MDR1 Genotype-Related Pharmacokinetics of Digoxin after Single Oral Administration in Healthy Japanese Subjects

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Purpose. To evaluate the *MDR1* genotype frequency in the Japanese population and to study the relationship between the *MDR1* genotype and the pharmacokinetics of digoxin after single oral administration in healthy subjects.

Methods. The *MDR1* genotype at exon 26 was determined in 114 healthy volunteers by polymerase chain reaction-restriction fragment length polymorphism. The serum concentration-time profile of digoxin was examined after single oral administration at a dose of 0.25 mg.

Results. It was found that 35.1 % (40/114) of subjects were homozygous for the wild-type allele (C/C), 52.6 % (60/114) were compound heterozygotes with a mutant T-allele (C3435T) (C/T), and 12.3 % (14/114) were homozygous for the mutant allele (T/T). There was no effect of gender or age on the distribution. The serum concentration of digoxin after a single oral administration increased rapidly, attaining a steady state in all subjects; however, it was lower in the subjects harboring the T-allele. $AUC_{0\text{-}4\ h}$ values (±SD) were 4.11 ± 0.57, 3.20 ± 0.49, and 3.27± 0.58 ng h/ml, respectively, with a significant difference between C/C and C/T or T/T.

Conclusions. The serum concentration of digoxin after single oral administration was lower in the subjects harboring a mutant allele (C3435T) at exon 26 of the *MDR1* gene.

KEY WORDS: MDR1; genotype; digoxin pharmacokinetics; single oral administration.

INTRODUCTION

The multidrug resistant transporter, MDR1 (P-glycoprotein; P-gp), was originally isolated from resistant tumor cells in 1976 (1). MDR1 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. MDR1 acts as an efflux pump to remove antitumor agents from cells; the magnitude of resistance depends on the MDR1 expression level (2,3). A gene that was overexpressed in multidrug-resistant KB carcinoma cells was isolated in 1986 and was demonstrated to encode human MDR1 (4,5). Over the last decade, it has been elucidated that human MDR1 is expressed in the liver, kidneys, small and large intestines, brain, testis, muscle tissue, placenta, and adrenals (6–9), and it is well-accepted that MDR1 confers intrinsic resistance to normal tissues by exporting unnecessary or toxic exogeneous substances or metabolites out of the body. MDR1 would be responsible for the pharmacokinetics of drugs that are substrates for MDR1, such as digoxin, amiodarone, quinidine, itraconazole, and cyclosporin A (10).

Hoffmeyer *et al.* reported that the mutant C3435T at exon 26 of *MDR1* gene is associated with a lower level of MDR1 expression in enterocyte preparations of the duodenum (11). They also suggested that this mutant results in a higher plasma concentration of digoxin because of weaker restriction of intestinal absorption based on data for rifampin-induction (12) and the maximum concentration in the steady state (13). However, the concentration of digoxin is thought to be a function of not only the absorption but also distribution and elimination. MDR1 is expressed throughout the body (6–9), and therefore, these findings encouraged us to conduct a pharmacokinetic study of digoxin to obtain basic data on the effect of the *MDR1* genotype. In this article, the *MDR1* genotype at exon 26 was determined in 114 healthy subjects by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and the serum concentration of digoxin was measured after single oral administration in 15 healthy subjects.

MATERIALS AND METHODS

Subjects

One-hundred and fourteen unrelated healthy subjects living in Kobe city and neighboring areas (65 males and 49 females, 19–76 years old) participated in the *MDR1* genotyping study. Fifteen of the 114 subjects further participated in the pharmacokinetic study of digoxin. The glutamic oxaloacetic transamase (GOT) activity and the concentration of creatinine in serum obtained before digoxin administration were measured by the Reitman–Frankel method and the Jaffé method, respectively, by using the diagnosis kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The aims of the study were fully explained to each subject, and written informed consent was obtained. The protocol was approved by the Institutional Review Broad of Kobe University Hospital, Kobe University, Japan.

