 58 samples, we analyzed 31 that were homozygotes for the –43T variant (–43T/T). The mean (± SD) hepatic expression level of OCT1 in homozygotes for 408Met (1222A/1222A), heterozygotes for

Table 1 Summary of *OCT1* and *OCT2* gene polymorphisms

Gene	Location	Position[a]	Allele[a]	Nucleotide sequence	Amino acid substitution	Allelic frequency (95% CI)	
						Responders (n = 24)	Non-responders (n = 9)
OCT1	Exon 1	123	C	tcttCctgg	41Phe > Leu	0.98 (0.94–1.02)	1.000
			G	tcttGctgg		0.02 (–0.02–0.06)	0.000
		156	T	agagTcctg	Ser52	0.58 (0.44–0.72)	0.44 (0.21–0.67)
			C	agagCcctg		0.42 (0.28–0.56)	0.56 (0.33–0.79)
		243	C	cgggCgagg	Gly81	1.000	0.94 (0.84–1.05)
			T	cgggTgagg		0.000	0.06 (–0.05–0.16)
		350	C	ctgcCgctg	117Pro > Leu	1.000	0.94 (0.84–1.05)
			T	ctgcTgctg		0.000	0.06 (–0.05–0.16)
	Intron 1	–43	T	atggTtctg	–	0.42 (0.28–0.56)	0.33 (0.12–0.55)
			G	atggGtctg		0.58 (0.44–0.72)	0.67 (0.45–0.89)
	Exon 2	480	C	tcttCtttg	160Phe > Leu	0.88 (0.78–0.97)	0.83 (0.66–1.01)
			G	tcttGtttg		0.13 (0.03–0.22)	0.17 (–0.01–0.34)
	Exon 6	1022	C	acgcCgcgc	341 Pro > Leu	0.81 (0.70–0.92)	0.89 (0.74–1.03)
			T	acgcTgcgc		0.19 (0.08–0.30)	0.11 (–0.03–0.26)
	Exon 7	1222	A	ggccAtgtc	408Met > Val	0.19 (0.08–0.30)	0.28 (0.07–0.49)
			G	ggccGtgtc		0.81 (0.70–0.92)	0.72 (0.52–0.93)
	Inron 7	+8	Deletion	(ggtaagtt)0		0.81 (0.70–0.92)	0.72 (0.52–0.93)
				(ggtaagtt)1		0.19 (0.08–0.30)	0.28 (0.07–0.49)
	Intron 10	+26	C	actcCgagg		0.98 (0.94–1.02)	1.000
			T	actcTgagg		0.02 (–0.02–0.06)	0.000
		–21	C	ccaaCttt		0.46 (0.32–0.60)	0.39 (0.16–0.61)
			T	ccaaTttt		0.54 (0.40–0.68)	0.61 (0.39–0.84)
OCT2	Exon 3	602	C	tataCgtgg	201Thr > Met	0.98 (0.94–1.02)	0.94 (0.84–1.05)
			T	tataTgtgg		0.02 (–0.02–0.06)	0.06 (–0.05–0.16)
	Exon 4	808	G	agttGctct	270Ala > Ser	0.92 (0.88–0.96)	0.94 (0.84–1.05)
			T	agttTctct		0.08 (0.04–0.12)	0.06 (–0.05–0.16)

[a] Position is relative to the ATG start site, and the reference allele for each gene was obtained from the GenBank accession numbers AL353625 for OCT1 and AL162582 for OCT2

408Met > Val (1222A/1222G), and homozygotes for 408Val (1222G/1222G) was 0.69 ± 0.43, 0.92 ± 0.53, and 1.01 ± 0.66, respectively. Although the hepatic expression of OCT1 tended to be lower in livers with the 408Met (1222A) variant, the differences did not reach the level of significance. In the –43T > G variant, the mean OCT1 expression level in –43T/T (n = 18), –43T/G (n = 8), and –43G/G (n = 10) samples (all harbored the 1222G/1222G allele) was 1.01 ± 0.70, 1.04 ± 0.34, and 1.46 ± 0.53, respectively.

