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Influence of *ABCB1* gene polymorphisms on the pharmacokinetics of azithromycin among healthy Chinese Han ethnic subjects

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Abstract:
The aim of this study was to evaluate the effects of *ABCB1* gene polymorphisms on azithromycin pharmacokinetics in Chinese Han ethnic subjects. In total, 20 healthy volunteers with various *ABCB1* genotypes (6 with 2677GG/3435CC, 8 with 2677GT/3435CT, 6 with 2677TT/3435TT) were enrolled. Each was given a single oral dose of 500 mg azithromycin. Plasma concentration was measured for up to 96 h by LC/MS/MS. As shown, C_{max} was significantly lower among individuals with 2677TT/3435TT genotype (468.0 ± 173.4 ng • h/ml) than those with 2677GG/3435CC (911.2 ± 396.4 ng • h/ml, p = 0.013). However, the t_{max} value was higher among subjects with 2677TT/3435TT (2.0 ± 0.5 h) than those with 2677GT/3435CT (1.6 ± 0.3 h) or 2677GG/3435CC (1.4 ± 0.4 h) genotypes (p = 0.068 and p = 0.026, respectively). Furthermore, the AUC_{last} tended to be higher among subjects with 2677GG/3435CC than those with 2677GT/3435CT or 2677TT/3435TT genotypes (5000.2 ± 1610.0 *vs*. 4558.0 ± 805.0 *vs*. 4131.0 ± 995.1 ng/ml). Our results showed for the first time that azithromycin pharmacokinetics may be influenced by particular polymorphisms of the *ABCB1* gene. Individualized dosage regimen design incorporating such information may improve the efficacy of the drug while reducing adverse reactions.

Keywords:
ABCB1, azithromycin, pharmacokinetics, single nucleotide polymorphism (SNP)

Introduction

Azithromycin is an azalide antibiotic, which belongs to a subclass of the macrolide family. Azithromycin is effective against respiratory and skin infections [2]. Since it has fewer adverse gastrointestinal effects than erythromycin, patient adherence to it is rising [9, 54]. In clinical use, azithromycin exhibits pharmacokinetic and pharmacodynamic variability between individuals [17]. Among the many factors potentially influencing the efficacy of azithromycin, genetic polymorphisms are thought to contribute substantially to variations in the drug's disposition between individuals [3, 40]. Therefore, the identification of genetic parameters predictive of optimal dosage is of great clinical interest.

Azithromycin is a substrate for P-glycoprotein (P-gp, a multi-drug resistance transporter), the product of the *ABCB1* (ATP-binding cassette B1) gene [35, 43]. P-gp was first identified for its overexpression in human tumor cells. It was subsequently discovered in various non-neoplastic human tissues, including the small and large intestinal epithelium, adrenal gland, placenta (trophoblasts), kidney (the brush border of the renal tubule), liver (the canalicular membrane of the hepatocyte), pancreas (pancreatic ductule cell), and capillary endothelial cells of brain and testes [7, 11, 16, 42, 47]. In such tissues, P-gp is located on the apical or luminal surface of the epithelial cells [7, 11, 12, 16, 37, 42, 47]. As a transporter, it plays a significant role in drug disposition, i.e., absorption, distribution, and excretion, and may also be involved in the secretion of steroids [28, 33, 39].

A number of single nucleotide polymorphisms (SNPs) have been identified in *ABCB1* gene between individuals and ethnic groups [34]. Three of the most frequently occurring SNPs within *ABCB1* include C1236T in exon 12, G2677T/A in exon 21 and C3435T in exon 26 [8]. Clinical studies have been conducted to investigate the association between such polymorphisms and the expression and function of P-gp as well as the pharmacokinetics of its substrates. However, discrepancies exist between the results [6, 19, 23, 25, 27, 29, 31, 38, 48, 49]. As genotypes comprising particular SNPs may be responsible for the alteration in the functions of P-gp [10, 25, 45], two particular SNPs, G2677T/A (in exon 21) and C3435T (in exon 26) are considered the main genetic factors in altered P-gp function. In this study, we attempted to assess the influence of such SNPs on the pharmacokinetics of azithromycin among Chinese Han ethnic subjects.

