MDR1 Genotype-Related Duodenal Absorption Rate of Digoxin in Healthy Japanese Subjects

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Purpose. Recent clinical studies suggest the importance of the *MDR1* genotype at position 3435 (C3435T) in terms of pharmacokinetics, but there is still no consensus in reports on the relationship between the genotype and plasma/serum concentration–time profiles of drugs after conventional oral administration. This study was performed to elucidate the effects of C3435T on the rate of duodenal absorption of digoxin in healthy Japanese subjects.

Methods. Digoxin solution was sprinkled directly over the surface of the duodenum using an endoscope, and its absorption rate was evaluated by serial monitoring of the serum concentration and by analysis of its initial 15-min increasing phase.

Results. The duodenal absorption rates of digoxin were 911 ± 91 ng/min and 506 ± 76 ng/min for C/C and T/T, respectively (±SE, $p = 0.007$).

Conclusions. The C3435T mutation of the *MDR1* gene is associated with suppression of duodenal absorption of digoxin.

KEY WORDS: *MDR1*; polymorphism; digoxin pharmacokinetics; absorption rate; intraduodenal administration.

INTRODUCTION

The multidrug-resistant transporter MDR1 (P-glycoprotein; ABCB1), originally isolated from resistant tumor cells, is a glycosylated membrane protein with 1280 amino acids (170 kDa) consisting of two similar regions containing six putative transmembrane segments and an intracellular binding site for ATP and acts as an efflux pump to remove exogeneous and unnecessary substances from cells (1). Human MDR1 is also expressed in normal tissues to confer intrinsic resistance (1). Human MDR1 expressed in the luminal membranes of renal proximal tubules and the biliary canalicular membrane of hepatocytes transports the substrates into urine and bile, respectively. MDR1 in the capillary endothelial cells of the brain and testis and in the epithelial cells of the small and large intestines restricts the substrate entry. MDR1 is responsible for the pharmacokinetics of various types of MDR1 substrate drugs, including digoxin, amiodarone, quinidine, itraconazole, and cyclosporin A (1).

In 2000, Hoffmeyer *et al.* reported that a silent mutation at exon 26, position 3435 of the *MDR1* gene (C3435T), was associated with a lower level of MDR1 expression in the duodenum in Caucasian volunteers (2). They also suggested that this mutation results in a higher plasma concentration of digoxin after rifampin induction (3) and in a higher maximum concentration after multiple oral administration (4) and suggested that these observations resulted from a weaker restriction of intestinal absorption (2). Recent investigations also suggested the importance of C3435T (5–9), but the relationship with the phenotype did not always agree with the report by Hoffmeyer *et al.* Plasma or serum concentrations of digoxin (5), fexofenadine (6), and efavirenz and nelfinavir (7) were lower in subjects homozygous for the mutant allele (T/T) than in those homozygous for the wild-type allele (C/C) after single or multiple oral administration; however, recent clinical investigations (8) supported the report by Hoffmeyer *et al.* MDR1 is expressed throughout the body, and thus, the pharmacokinetic profile of the probe drug used and/or mode of administration might affect the apparent relationship between the genotype and phenotype. In this study, the effects of the *MDR1* genotype at exon 26, position 3435 on the duodenal absorption of digoxin were examined in healthy Japanese subjects. Digoxin solution was sprinkled directly over the surface of the duodenum using an endoscope, and the rate of absorption of digoxin was evaluated by serial monitoring of the serum concentration through an indwelling catheter set in the cephalic vein on the right forearm in advance and by analysis of the initial increasing phase of serum concentration.

MATERIALS AND METHODS

Subjects

Eleven unrelated healthy Japanese subjects living in Kobe city and neighboring areas participated in this study. The *MDR1* genotype at exon 26, position 3435 was homozygous for the wild-type allele (C/C; $n = 5$) or homozygous for the mutant T-allele (T/T; $n = 6$). There were no differences in demographic data between C/C and T/T groups. The glutamic oxaloacetic transaminase (GOT) activity and the concentration of creatinine (Scr) in serum obtained before digoxin administration were measured by the Reitman–Frankel method and the Jaffé method, respectively, using diagnosis kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan). All subjects participating in this study showed normal levels of GOT and Scr. The aims of the study were fully explained to all subjects, and written informed consent was obtained. The protocol was approved by the Institutional Review Board of Kobe University Hospital, Kobe University, Japan.

Genotyping

Genomic DNA was extracted from 0.5 ml of whole blood as described (5). Approximately 500 ng of genomic DNA was used for PCR amplification. The *MDR1* genotype at exon 26, position 3435 was determined by polymerase chain reaction–

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restriction fragment length polymorphism (PCR-RFLP) analysis as described previously (5).