Isolation of Genomic DNA

Peripheral blood (2.0 ml) was drawn into a sampling tube containing EDTA-2Na (3.0 mg). Genomic DNA was extracted from whole blood (0.5 ml) in the presence of concentrated sodium iodide (NaI) and sodium dodecyl sulfate (SDS) using a DNA Extractor WB Kit® (Wako Pure Chemical Industries Ltd., Osaka) (14). Briefly, an equal volume of lysis solution containing 1% Triton X-100 was added to 0.5 ml of blood, and the nuclei were isolated according to Buffone and

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Darlington (15). Isolated nuclei were suspended in enzyme reaction solution containing 1% SDS and digested with 0.8 mg/ml proteinase K to liberate DNA from nuclear proteins. After a 1-h incubation, NaI solution was added to the nuclear lysate to final concentrations of 4.5 M NaI and 0.4% SDS, then isopropanol was added. The mixture was shaken until a whitish precipitate appeared. The precipitate was collected by centrifugation and washed twice with 40% isopropanol and 70% ethanol. Total genomic DNA was suspended in 50–100 μl of TE buffer (pH 8) containing 10 mM trisaminomethane and 1 mM EDTA.

Determination of *MDR1* Genotype at Exon 26

A polymorphism at exon 26 of the *MDR1* gene has been described by Hoffmeyer *et al.* (11). The wild-type normal gene is called the C-allele, and the mutant gene, the T-allele, carries nucleic acid replacements (C3435T). The following PCR primers synthesized by Nisshinbo Co. (Tokyo, Japan) were used in this study: Forward (5′-TGA TGG CAA AGA AAT AAA GCG A-3′), Reverse (5′-TGA CTC GAT GAA GGC ATG TAT GT-3′). A 193-bp sequence of the *MDR1* gene was amplified by PCR with the oligonucleotide primers and a Gene Amp PCR Reagent Kit (Takara Shuzo Co., Kyoto, Japan). The DNA (approximately 500 ng) was amplified by PCR. The PCR consisted of an initial de-naturation step at 94°C for 3 min, 35 cycles of 94°C for 20 s, 55°C for 20 s, and 72°C for 30 s, and 1 cycle of 72°C for 5 min. The temperature was controlled by a programmable heat block (GeneAmp PCR System 9700, PE Applied Biosystems, Foster City, CA) (Fig. 1).

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 a 193-bp PCR product into 49 and 144 bp, 5 units of *Mbo* I (Takara Shuzo Co.) was added to the basal buffer at 37°C for 1 h. The fragments digested by *Mbo*I were separated by agarose gel electrophoresis (3%, 100V) along with a DNA molecular weight marker (pUC18 *Msp* I Digest, Sigma Chemical Co., St. Louis, MO) for reference. Because the T-allele lacks a *Mbo* I site, its PCR product is resistant to *Mbo* I digestion, and the genotype of each individual was determined by this restriction enzyme digestion (Fig. 2).

Serum Concentration-Time Profiles of Digoxin after Single Oral Administration

Among those subjects whose genotype was determined, 15 participated in the pharmacokinetic study of digoxin. The genotypes of the subjects were C/C ($n = 5$), C/T ($n = 4$), and T/T ($n = 6$). The subjects were prohibited from taking any drugs for 1 week before the administration of digoxin until the end of the study. After fasting overnight, they were given a single oral dose of digoxin (0.25 mg) as a tablet (Digosin®, Chugai Pharmaceutical Co., Ltd., Japan). They were cautioned not to eat or drink anything for 3 h after the administration. Venous blood samples were drawn at 0.5, 1, 2, 4, 6, 8, 12, and 24 h after digoxin administration, and serum samples were prepared. Serum samples were stored at –20°C until analysis.

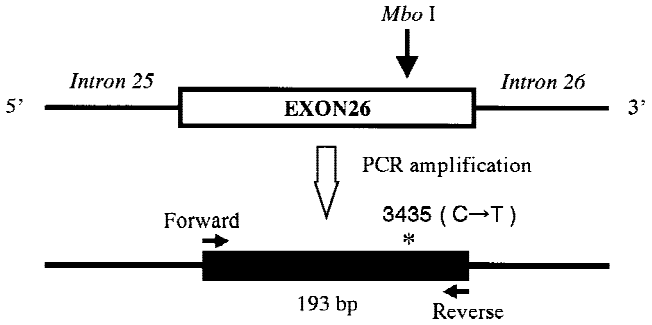

Fig. 1. A summary of the human *MDR1* genes and polymerase chain-reaction restriction fragment length polymorphism. The arrow represents the restriction enzyme site; the asterisk indicates the nucleotide substitution in each allele.