Materials and Methods

Subjects

Among 202 subjects who had been previously genotyped for *ABCB1* exons including polymorphisms G2677T/A and C3435T, 20 healthy males (Han ethnics) were enrolled. These included 6 individuals with 2677GG/3435CC, 6 with 2677TT/3435TT and 8 with 2677GT/3435CT genotypes. The study protocol was approved by the ethics committee of Shengjing hospital affiliated with China Medical University and performed according to the rules of Helsinki. Prior to drug administration, all participants had given written consent to participate in the study after having been informed about the nature and implications of the trial.

All subjects were nonsmokers and in good health as determined from medical history, physical examination, ECG evaluation and routine laboratory tests (blood chemistry, hematology and urine analysis). Participants were instructed not to take any prescription or nonprescription medication for 2 weeks prior to and throughout the duration of the study. Participants were also instructed to abstain from grapefruit, grapefruit juice, herbal dietary supplements, and caffeine-containing beverages including coffee and green tea for three days prior to and throughout the study. Only subjects fulfilling such criteria were included.

Determination of the *ABCB1* gene polymorphism

First, 2 ml of peripheral blood was obtained from each subject, and their DNA was extracted using an EZ-10 Spin Column Genomic DNA Miniprep Kit (Bio Basic Inc.). The genotype of C3435T was determined by polymerase chain reaction-restriction fragment length polymorphism analysis (RFLP-PCR) as described elsewhere [32] (Genbank accession numbers NC000007, NM000927). The G2677T/A genotype was determined by sequencing. The primers and conditions for PCR amplification are shown in Table 1.To verify the results of RFLP-PCR, samples for each genotype (homozygous wild-type, heterozygous, and homozygous polymorphism; total of 9 samples) were sequenced.

Azithromycin pharmacokinetic study

After overnight fasting for 10 h, all subject received a single oral dose of 500 mg of azithromycin with 240 ml of water. Standardized meals were served 4 and 10 h after dosing. Venous blood samples were collected at multiple timepoints beginning before drug administration (0 h) and continuing at timepoints 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 48.0, 72.0 and 96.0 h post dosing. All samples were collected into lithium heparin tubes and centrifuged im-

Tab. 1. Primers and conditions of *ABCB1* SNP for PCR amplification

Exon	Primer(5'–3')	Alleles and products	Annealing temperature	Digestive enzyme
21	F: AGT TTT CAG AAA ATA GAA GCA TGA GT R: GGG AGT AAC AAA ATA ACA CTG ATT AGA	G/A/T, 351 bp	51.0 C	–
26	F: TGT GCT GGT CCT GAA GTT R:TAG GCA GTG ACT CGA TGA A	C/T, 246 bp	52.0 C	Dpn

mediately. Separated plasma samples were stored at –20°C until analysis.

Plasma azithromycin concentrations were analyzed with the LC/MS/MS method as described elsewhere with slight modification. The assay was linear in the range of 10–1000 ng/ml for plasma; calibration curves yielded a correlation coefficient greater than 0.99. Criteria for validation were that intra- and inter-assay coefficient of variation were less than 7.58% and 8.96%, at a level of 800 μg/ml, respectively. Mean recovery of azithromycin from plasma was 68.6%, 67.2% and 67.3% at level of 30, 300 and 800 ml/L, respectively.

The peak plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) were estimated directly from the observed data. Plasma concentration-time curve from time 0–96 h (AUC_{last}) was calculated using the linear trapezoidal rule. The AUC from time 0 to infinity (AUC_{inf}) was calculated as $AUC_{inf} = AUC_{last} + C_t/Ke$, where C_t is the last plasma concentration measured and Ke was determined using linear regression analysis of the logarithm-linear part of the plasma concentration-time curve. The half-life ($t_{1/2}$) of azithromycin was calculated as $t_{1/2} = \ln 2/Ke$. Oral clearance (CL/F) of azithromycin was calculated as $CL/F = dose/AUC_{inf}$.

Statistical analysis

All data were expressed as the mean ± SD. Normally distributed data were analyzed with one-way ANOVA. The general linear model for univariate was used to test the interactions of the various pharmacokinetics parameters between different genotypic groups. Non-normally distributed data were analyzed with Mann-Whitney *U*-test (for two-group comparison) or Kruskal-Wallis *H*-test (for multi-group comparison). Statistical analysis was carried out using the SPSS package (version 11.0; SPSS Inc., Chicago, IL, USA); p value < 0.05 was considered to be statistically significant.