In this study, the *MDR1* genotype at exon 21, position 2677 was also determined. The wild-type normal gene is called the G-allele, and the mutant gene, the A- or T-allele, carries nucleic acid replacements (G2677(A,T)). The following PCR primers synthesized by Hokkaido System Science, Co., Ltd. (Sapporo, Japan) were used in this study (10,11): Forward 5′-TTT GCA GGC TAT AGG TTC CAG-3′; Reverse 1 (for G2677A) 5′-GTT TGA CTC ACC TTC CCA G-3′; Reverse 2 (for G2677T) 5′-TTT AGT TTG ACT CAC CTT CCC G-3′. A 222-bp sequence of the *MDR1* gene was amplified for G2677A by PCR with the oligonucleotide primers and a Gene Amp PCR Reagent Kit (Takara Shuzo Co., Kyoto, Japan), and the fragment for G2677T was 226 bp. PCR consisted of an initial denaturation step at 94°C for 3 min; 40 cycles of 94°C for 20 s, 55°C for 20 s, and 72°C for 30 s; and a final extension step at 72°C for 5 min. The temperature was controlled with a programmable heat block (GeneAmp PCR System 9700, PE Applied Biosystems, Foster City, CA). After amplification, the PCR product (5 μl) was taken directly from the aqueous phase. DNA was digested with restriction endonucleases in 10 μl of the appropriate basal buffer. To digest the 222-bp PCR product into 14- and 208-bp fragments, 5 U of *Bsr* I (Takara Shuzo Co.) was added to the basal buffer at 65°C for 1 h. PCR products from the G-allele or T-allele were resistant to *Bsr* I digestion. Five units of *Ban* I (Takara Shuzo Co.) was used to digest the 226 bp PCR product into 26- and 200-bp fragments at 37°C for 1 h. The PCR product from T-allele was resistant to *Ban* I digestion. The fragments produced by *Bsr* I or *Ban* I digestion were separated by agarose gel electrophoresis (5%, 100V) along with a DNA molecular weight marker (pUC18 *Msp* I Digest, Sigma Chemical Co., St. Louis, MO) as a reference.

These genotypes were also confirmed by direct sequencing (ABI PRISM® 310 Genetic Analyzer, PE Applied Biosystems).

Serum Concentration–Time Profiles of Digoxin after Oral Administration of Tablets or Intraduodenal Administration as Saline Solution

The subjects were prohibited from taking any drugs for at least 1 month before the first administration of digoxin until the end of the study. They were cautioned not to eat or drink anything for 3 h after the administration. After fasting overnight, they were given a digoxin tablet (0.25 mg/T, Digosin® tablet, Chugai Pharmaceutical Co., Ltd., Japan) with 200 ml of tap water orally or digoxin saline solution (0.25 mg per total 5 ml) intraduodenally, which was prepared by dilution of the digoxin injection dose (0.25 mg/V, Digosin® injection, Chugai Pharmaceutical Co., Ltd.) with saline.

In the case of the oral administration of a tablet, serum samples were collected at 0 (blank), 30, 60, 120, 240, 360, 480, and 720 min from the cephalic vein on the right forearm. For intraduodenal administration, the digoxin solution was sprinkled directly over the surface of the duodenum using an endoscope (GIF type XQ200, Olympus, Tokyo, Japan) equipped with an EVIS system (light source: CLV-U40D, Olympus, processor: CV-240, Olympus), real-time TV monitor (OEV141, Olympus), and dye-sprinkling tube for a 2.8 mm channel (PW-5L-1, Olympus). The appearance of digoxin in serum was monitored by serial sampling of blood at 0 (blank), 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, and 240 min through an indwelling catheter set in the cephalic vein on the right forearm in advance.

Determination of Serum Concentration of Digoxin

The serum concentration of digoxin was measured on the day of sampling by fluorogenic enzyme immunoassay (FEIA) using an OPUS analyzer (Dade Behring, Inc., Newark, DE). The determination was routinely validated to confirm the precision and accuracy. All assays were performed in duplicate for a serum sample, and mean values were calculated. The limit of quantification (LOQ) was 0.25 ng/ml. For those samples with a concentration below the LOQ, a value of zero was recorded. Three serum samples were prepared for a blood sample, and the average value was adopted as the data. For example, a value of 0.18 ng/ml was adopted for a blood sample with values of 0.27 ng/ml, 0.27 ng/ml, and below the LOQ. It was confirmed that there was no detectable digoxin in the blank serum.

