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IMPACT OF CYP3A5 AND P-gp POLYMORPHISMS ON THE PHARMACOKINETIC INTER-INDIVIDUAL VARIABILITY OF A SINGLE-DOSE OF A QUETIAPINE IMMEDIATE-RELEASE TABLET: A RANDOMIZED, OPEN-LABEL, TWO-PERIOD CROSSOVER STUDY IN HEALTHY JORDANIAN VOLUNTEERS

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ABSTRACT

This study aimed to investigate the impact of the CYP3A5 and ATP-binding cassette sub-family B member 1 (ABCB1) C3435T polymorphisms on the inter-individual variability of quetiapine pharmacokinetics in a Jordanian population. Quetiapine plasma concentrations were measured in 34 healthy Jordanian Arabic volunteers. Twenty blood samples were collected over a 24-hour period following the administration of a 25 mg immediate release tablet. The pharmacokinetic parameters were determined from the plasma concentration-time profiles using the WinNonlin® software. The CYP3A5 and ABCB1 C3435T genotypes were determined by polymerase chain reaction. With regard to the CYP3A5 polymorphism, the *3*3 genotype carriers showed consistently higher exposure index values in comparison to the non-carriers, with differences ranging from 1.34- to 1.6-fold. While, the TT genotype subjects displayed a trend toward lower exposure and higher disposition (up to 2.2-fold) indexes when compared to the CC genotype carriers. These results may provide useful data that clinicians can utilize to optimize the quetiapine dose administered to psychotic patients.

Keywords: Quetiapine, antipsychotics, ABCB1, P-glycoprotein, CYP3A5, polymorphisms

INTRODUCTION

Quetiapine, an atypical antipsychotic drug, was recently approved by the U.S. Food and Drug Administration (FDA) for the treatment of schizophrenia and depressive episodes of bipolar disorder.[1-3] Quetiapine is available in tablet strengths ranging from 25 to 300 mg,[1] and data from routine therapeutic drug monitoring (TDM) have demonstrated extensive inter-individual variability in quetiapine pharmacokinetics.[4,5] In addition, the efficacy concentrations have been shown to vary between 0.02 and 0.3 mg/L.[6,7] To date, few studies with short-term follow-up periods have been published regarding the relationship between the dose and serum concentration of quetiapine and/or the relationship between the serum concentration and the treatment response (using standardized psychiatric rating scales),[6-8] and these studies have generated controversial results.[1,2] However, the contemporary consensus guidelines proposed by a universal pharmacopsychiatric group recommended TDM as a useful tool to optimize the clinical response in patients treated with low drug concentrations.[9] Moreover, in vitro experiments have indicated that quetiapine is extensively metabolized in the liver by the cytochrome P450 (CYP) system, primarily by CYP3A4.[10,11] CYP3A4 and CYP3A5 are the two primary human CYP3A isoforms, and CYP3A5 contributes to more than 50% of the total CYP3A liver content.[10] The large inter-individual variability in the pharmacokinetic behavior and clinical response of many CYP3A substrates has been primarily attributed to the impact of a genetic polymorphism in CYP3A5; however, these substrates display higher affinities for CYP3A4 when forming their major metabolites.[12,13] Interestingly, the role of CYP3A5