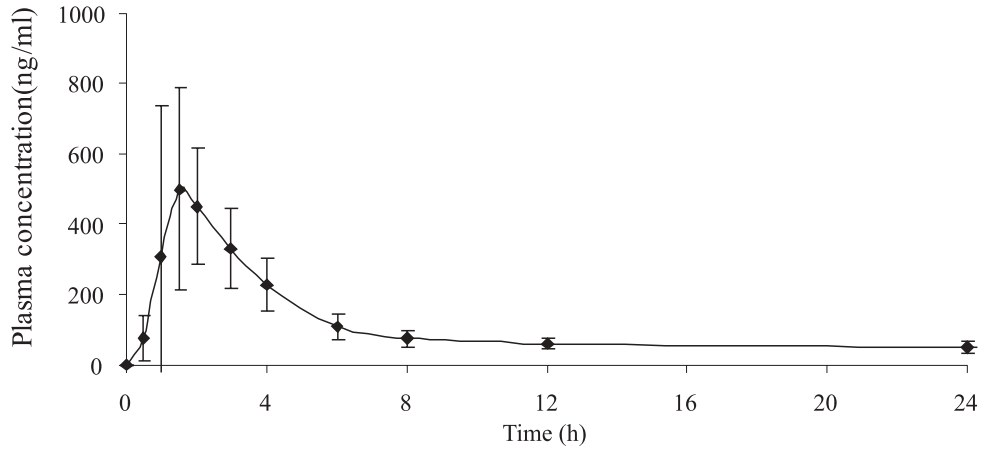

Fig. 1. Plasma concentration of azithromycin over time following oral administration of a 500-mg dose in 20 ethnic Han Chinese healthy subjects. Values are given as the mean ± SD

Results

All subjects completed the study without clinically significant adverse effects.

Pharmacokinetics of azithromycin

The average azithromycin plasma concentration *vs.* exposure time is shown in Figure 1, the typical value of C_{max} (668.4 ± 316.4 ng/ml), AUC (4563.6 ± 1141.5 ng • h/ml), t_{max} (1.7 ± 0.5 h), and $t_{1/2}$ (44.5 ± 14.4 h), while azithromycin disposition was highly variable in the cohort, ranging from 1223 to 7205 L/h. The above data were consistent with current product labeling for azithromycin, but much longer than for other macrolides [4, 17].

Genotype-phenotype correlations

The main pharmacokinetic parameters of azithromycin of different genotypic groups are summarized in Table 2, and the profiles of azithromycin *vs.* time in plasma in different genotypic groups are shown in Figure 2. A significant decrease in plasma C_{max} was observed between the 2677TT/3435TT and 2677GG /3435CC groups (468.0 ± 173.4 *vs*. 911.2 ± 396.4 ng/ml, p = 0.013), and between the 2677GT/3435CT and 2677GG/3435CC groups (636.6 ± 227.8 *vs*. 911.2 ± 396.4 ng/ml, p = 0.083). Average values of t_{max} were the highest in 2677TT/3435TT group and lowest in 2677GG/3435CC group (2.0 ± 0.5 vs. 1.4 ± 0.4 h, p = 0.026). In addition, the AUC_{last} of azithromycin also tended to be greater in the 2677GG/3435CC group than 2677GT/3435CT and 2677TT/3435TT groups (5000.2 ± 1610.0 *vs*. 4558.0 ± 805.0 *vs*. 4131.0 ± 995.1 ng • h/ml). This difference, however, was not statistically significant possibly due to interindividual variability as well as the small number of recruited subjects (p > 0.05) and may be dependent on variability of the *ABCB1* SNP. The Vd/F of azithromycin in plasma showed modest differences among the 2677TT/3435TT, 2677GT/3435CT and 2677GG/3435CC groups (3506.0 ± 1983.4 *vs*. 2662.5 ± 725.3 *vs*. 3255.5 ± 2126.8). No significant difference was found in regard to other pharmacokinetic parameters of azithromycin among the three genotypic groups.