Data Analysis

The maximum serum concentration of digoxin (C_{max}) and the time to reach C_{max} (T_{max}) were obtained from the raw data. The area under the serum concentration–time curve and mean residence time of digoxin were calculated using the data with the sampling time of 0–4 h by the trapezoidal method (AUC(0–4) and MRT(0–4), respectively).

The initial phase of increase in the serum concentration after the intraduodenal administration was fitted to a linear plot to obtain the rate of increase in serum concentration per unit of time, dC/dt (ng/ml/min). The absorption rate of digoxin was calculated as $dX_{abs}/dt = dC/dt \times$ (volume of serum). Volume of serum was calculated as total body weight (kg) × 0.040 (L/kg). The concentration data were also fitted to the following equation to estimate the actual values of T_{max} and C_{max}: $C = -A \times \exp[-\alpha \times (t - t_{lag})] + B \times \exp[-\beta \times (t - t_{lag})] + C \times \exp[-\gamma \times (t - t_{lag})]$, where t_{lag} was the lag time.

Statistical Analysis

The data are presented as the means ± SE. The unpaired *t*-test was used for statistical analysis. Statistical significance was assumed at $p < 0.05$.

RESULTS

Figure 1 shows the serum concentration–time profiles of digoxin after oral administration of digoxin tablets in the subjects homozygous for the wild-type allele C/C and homozygous for the mutant allele T/T at exon 26, position 3435. The serum concentration increased slowly, and AUC(0–4) values were 3.87 ± 0.24 ng h/ml and 3.27 ± 0.24 ng h/ml, respectively. The systemic exposure was lower by 16% in T/T than C/C, but there was no statistical difference between C/C and T/T ($p = 0.105$).

Figure 2 indicates the serum concentration–time curves of digoxin after intraduodenal administration of digoxin saline solution in the subjects with C/C and T/T. The digoxin appeared rapidly in the serum when compared with the oral administration. The serum concentration increased more rap-

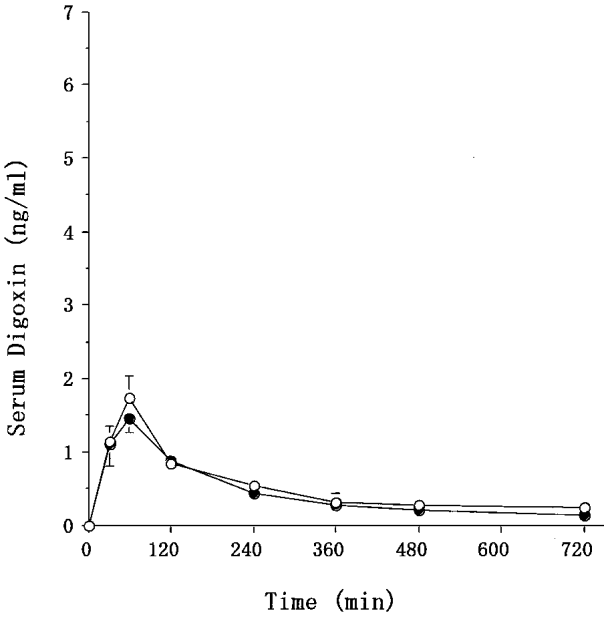

Fig. 1. Effects of C3435T polymorphism on serum concentration–time curves of digoxin after oral administration of a digoxin tablet in healthy Japanese subjects. *Open circles,* C/C (n = 5); *closed circles*, T/T (n = 6). The values are the means ± SE. There was no significant difference in concentrations at any time points.

idly in the subjects with C/C than in T/T. Table I summarizes the pharmacokinetic parameters after intraduodenal administration. Nine of 11 subjects showed a T_{max} of 15 min, and the other two whose genotype was T/T had values of 30 min. Estimated values of T_{max} were 16.9 ± 1.6 min and 19.6 ± 1.6 min in C/C and T/T, respectively (p = 0.268). Compared with the C/C subjects (4.70 ± 0.41 ng/ml), the C_{max} was decreased in T/T (2.55 ± 0.39 ng/ml) (p = 0.005). Estimated values were 4.70 ± 0.41 ng/ml and 2.54 ± 0.40 ng/ml in C/C and T/T, respectively (p = 0.005). AUC(0–4) values were also decreased in T/T, suggesting lower systemic exposure: 5.07 ± 0.29 ng h/ml and 3.78 ± 0.27 ng h/ml in C/C and T/T, respectively (p = 0.010). MRT(0–4) values were 71.1 ± 1.8 min and 82.5 ± 2.6 min in C/C and T/T, respectively (p = 0.007). The duodenal absorption rates were 911 ± 91 ng/min and 506 ± 76 ng/min, respectively (p = 0.007), both indicating the restriction of absorption in T/T.