in the metabolism of quetiapine is poorly understood in vitro[11] and is virtually unknown in vivo. Like several other psychoactive drugs, quetiapine has been shown to be a substrate of p-glycoprotein (P-gp), an efflux pump encoded by the ATP-binding cassette sub-family B member 1 gene (ABCB1), in the intestine.[14] The following three single nucleotide polymorphisms (SNPs) have been primarily associated with P-gp activity and expression: C1236T, G2677TA, and C3435T.[15,16] ABCB1 is expressed in numerous organs, such as the brain, liver, and kidney,[17,18] which suggests that potential substrates may be transported out of these tissues via P-gp, thereby greatly affecting substrate absorption, distribution, and excretion.[19] Additionally, P-gp substrates are thought to act as competitive inhibitors of the pump itself (to variable extents and potencies),[20] and the modulation of P-gp function by P-gp inhibitors or inducers has been shown to be involved in clinically significant drug-drug interactions.[21] For example, when the management of tumor multidrug resistance was investigated, some of these substrates, such as cyclosporine A and verapamil, displayed a potent inhibitory effect on P-gp function.[22,23] In subsequent in vitro studies, the inhibitory effects of atypical antipsychotic agents (including quetiapine) on P-gp function were significant enough to induce pharmacokinetic interactions at the cellular level.[24,25] Moreover, these effects were deemed to be particularly valid for in vivo settings if these drugs were to accumulate at high concentrations in the gastrointestinal tract or at the blood-brain barrier.[25] Additionally, previous findings from a clinical pharmacogenetic study of the potent P-gp inhibitor cyclosporine revealed that potential increases in the extent of its bioavailability and distribution due to this inhibitory impact could be observed at equal degrees in ABCB1 expressors and non-expressors.[26] As a moderate inhibitor of P-gp in vitro,[25] quetiapine can be assumed to similarly inhibit its own bioavailability and distribution, and this assumption drove the initial objectives of the current study. In vitro studies and in vivo animal model studies recently demonstrated that P-gp located at the blood-brain barrier could influence antipsychotic drug concentrations in the brain.[27] Therefore, it was postulated that the pharmacological and adverse effects of these drugs may be specifically affected by the P-gp expression levels in individual patients. However, with the exception of risperidone, most psychotropic drugs have displayed only moderate increases in penetration into the brains of P-gp knock-out mice as compared to wild-type controls.[28] Most other studies have utilized various in vitro models and have obtained controversial results. For example, in a P-gp-mediated ATP-ase activity model, only quetiapine and risperidone were classified as good P-gp substrates and potent inhibitors, whereas other antipsychotics were identified as intermediate or poor P-gp substrates.[14] In another in vitro model, El Ela et al.[24] measured the P-gp-mediated efflux of 14 psychoactive drugs across a human cell monolayer and determined that quetiapine was a poor substrate. Therefore, it is noteworthy that the experimental conditions employed for in vitro studies may alter a drug's effects on the functional consequences of P-gp or CYP3A5. As conflicting results are likely to be obtained between in vitro acute drug administration studies and chronic dosing methods in real clinical practice, further clinical evidence regarding the impact of P-gp polymorphisms on the pharmacokinetics and pharmacodynamics of antipsychotics is required in humans. In addition, pharmacokinetic studies characterizing quetiapine behavior and its association with genetic polymorphisms in different ethnic groups remain limited. To our knowledge and with regard to quetiapine, only one recent pilot study, which involved 22 schizophrenic patients, has been conducted to evaluate its pharmacokinetic- and pharmacodynamic-pharmacogenetic relationship.[29] Although this previous study examined the impact of various CYP3A and ABCB1 SNPs, it only revealed associations between the ABCB1 SNPs (C3435T, G2677AT, and C1236T) and decreased quetiapine plasma concentrations and clinical response, which were able to explain 41% and 48% of the variability in these parameters, respectively. In contrast, three similar studies involving psychophrenic patients reported an association of some (but not all) ABCB1 SNPs with increased plasma concentrations and clinical treatment outcomes, but these studies evaluated other atypical antipsychotics such as risperidone (C1236T),[30] olanzapine (C3435T),[31] and bromperidol (C3435 and G2677AT).[32] In one study, higher plasma levels of risperidone were significantly associated with CYP3A5 non-expressors as compared to expressors.[33] Therefore, these studies reported inconsistent conclusions. However, one consistent finding among the four ABCB1 association studies[29-32] is that the presence of at least one of the variant T alleles in a patient may represent a significant predictive value of drug CNS concentrations and/or the subsequent antipsychotic response. However, all of these findings were considered to be preliminary due to small sample sizes, the open approach in which the medication was administered, and the retrospective nature of these clinical investigations. In the current study, we sought to investigate the impact of the CYP3A5 and ABCB1 C3435T polymorphisms on the inter-

individual variability of quetiapine pharmacokinetics in a healthy Jordanian volunteer population. As a secondary objective, we attempted to examine the impact of the quetiapine P-gp inhibitory effect on its oral bioavailability and systemic clearance.

MATERIALS AND METHODS

Study design: The volunteers included in this report were participants in an official bioequivalence study of two quetiapine products, QUETIAPINE 25 mg tablets (Pharma International Company, Jordan), as a test product, and SEROQUEL 25 mg tablets **(**AstraZeneca UK Limited, Macclesfield Cheshire SK10 2NA, United Kingdom), as a reference product. This study was an open-label, single-dose, randomized, two-treatment, two-period, two-sequence, fasting, crossover (with a washout period of 1 week between doses) trial conducted in healthy participants who were hospitalized in the PharmaquestJo, a Jordanian clinical research center, during the study. The study was conducted according to the International Conference on Harmonization/Good Clinical Practice standards and the Declaration of Helsinki. The protocol was approved by the Institutional Review Board/Independent Ethics Committee at PharmaquestJo.