Tab. 2. Pharmacokinetic profiles of azithromycin in different genotypic groups following administration of a single dose of the drug (500 mg)

Parameter	Genotype Group			Multiple Comparison		
	2677GG/3435CC Group (n = 6)	2677GT/3435CT Group (n = 8)	2677TT/3435TT Group (n = 6)	*vs.*	*vs.*	*vs.*
C_{max} (ng/ml)	911.2 ± 396.4	636.6 ± 227.8	468.0 ± 173.4	0.083	0.013	0.274
(95% CI)	(495.2, 1327.2)	(446.2, 827.1)	(286.1, 649.9)			
t_{max}	1.4 ± 0.4	1.6 ± 0.3	2.0 ± 0.5	0.524	0.026	0.068
(95% CI)	(1.0, 1.8)	(1.3, 1.8)	(1.4, 2.6)			
Half-life (h)	48.1 ± 20.3	41.5 ± 12.3	44.9 ± 11.8	0.421	0.712	0.678
(95% CI)	(26.9, 69.4)	(31.2, 51.8)	(32.5, 57.3)			
AUC_{last} (ng·h/ml)	5000.2 ± 1610.0	4558.0 ± 805.0	4131.0 ± 995.1	0.486	0.208	0.501
(95% CI)	(3310.5, 6689.8)	(3885.0, 5231.0)	(3086.7, 5175.3)			
AUC_{inf} (ng·h/ml)	5978.3 ± 1415.0	5379.4 ± 1130.8	4947.3 ± 939.6	0.358	0.146	0.504
(95% CI)	(4493.4, 7463.3)	(4434.0, 6324.8)	(3961.3, 5933.4)			
CL/F(L/h)	43.3 ± 12.7	45.1 ± 8.1	51.4 ± 15.3	0.781	0.256	0.344
(95% CI)	(30.0, 56.6)	(38.4, 51.9)	(35.4, 67.5)			
Vd/F (L)	3255.5 ± 2126.8	2622.5 ± 725.3	3506.0 ± 1983.4	0.513	0.795	0.356
(95% CI)	(1023.5, 5487.5)	(2056.2, 3268.9)	(1424.5, 5587.5)			

Data are shown as the mean ± SD. C_{max}: Peak plasma concentration; t_{max}: time to C_{max}; AUC_{last}: area under the time-concentration curve from 0 to 96 h; AUC_{inf}: area under the time-concentration curve from 0 to infinity; CL/F, oral clearance

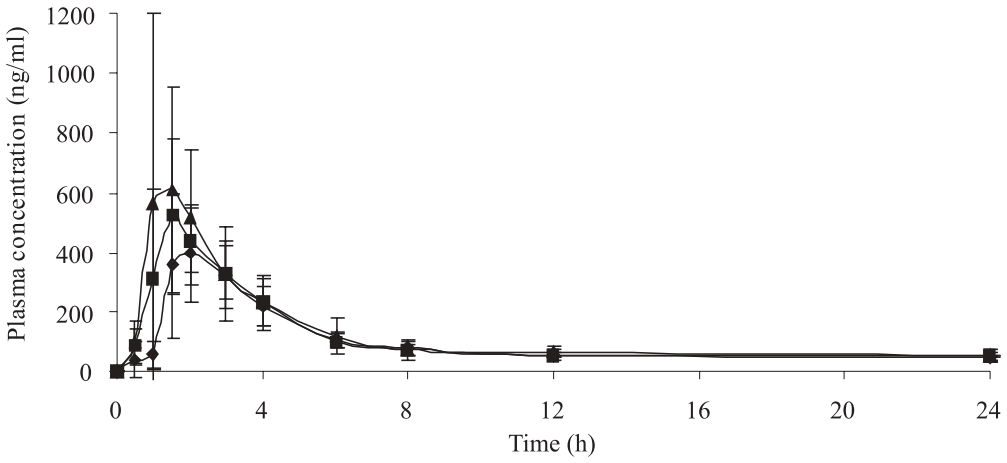

Fig. 2. SNP variability in plasma concentration of azithromycin over time following oral administration of a 500-mg dose. Values are given as the mean ± SD. 2677GG/3435CC (▲); 2677GT/3435CT(■); 2677TT/3435TT(◆)