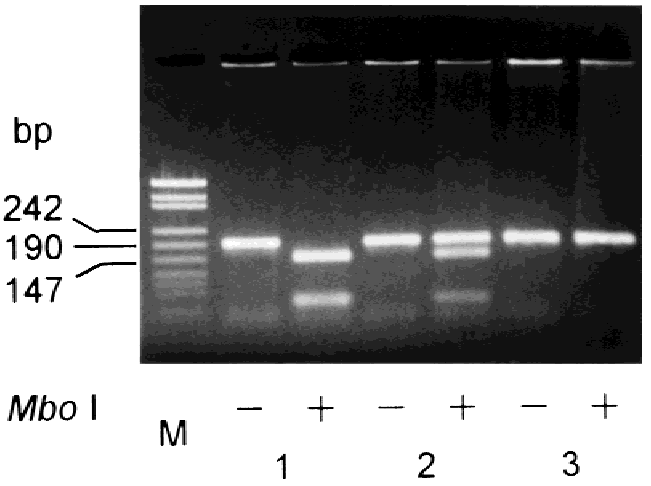

Fig. 2. Determination of polymorphic *MDR1* gene. Polymerase chain reaction amplification and digestion with *Mbo* I are described in Materials and Methods. Lane 1 shows the digestion of the polymerase chain reaction product into two smaller fragments (49 and 144 bp), and the absence of the T-allele in this subject. Lane 2 shows incomplete digestion, indicating that this subject carries the T-allele. Lane 3 reveals no digestion, which indicates the subject is homozygous for the T-allele. M, Molecular weight marker (pUC18 in *Msp*I Digest); + and –, treatment with or without the restriction enzyme, respectively.

Determination of Serum Concentration of Digoxin

The serum concentration of digoxin was measured by fluorogenic enzyme immunoassay using an OPUS analyzer (Behring Diagnostics, Westwood, MA). The determination was validated routinely 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, the data of 0.18 ng/ml was adopted for a blood sample with 0.27 ng/ml, 0.27 ng/ml, and below LOQ.

Data Analysis

The area under the serum concentration-time curve of digoxin was calculated by the trapezoidal method from 0 to 4 h or to 24 h ($AUC_{0-4\ h}$ or $AUC_{0-24\ h}$). The serum concentration of digoxin was close to LOQ at 6 hr and after (see Fig. 3), and the values of $AUC_{0-24\ h}$ were calculated as reference data.

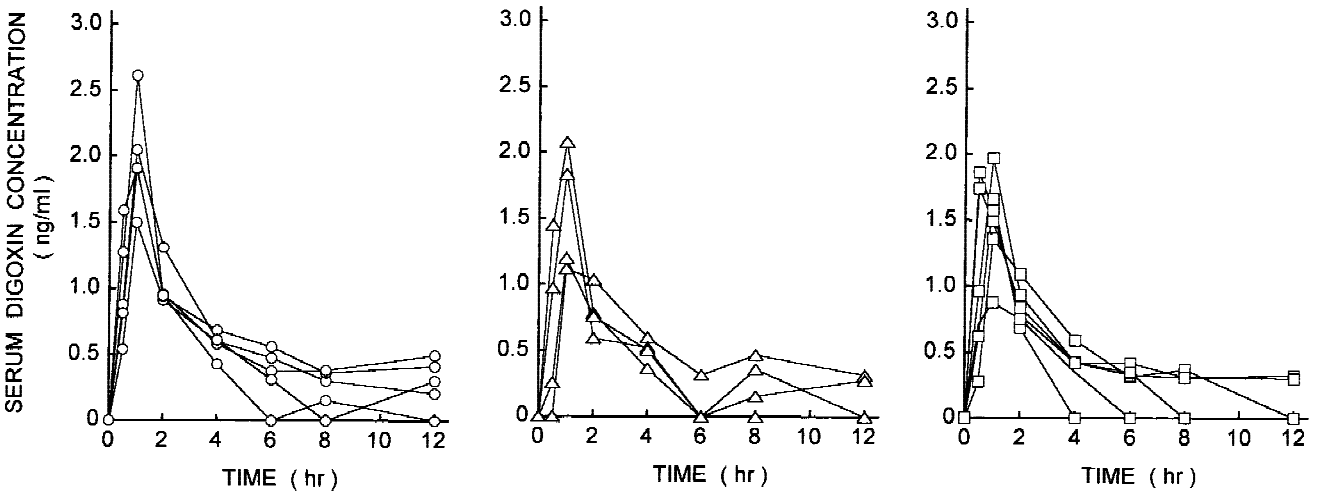

Fig. 3. Serum concentration-time profiles of digoxin for three genotype groups: left panel (○), C/C, $n = 5$; central panel (△), C/T, $n = 4$; right panel (□), T/T, $n = 6$ after a single oral administration of digoxin.