Table 2 Stepwise discriminant functional analysis of the efficacy of metformin

Variable	Coefficient	χ^2 value	P
Age	0.09	5.59	0.05
BMI	0.23		
Treatment with lipid-lowering agents	2.25		
–43T > G (intron 1)	–2.35		
408Met > Val (exon 7)	2.51		

Predictive accuracy = 55.5%

Discussion

In this study we first analyzed mutations in *OCT1* and *OCT2* and then examined the association between polymorphisms in these two genes and the efficacy of metformin, because in vitro studies have indicated that OCT1 and OCT2 are responsible, respectively, for the hepatic and renal transport of metformin (Kimura et al. 2005; Wang et al. 2002, 2003). In contrast to studies in vitro and with animals, there are no data from human studies on the contribution of these polymorphisms to the phenotypes of metformin.

In the *OCT1* gene, all non-synonymous variants except 41Phe > Leu and 117Pro > Leu have already been identified in some racial populations, with a frequency of 0.005–0.81 (Kerb et al. 2002; Shu et al. 2003). The 41Phe > Leu and 117Pro > Leu allele frequencies were relatively low (0.004), and they have already been reported in a Japanese population (Itoda et al. 2004). Recent expression studies have indicated that 341Pro > Leu had decreased ability to transport test compounds, while 160Phe > Leu and 408Met > Val were unchanged (Kerb et al. 2002; Sakata et al. 2003;

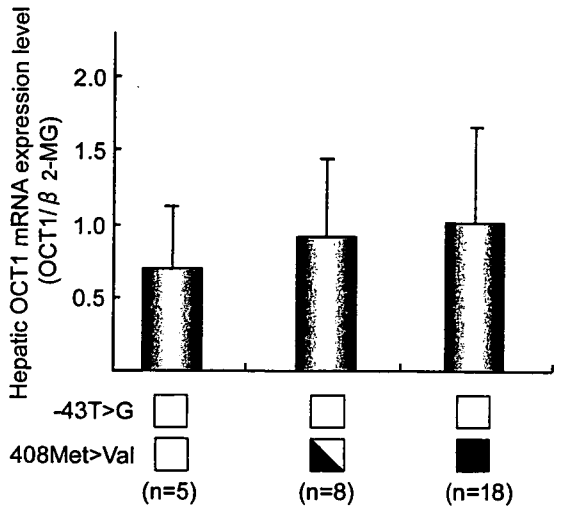

Fig. 2 Hepatic OCT1 mRNA expression levels with regard to the 408Met > Val (1222A > G) variant. Among 58 samples, 31, which were homozygotes for the –43T variant (–43T/T), were analyzed. *Open squares*, *partially filled squares* and *closed squares* correspond to patients homozygous for the 408Met (1222A) allele and heterozygous and homozygous for the 408Val (1222G) allele

Shu et al. 2003). Interestingly, the 341Pro > Leu variant was observed in Asian and African American populations but not in Caucasians (Shu et al. 2003); however, there was no difference in the allele frequency of 341Pro > Leu between responders and non-responders to metformin therapy in this study.

In contrast to those in the *OCT1* gene, it appears that the number of non-synonymous variants in the *OCT2* gene and their allelic frequencies were lower than in other known drug transporter genes such as *MDR1*, *MRP1*, *MRP2*, and *OATP-C* (Nishizato et al. 2003). These observations are consistent with the finding of a lower frequency of non-synonymous variants in ethnically diverse genomic DNA samples (Leabman et al. 2002). Recent population-genetic analysis has demonstrated that selection has acted against amino acid changes in OCT2 (Leabman et al. 2002), suggesting that OCT2 is relatively intolerant of non-synonymous changes. In general, the less frequent non-synonymous variants resulted in more significant and deleterious functional changes. However, the 270Ala > Ser variant was reported to exhibit subtle functional differences from the reference form of OCT2 (Leabman et al. 2002).