Participant recruitment: Prior to enrollment and during the recruitment phase, approximately 60 potential participants went through the screening procedures, which included the collection of demographic and medical history information and the implementation of physical and laboratory examinations (including complete blood count, urine analysis, virology, endocrine disorders, liver function, alcohol consumption and drug abuse tests) and a 12-lead electrocardiogram (ECG).

Drug administration: The participants were asked to fast, starting at 21:00 or 11 hours prior to drug intake, and a standardized meal was provided at 4 and 10 hours after dosing. Until 3 hours before dosing (i.e., 5:00), no excessive fluid intake (>120 mL/hour) was allowed, and no fluid was allowed 1 hour before and 1 hour after drug administration. From approximately 8:00 to 8:20, the drug (test or reference) was administered to the participants in the fasting state with a standardized quantity of water (240 mL). Because the drug may cause hypotension and dizziness, the participants were not allowed to leave their beds for the first 4 hours after dosing.

A total of 34 male subjects were determined to be eligible for inclusion in the study because they met all of the following criteria: age of 18-50 years inclusive, body mass index of 19 to 30 kg/m^2 inclusive, systolic blood pressure (BP) equal or greater than 110 mmHg, diastolic BP equal or greater than 70 mmHg, heart rate (HR) within the normal range (60-100 beats/min), and Oral body temperature within the normal range (35.9-37.6 ºC). Participants who met any of the following criteria were excluded from the study: a history of hypersensitivity to quetiapine or a similar compound; smoking the equivalent of 10 or more cigarettes per day; the use of any known enzyme inducers or inhibitors (e.g., barbiturates, carbamazepine, phenytoin, and rifampin) within 30 days prior to study entry; any known condition that could interfere with the absorption, distribution, metabolism, or excretion of drugs; blood donation or participation in another clinical study within 60 days prior to the initiation of period I; or obvious signs of renal, gastrointestinal, cardiovascular, hepatic, respiratory, neurological, musculoskeletal, or endocrine disorders, as evidenced by physical examination and/or clinical laboratory tests.

Assessment of safety: Throughout the hospitalization periods (following dosing and at the end of the study or within 72 hours after the completion of period II), all of the participants passed a follow-up examination, which included a physical examination identical to the initial examination, vital sign monitoring, ECG examination, hematology, and clinical chemistry laboratory tests. During the hospitalization periods, sitting BP and HR were measured using a mercury sphygmomanometer prior to dosing (-2 hours) and at the following time points on both treatment days: 1, 2, 3, 4, 5, 6, 8, 12, 14, and 24 hours after dosing. Any changes in vital signs (BP, HR, or oral body temperature) outside the normal ranges were judged by the attending physician on a case-by-case basis in accordance with the baseline values and were assessed as clinically significant or non-significant. Normal saline was infused intravenously at any time after drug intake to any participants who become hypotensive. At scheduled intervals of 1, 2, 3, 4, 5, 6, 8, 12, 14, and 24 hours after dosing, the participants were queried regarding the presence of any adverse events, and any adverse events that were volunteered were reported. All of the adverse events and serious adverse events were followed up until an outcome was determined.

Special sampling techniques: Blood samples were collected before dosing and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 14, and 24 hours after dosing. All of the samples were collected via an indwelling catheter into labeled Heparin blood tubes (10.0 mL) and centrifuged

(4,000 rpm/4.00 min). The plasma samples were transferred with disposable polypropylene droppers into the labeled polypropylene tubes, and they were then capped and stored at -80 °C until analysis.