Statistical Analysis

The χ square test or Fisher's exact probability test was used for statistical analysis. Statistical significance was at $P < 0.05$.

RESULTS

MDR1 Genotype Distribution in Healthy Subjects

Figure 2 shows the identification of the *MDR1* genotype according to PCR-RFLP. Because the T-allele lacks an *Mbo* I site, the 193-bp PCR product is resistant to *Mbo* I digestion. Lane 1 shows the digestion of the PCR product into two smaller fragments (49 and 144 bp), and the absence of a T-allele in this subject. Lane 2 shows incomplete digestion, indicating that this subject is heterozygous for the T-allele. Lane 3 reveals no digestion, which indicates the subject is homozygous for the T-allele.Table I shows the *MDR1* genotypes. Of the 114 subjects, 35.1% (40/114) were homozygous for wild-type allele (C/C), 52.6% (60/114) were compound heterozygotes (C/T), and 12.3 % (14/114) were homozygous for the mutant allele (T/T). There was no effect of gender or age on the genotype distribution.

Serum Concentration-Time Profiles of Digoxin after Single Oral Administration

Figure 3 shows the serum concentration-time profiles of digoxin in 15 healthy subjects, harboring C/C, C/T, and T/T. Table II lists the demographic data and pharmacokinetic parameters. There was no statistical difference of demographic data among the subjects harboring C/C, C/T, and T/T. The serum concentration of digoxin after single oral administration increased rapidly and subsequently attained a steady-state in all subjects, though it was higher in the subjects with C/C than C/T or T/T. At 12 h after administration, the serum concentration of digoxin was below the limit of quantification for one subject (20 %) with C/C, two subjects (50 %) with C/T, and four subjects (67 %) with T/T. $AUC_{0-4\ h}$ values (±SD) were 4.11 ± 0.57, 3.20 ± 0.49, and 3.27 ± 0.58 ng h/ml, respectively, with a significant difference between C/C and C/T or T/T. $AUC_{0-24\ h}$ values were 8.84 ± 3.28, 5.80 ± 1.94, and 5.74 ± 3.04 ng h/ml, respectively.

Table I. *MDR1* Genotype at Exon 26 in Healthy Subjects

Genotype	C/C		C/T		T/T	
Male	20/65	(30.8%)	37/65	(56.9%)	8/65	(12.3%)
Female	20/49	(40.8%)	23/49	(46.9%)	6/49	(12.2%)
Total	40/114	(35.1%)	60/114	(52.6%)	14/114	(12.3%)

DISCUSSION

The major pathway for the elimination of digoxin from the human body is proximal tubular secretion and glomelular filtration, the contribution of metabolism being neglectable (16). Tubular secretion was found to be mediated via human MDR1 in LLC-GA5-COL150 cells, established by transfection of human *MDR1* cDNA into porcine kidney epithelial LLC-PK_1 cells (17,18). Thus, digoxin is a useful probe for examining alterations of MDR1 *in vivo* (11).

Human MDR1 expressed in the luminal membranes of

Table II. Demographic Data and Pharmacokinetic Parameters after Single Oral Administration of Digoxin in Healthy Subjects

Genotype N	C/C 5	C/T 4	T/T 6
Age (years)	28 ± 4	28 ± 4	30 ± 6
Height (cm)	166 ± 5	169 ± 6	172 ± 5
Weight (kg)	57.4 ± 7.6	60.3 ± 6.1	63.8 ± 8.4
BMI (kg/m^2)	20.7 ± 1.6	21.0 ± 0.7	21.5 ± 2.5
GOT (IU/ml)	21 ± 6	19 ± 7	20 ± 6
Serum creatinine (mg/dl)	0.92 ± 0.31	0.87 ± 0.34	0.78 ± 0.23
$C_{1\ h}$ (ng/ml)	2.00 ± 0.40	1.56 ± 0.47	1.49 ± 0.36
$C_{4\ h}$ (ng/ml)	0.58 ± 0.09	0.50 ± 0.10	0.37 ± 0.20
$C_{12\ h}$ (ng/ml)	0.28 ± 0.19	0.15 ± 0.17	0.11 ± 0.16
$AUC_{0-4\ h}$ (ng h/ml)	4.11 ± 0.57	3.20 ± 0.49*	3.27 ± 0.58*
AUC_{0-24h} (ng h/ml)	8.84 ± 3.28	5.80 ± 1.94	5.74 ± 3.04