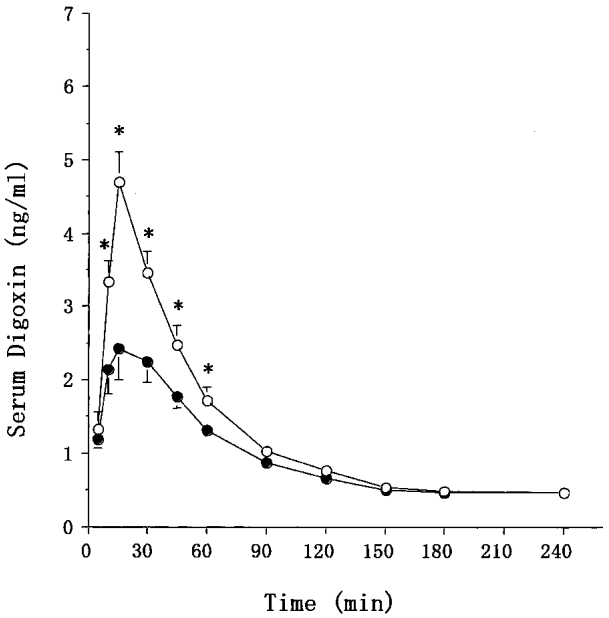

Fig. 2. Effects of C3435T polymorphism on serum concentration–time curves of digoxin after intraduodenal administration of a digoxin saline solution in healthy Japanese subjects. *Open circles,* C/C (n = 5); *closed circles*, T/T (n = 6). The values are the means ± SE. *Statistically significant difference, p < 0.05, between C/C and T/T by the unpaired *t*-test.

Three of five subjects with C/C at exon 26, position 3435 were accompanied with G/G at exon 21, position 2677, and four of six subjects with T/T at 3435 had T/T at 2677 (Table I). The serum concentrations were lower in the subjects with T/T than G/G. AUC(0–4) values were 5.40 ± 0.07 ng h/ml and 3.94 ± 0.30 ng h/ml in G/G and T/T, respectively (p = 0.022), suggesting lower systemic exposure in T/T. G2677T was also suggested to be associated with a lower rate of duodenal absorption (898 ± 119 ng/min and 541 ± 91 ng/min for G/G and T/T, respectively) (p = 0.110).

DISCUSSION

Hoffmeyer *et al.* reported that mutation at exon 26, position 3435 of the *MDR1* gene (C3435T) is associated with a lower level of MDR1 expression in the duodenum (2). A substudy with a smaller study population was also conducted to assess the effects of rifampin induction on the plasma concentration of digoxin after single oral administration (3). The results also suggested that the plasma concentration of digoxin after rifampin induction was higher in subjects with the mutant T-allele, suggesting that the mutant T-allele resulted in a lower level of MDR1 expression before rifampin treatment and/or suppression of MDR1 induction by rifampin. The maximum digoxin plasma concentration was also higher in T/T than C/C after multiple oral administration (4). These observations were explained by a weaker restriction of intestinal absorption in T/T (2).

The report by Hoffmeyer *et al.* strongly suggested the importance of *MDR1* genotyping. To date, 28 SNPs have been found in the *MDR1* gene (1). The frequency of the C3435T mutation has been reported to vary significantly among different ethnic populations (12). However, the relationship with the phenotype in recent investigations did not always agree with the report by Hoffmeyer *et al.* MDR1 expression in Japanese placenta was independent of C3435T (13), and MDR1 mRNA levels in duodenal biopsies taken from healthy Japanese subjects were higher in T/T than C/C (14). Plasma or serum concentration of digoxin (5), fexofenadine (6), and efavirenz and nelfinavir (7) were lower in T/T than in C/C. Presumably the discrepancy was related to other SNPs, ethnic differences, pharmacokinetic profiles of the probe drug used, and mode of administration, e.g., single or multiple. MDR1 is expressed throughout the body and is susceptible to down-regulation and induction. It is generally accepted that the C3435T mutation is related to MDR1 expression throughout the body and affects the renal or biliary secretion and tissue distribution. Thus, it is important to perform clinical investigations to elucidate the effects of this mutation on each process of the pharmacokinetics. Here, the effects of C3435T on the duodenal absorption of digoxin were

Table I. Pharmacokinetic Parameters after Intraduodenal Administration of 0.25 mg of Digoxin as a Saline Solution in Healthy Japanese Subjects