Quetiapine analytical method: Quetiapine concentrations determination in human plasma were performed on high performance liquid-chromatography system (1200, Agilent, USA). Detection was achieved on an API 5000 mass spectrometer (Sciex/Applied Biosystems, Canada) with turbo ion spray ionization (Sciex/Applied Biosystems, Canada). The method was validated for its application to the analysis of quetiapine in authentic plasma samples harvested after an oral dose of 25 mg tablets. The drug was eluted at a flow rate of 1000 µL/min by a mobile phase gradient consisting of 5% to 32% ammonium acetate (10 mM, pH 8.88) and acetonitrile (Merck, Germany) for a total run time of 4.0 minutes. Chromatographic separation was carried out on Merck C18 analytical column (Germany, 55 × 4.0 mm) with 3 µm particle size. Quetiapine-d8 (PQJ code As-105, Germany) was employed as the internal standard. Extraction was achieved by protein precipitation using acetonitrile. A lower limit of quantification of 1.000 ng/ml was achieved. The method was linear between 1.000 ng/ml and 120.000 ng/ml for quetiapine. The method was selective for both the drug and the internal standard with an absolute recovery of 92.67% for the drug and 72.06% for the internal standard. Accuracy and precision (intraday and interday) were assessed. QC precision range (%) was 0.42 - 8.09, QC accuracy range (%) was (-6.05) – (13.18). The analytical procedures and full validation were conducted and operated in accordance with written PharmaquestJo SOP's and FDA guidance. Blank samples of human plasma (the matrix) were obtained from Central Blood Bank in Amman-Jordan.

Genotyping methods

Genotyping of CYP3A5*3 and *1: Genomic DNA was extracted from 400 µL of whole blood using a phenol-chloroform kit (PIERCE, Rockford, IL, USA). Polymerase chain reaction (PCR) was performed in 20 µL of a solution containing 2 µL of 10x PCR Gold Buffer, 2 mM MgCL2, 80 µM each of dNTPs, 50 pmol each of primers, 50 ng of genomic DNA, and 0.6 U of AmpliTaq Gold (Applied Biosystems, Carlsbad, California, USA). The forward primer sequence was 5'-ATGGAGAGTGGCATAGGA-GATA-3', but a modified reverse primer (5'-TGTGGTCCAAACAGGGAAGAGAT-3') was used based on the reported sequence (GenBank accession number: AF355800). The PCR conditions consisted of 8 min at 94 °C; 40 cycles of 30 s at 94 °C, 30 s at 59 °C and 30 s at 72 °C; and a final extension for 10 min at 72 °C. The PCR product was detected on a 2% agarose gel using ethidium bromide staining.

Genotyping of MDR1 at exon 26: The genotyping of MDR1 at exon 26 for the C3435T SNP was performed using forward (5'-TGCTGGTCCTGAAGTTGATCTGTGAAC-3') and reverse (5'-ACATTAIGGCAGTGACTCGATGAAGGCA-3') primers with the Mbol endonuclease.

Pharmacokinetic analysis: Based on the measurements of quetiapine in the plasma, the pharmacokinetic parameters for the test and reference product were calculated using the Phoenix™ WinNonlin® 6.1 software program with the noncompartment model. The individual plasma concentration-time profiles for each volunteer and the mean values for each sampling time were drawn. The maximum plasma concentration (C_{max}) and the time of the peak concentration (t_{max}) were derived directly from the raw plasma concentration-time data. Based on the assumption that the terminal elimination phase is reached within the sampling period, the terminal half-life ($t_{1/2}$) values were estimated from the slope (terminal rate constant k_e) of the linear regression of the semi-logarithmic plot of the terminal phase of the plasma concentration curve ($t_{1/2} = \ln 2 / k_e$). The area under the plasma concentration-time curve (AUC_{last}) was estimated using the linear trapezoidal rule. The AUC_∞ was calculated as the sum of the estimated and extrapolated parts ($AUC_\infty = AUC_{last} + AUC_{last\to\infty}$). The extrapolation was performed by dividing the last measurable plasma concentration C_{last} by the terminal rate constant k_e ($AUC_{last\to\infty} = C_{last} / k_e$). The residual areas were determined as percentages using the following equation: $\{(AUC_\infty - AUC_{last}) / AUC_\infty\} * 100$. The mean residence time from zero to infinity ($MRT_{0\to\infty}$) was determined**.** The apparent oral clearance (CL/F) and volume of distribution (V_d/F) were evaluated as secondary parameters.

Statistical analysis: The arithmetic means, medians, minimum and maximum values, standard deviations, and coefficients of variations for the parameters were reported. When appropriate, the subject demographic data were examined for statistical significance using the Mann-Whitney *U*, Fisher exact or Chi-square test. ANOVA and post-hoc multiple comparisons with Tukey's and Bonferroni tests were employed to compare the mean values of the log-transformed pharmacokinetic parameters associated with various genotypes. All of the tests were performed using the SPSS software program (version 16, SPSS Inc.,

Chicago, IL), and a p value < 0.05 was considered statistically significant.