The values are the mean ± standard deviation.
$C_{1\ h}$, $C_{4\ h}$, and $C_{12\ h}$ mean the serum concentration of digoxin at 1 h, 4 h, and 12 h after single oral administration, respectively.
* Statistically significant at $P < 0.05$ compared with C/C.

renal proximal tubules and the biliary canalicular membrane of hepatocytes transports the substrate energy-dependently into urine and bile, respectively (6–9). MDR1 in the capillary endothelial cells of the brain and testis, and in the epithelial cells of the small and large intestines, restricts the entry of the substrate (6–9). MDR1 in skeletal muscle tissues is understood to restrict the distribution of xenobiotics throughout the body (19). It is accepted that MDR1 acts as a molecular barrier to the exogeneous toxic substances (6–9).

Hoffmeyer *et al.* have reported that mutation (C3435T) at exon 26 of *MDR1* gene is associated with a lower level of MDR1 expression in the duodenum (11). This finding was obtained using the samples from the study at another institute in Germany (12). The MDR1 expression had been determined by quantitative immunohistochemistry and Western blots of biopsies and enterocyte preparation of duodenum (12). A substudy with smaller size of subjects had also been conducted to assess *in vivo* MDR1 activity with or without rifampin induction by measuring the plasma concentration after single oral administration of digoxin (12), and they also suggested that the plasma concentration of digoxin after rifampin induction was higher in subjects harboring the mutant T-allele (11). Unfortunately, the data without rifampin induction were not presented, and it is still unclear whether this T-allele-related elevation of plasma concentration is due to lower level of MDR1 expression before rifampin treatment or suppression of MDR1 induction by rifampin. In the report of Hoffmeyer *et al.* (11), to validate further the correlation of *MDR1* genotype with intestinal absorption of digoxin *in vivo,* additional volunteers of another clinical study were evaluated, in which plasma levels of digoxin after multiple oral application was addressed under no rifampin induction (13). They suggested that the maximum concentration after attaining the steady-state, Cmax,ss, was higher in the subjects of T/T than C/C (11). They explained this finding by lower level of MDR1 in the duodenum. However, the value of Cmax,ss was well-known to be defined by a complicated function of absorption, distribution, metabolism, and excretion, thus the correlation with Cmax,ss can not attribute only to lower level of MDR1 in the duodenum.

The simulative report by Hoffmeyer *et al.* (11) strongly has suggested the importance of *MDR1* genotyping. In the subjects with mutation (C3435T) at exon 26 of *MDR1* gene, renal or biliary secretion of digoxin would be slower and the elimination rate decreased, and this also could explain the higher concentration of digoxin. In contrast, the distribution of digoxin was enhanced in such subjects, that is, the concentration is not easily increased, since the total weight of the muscle tissues is about 50% of the body weight. The serum concentration of digoxin is a function of not only weaker restriction of absorption but also decreased secretion via renal and biliary canalicular route, and enhanced distribution. It is important to elucidate separately the effect of *MDR1* genotype on each process underlying the biological fate of drugs. In this study, the serum concentration-time profile was examined following single oral administration without any treatment including rifampin. It was found to be lower in subjects harboring the mutant T-allele. This could be explained by alteration of the distribution, that is, the enhanced rate of distribution, was more predominant for single administration of digoxin when compared with the increase in the absorption rate or decrease of biliary or renal secretion, however, further evaluation is necessary to ensure this speculation.

In summary, the distribution of the *MDR1* genotype at exon 26 was 35.1 % C/C, 52.6 % C/T, and 12.3 % T/T in healthy Japanese subjects. There was no effect of gender or age on the distribution. The serum concentration of digoxin after single oral administration was lower in the subjects harboring a mutant T-allele at exon 26 of the *MDR1* gene.

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