Subject	Genotype 3435	Genotype 2677	T_{max} (min)		C_{max} (ng/ml)		AUC (0–4) (ng · h/ml)	MRT (0–4) (min)	dX_{abs}/dt (ng/min)
7468	C/C	G/G	15	13.5[a]	6.10	6.16[a]	5.28	69.4	1159
6962	C/C	G/G	15	22.1	3.70	3.85	5.57	77.2	627
7664	C/C	G/G	15	17.7	4.26	4.30	5.35	73.2	909
6757	C/C	G/A	15	17.6	5.09	4.94	5.23	68.6	821
7570	C/C	T/A	15	13.5	4.35	4.22	3.95	67.2	1042
Average			15	16.9	4.70	4.70	5.07	71.1	911
SE			0	1.6	0.41	0.41	0.29	1.8	91
7470	T/T	T/T	30	25.6	2.48	2.38	3.58	80.9	465
6556	T/T	T/T	15	16.9	4.14	4.16	4.80	74.5	777
7962	T/T	T/T	15	17.3	3.07	3.10	4.25	81.4	655
6349	T/T	G/T	30	21.6	2.11	2.09	3.80	84.6	378
7260	T/T	T/T	15	20.9	1.32	1.35	3.12	93.6	266
7061	T/T	G/T	15	15.1	2.19	2.19	3.10	80.2	495
Average			20	19.6	2.55	2.54	3.78	82.5	506
SE			3	1.6	0.39	0.40	0.27	2.6	76
p			0.186	0.268	0.005	0.005	0.010	0.007	0.007

Note: C_{max}, maximum serum concentration; T_{max}, time to reach C_{max}; AUC (0–4), area under serum concentration–time curve from 0 h to 4 h; MRT (0–4), mean residence time from 0 h to 4 h; dX/dt, absorption rate (ng/min).
[a] Estimated values of T_{max} and C_{max}.

examined. The absorption rate was quantitatively evaluated by sprinkling a digoxin saline solution directly over the surface of the duodenum using an endoscope and by analysis of the initial increasing phase of the serum concentration. As shown in Figs. 1 and 2, conventional oral administration of a digoxin tablet was inappropriate for discussing the effect of *MDR1* genotype on the absorption, as the appearance of digoxin in the serum was delayed by disintegration of the tablet, dissolution of digoxin, and gastric emptying. Digoxin should be administered as a saline solution directly over the surface of the duodenum. It was clearly demonstrated that the C3435T mutation was associated with suppression of the duodenal absorption of digoxin. The rate of duodenal absorption of digoxin was 506 ± 76 ng/min in T/T, which was about 56% of the rate in C/C.

Plasma/serum concentration–time profiles are usually biphasic after intravenous administration (15). The first phase, lasting 4–8 h, represents the time required for drug distribution throughout the body, and the steady-state volume of distribution and half-life in β phase are 5–7.3 L/kg and 36 h, respectively (15). However, frequent blood sampling suggests three compartments in the body, and two small compartments representing intravascular or extracellular fluid spaces and highly vascularized tissues are proposed in addition to one large peripheral compartment (15). Here, the serum concentration–time profiles up to 4 h after intraduodenum administration (Fig. 2) could not be described by a one-compartment model with first-order absorption, suggesting that the profiles were defined by compartments representing the intravascular or extracellular fluid spaces and highly vascularized tissues. In this analysis, the apparent volume of distribution divided by bioavailability was estimated to be 0.50 ± 0.08 L/kg and 1.33 ± 0.37 L/kg in C/C and T/T, respectively ($p = 0.074$), being smaller than the steady-state volume of distribution, that is, 5–7.3 L/kg, which was obtained by blood sampling for more than 1 day after administration. Although there was no statistical difference, the digoxin distribution into highly vascularized tissues tended to be more extensive in T/T than C/C.

In this study, the effect of mutation at exon 21, position 2677 (G2677(A,T)) was also examined. The mutation was suggested to be linked with C3435T (6,13,16). The G2677(A,T) mutation resulted in $Ala^{893}Thr$ and $Ala^{893}Ser$, respectively, and the effect of the C3435T mutation was suggested to be caused by linkage. Herein, AUC(0–4) values were clearly demonstrated to be lower in T/T than G/G after intraduodenal administration as a saline solution.

In summary, conventional oral administration of a digoxin tablet was inappropriate for discussing the effect of *MDR1* genotype on the absorption. By intraduodenal administration of digoxin solution, it was clearly demonstrated that the C3435T mutation of the *MDR1* gene is associated with suppression of the duodenal absorption of digoxin.

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