Although there were no remarkable differences in the prevalence of any mutation sites between responders and non-responders, we next carried out discriminant functional analysis including not only genetic polymorphisms but also the patients' background. As shown in Table 2, age, BMI and treatment with lipid-lowering agents were demonstrated as positive predictors of metformin efficacy. These observations are partially in agreement with the findings by Knowler et al. (2002), that metformin was less effective in subjects with lower BMI or a lower fasting plasma glucose concentration. BMI > 25 kg/m^2 is defined as obesity in Japan; 66.7% of responders and 44.4% of non-responders were obese in this study. Although the precise mechanism is unknown, these data suggest that metformin is more effective in the case of obesity-induced insulin resistance that is higher fasting plasma glucose. The contribution of lipid-lowering agents was somewhat unexpected, because metformin therapy has been reported to improve both glycemic control and lipid concentrations (i.e., plasma total and low-density lipoprotein cholesterol and triglyceride) in patients with non-insulin-dependent diabetes mellitus (DeFronzo and Goodman 1995). However, in our study, 12 responders and two non-responders were treated with lipid-lowering agents, and most of these patients (11/12 responders and 1/2 non-responders) used HMG-CoA reductase inhibitors (statins). Several studies have shown that low-density lipoprotein (LDL) size rather than plasma LDL level is more correlated with insulin resistance and eventual progression of coronary heart disease (Rizzo and Berneis 2006). Although the efficacy of modifying LDL size is different among agents (fluvastatin and atorvastatin seem to be much more effective agents than pravastatin and simvastatin), statins moderately lower all LDL subclasses, and, somehow, this process seems to make metformin more effective.

Since –43T > G and 408Met > Val (1222A > G) variants were identified as negative and positive predictors, respectively, for the clinical effectiveness of metformin, we evaluated the functional significance of the latter non-synonymous variant in the expression of OCT1 mRNA, using human liver samples. Our findings indicate that samples with the 408Met (1222A) allele tended to be associated with a reduced expression level, as compared with those without the 408Met allele; however, the difference did not reach significance. A recent study using site-directed mutagenesis has indicated that point mutations in the predicted ninth transmembrane domain such as 1222A > G (408Met > Val) do not lead to functional changes (Kerb et al. 2002). We also measured OCT1 mRNA expression with regard to the non-coding –43T > G variant; however, no significant effect was observed. In the present study, the predicted accuracy is still insufficient for its clinical application (i.e., 55.5%). Thus, if these observations are taken into consideration, the contribution of polymorphisms in

OCT1 and *OCT2* genes to metformin efficacy may not be as significant as our expectations had led us to believe. However, since a non-synonymous variant 408Met > Val is often observed simultaneously with other non-synonymous variants (Shu et al. 2003), further in vitro and in vivo studies with regard to the haplotypic consideration, including the non-coding region, are needed to elucidate the functional properties of the variants identified in this study.

While data from only 24 responders and nine non-responders were used, this preliminary investigation is the first study addressing the genotype–phenotype relationship of OCTs in the efficacy of metformin. However, obviously, the small number of patients is a drawback in our study. For example, co-medication of other anti-hyperglycemic drugs in both groups made it difficult for us to judge whether the decreases in HbA_{1c} levels in the responders are attributable to the metformin effect. Clearly, definition of the clinical cut-off point is also essential to divide patients into the two groups correctly. In order to overcome these problems, it is clear that the results in this study should be confirmed in a population study involving large numbers of patients. Nevertheless, this report provides for the possibility of OCTs' functions in humans.

Acknowledgments This work was supported by Health and Labor Sciences Research grants from the Ministry of Health, Labor, and Welfare for Research on Advanced Medical Technology. None of the authors claims any conflict of interest.

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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

Expert Opin. Drug Metab. Toxicol. (2006) 2(5):651-674

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

10.1517/17425255.2.5.651 ISSN 1742-5255

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.