Discussion

Inter-individual variability in drug efficacy and toxicity, which may result in unexpected drug responses, is common in clinical settings [13, 14]. This may be due to sequence variants in genes encoding metabolism enzymes, transporters and/or drug target proteins [15]. Some SNPs have been associated with an altered expression of P-gp in human tissues (e.g., intestinal epithelium and placental trophoblast) and pharmacokinetic profiles of certain drugs [4, 10, 29]. Among such, G2677T/A and C3435T have been most extensively studied in terms of gene expression and function. Results of such studies, however, have been inconsistent [6, 22, 24, 29, 31, 36, 46, 48]. As suggested by several recent studies, the primary determinant of functional differences in P-gp resides not in single SNP differences, but rather in particular genotypes of the *ABCB1* gene [30]. Given the known inter-populational differences in drug response for azithromycin, it may be important to consider variability among ethnic groups by characterizing variability in genotype structure, linkage disequilibrium and recombination within and between ethnic populations. As polymorphisms G2677T/A and C3435T are closely associated in the majority of subjects [25, 26], we have recruited subjects simultaneously harboring wild type, heterozygous or mutant types of above polymorphisms in this study.

The objective of this study was to evaluate whether genetic polymorphism(s) of the human *ABCB1* gene has a significant impact on the pharmacokinetics of azithromycin. The most important findings concluded that: 1) significant differences in C_{max} and t_{max} between subjects from different genotypic groups; 2) heterozygous carriers (2677GT/3435CT genotype) for the mutant alleles yielded intermediate values for certain pharmacokinetic parameters when compared with homozygous carriers (e.g., 2677GG/3435CC and 2677TT/3435TT genotypes). As suggested by our results, individuals carrying the wild type *ABCB1* gene (2677GG/3435CC) have an increased C_{max} of azithromycin when compared with heterozygotes (2677GT/3435CT) or mutant-types (2677TT/3435TT). Meanwhile, t_{max} values in such subjects were, respectively, 25.0% and 42.9% higher than those with 2677GT/3435CT or 2677GG/3435CC genotypes. Above findings indicate that genetic variants of the *ABCB1* gene are likely an important contributor for the interindividual variability in pharmacokinetics of azithromycin, and thereby pharmacodynamics of substrate drugs.

Consistent with our results, it has been reported that nelfinavir, a P-gp substrate, can increase the intestinal absorption of azithromycin by inhibiting P-gp in humans, and that P-gp inhibitors can increase cellular accumulation of azithromycin in J774 murine macrophages [3, 40].

Substrates of CYP3A4 and P-gp overlap substantially [20, 50]. Unlike erythromycin and clarithromycin, which are P-gp inhibitors, some studies have reported that the pharmacokinetics of azithromycin are not influenced by the CYP3A4 inhibitor, zafirlukast,

and that azithromycin has no effect on the pharmacokinetics of triazolam, a substrate for CYP3A4 [18, 21]. In human, azithromycin is mainly eliminated in unchanged form in the feces *via* biliary excretion and intestinal secretion, whereas urinary excretion is the minor elimination route [41]. Once absorbed, it becomes a weak substrate for phase I metabolism by the CYP3A system where it undergoes minimal metabolism and neither induces or inhibits the system [1, 5, 52, 53]. Therefore, the influence of CYP3A polymorphism was not considered in the present study.

Pharmacokinetic parameters also exhibit great variability between the 2677GG/3435CC, 2677GT/3435CT and 2677TT/3435TT groups (e.g., the values of C_{max} had ranged 552.0 to 1628.0 ng/ml, 285 to 976 ng/ml and 250.0 to 726.0 ng/ml, respectively). Further studies involving larger sample size and stratification based on haplotypes may be necessary for understanding the influence of *ABCB1* gene variants on the disposition, therapeutic response, and toxicity of azithromycin. To reduce the risk of spurious association between *ABCB1* genotypes and *in vivo* phenotypes, a subjects' SNPs as well as population sample size and environmental factors should also be taken into consideration. Notably, other factors not included in this analysis may also contribute to the substantial inter-individual variations as observed. Although most nucleotide variants, including the studied ones, are located within the coding regions of the *ABCB1*, variations in the promoter region may account for inter-individual differences in P-gp expression, enzymatic activity, as well as placental and hepatic mRNA levels [44]. Further studies taking into consideration such factors are required for a full understanding of the pharmacokinetic activity of azithromycin.

Taken together, we have for the first time demonstrated that polymorphisms of the *ABCB1* gene may have a considerable impact on the pharmacokinetics of azithromycin among healthy Chinese Han ethnic subjects. Genotyping may prove an essential tool for individualized treatment by optimizing the drug dosage for an individual's genetic variability.

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