RESULTS

Study subjects: A total of 34 male subjects were included in the present study. These subjects demonstrated no clinically important findings regarding their medical history, physical examinations, ECG results, and laboratory test results. The subjects were between the ages of 19 and 46 years (28.3 ± 7.6), and their weights ranged from 57 to 94 kg (72.4 ± 10.7). Eleven subjects were non-smokers, and 23 were moderate smokers (smokers of less than 10 cigarettes per day). Table I lists the pharmacokinetic parameters of quetiapine following the administration of a single oral dose the 25 mg Quetiapine® or Seroquel® tablets to the 34 healthy volunteers. The observed genotype frequencies and distribution for CYP3A5 and ABCB1 in the Jordanian subjects were consistent with Hardy-Weinberg equilibrium (p > 0.1). Approximately 73.5% (n = 25) of the individuals had the homozygous *3/*3 variant genotype, 17.6% (n = 6) were heterozygous *1/*3, and 8.8% (n = 3) had the wild-type expressor genotype *1/*1. With regard to ABCB1 C3435T, the most common ABCB1 genotype (heterozygous CT) was observed in 50% (n = 17) of the subjects and was followed in frequency by the homozygous wild-type CC, which was observed in 32.4% (n = 11) of the subjects. The homozygous mutant genotype was identified in 17.6% (n = 6) of this population. The distribution of the baseline patient characteristics (Table II) did not differ significantly within the subcategories of the two polymorphisms. Interestingly, the majority of the ABCB1 3435CT patients were also of the CYP3A5 *3/*3 genotype (Table III), and this result may be attributed to random chance because the majority of the individuals in this population were CYP3A5 non-expressors. All of the volunteers completed the study. During both the study period and the post-study follow-up visits, none of the subjects experienced any serious or unexpected adverse events. There were no clinically significant findings upon physical examination or changes in the laboratory tests during the follow-up assessments.

The impact of the CYP3A5 and ABCB1 genotypes on quetiapine pharmacokinetics: The quetiapine noncompartmental pharmacokinetic parameters of the reference product (SEROQUEL 25 mg Tablets) stratified according to the CYP3A5 and ABCB1 genotypes are presented in Table IV and Table V, respectively. As shown in Table IV, the CYP3A5 *3*3 genotype carriers showed consistently higher values for AUC_{last} (1.6-fold), AUC_{∞} (1.5-fold), residual area (1.3-fold), $MRT_{0\rightarrow\infty}$ (1.14-fold), and C_{max} (1.34-fold) as compared to the non-carriers. However, comparison of these absorption pharmacokinetic parameters across the different CYP3A5 genotypes in the ANOVA did not reveal any statistically significant differences (p < 0.05). The T_{max} of the CYP3A5 homozygous expressors (*1*1) was significantly longer (1.9-fold) than that of the non-expressors (*3*3) (p = 0.017). Additionally, the elimination parameters (K_e, CL/F) were (1.5- to 2.4-fold) greater whereas the elimination $t_{1/2}$ value was (1.4- to 1.7-fold) smaller among expressors as compared to the other two genotypes, which indicated that faster elimination occurred in this group. However, this effect was not statistically significant. With regard to the ABCB1 impact on the absorption parameters, the AUC_{last}, AUC_{∞}, C_{max}, and C_{max}/AUC_{∞} values were consistently higher in subjects with the CC genotype as compared to the CT and TT genotypes, although the differences were not statistically significant (Table V). Surprisingly, the elimination parameters (K_e and CL/F) were greater whereas the elimination $t_{1/2}$ value was smaller in the TT group as compared to the other two genotypes, which suggested that more rapid elimination occurred in this group. Nevertheless, statistical analysis revealed no significant differences between the different ABCB1 genotypes. Moreover, the results of the SNP associations with various pharmacokinetic parameters were consistent for the test (QUETIAPINE®) and the reference (SEROQUEL®) quetiapine products (the data shown in Table IV and Table V are those for the reference product).

The impact of genotype combinations on quetiapine pharmacokinetics: Following the combination of both the CYP3A5 and MDR1 C3435T genotypes, only seven of the nine possible genotype combinations were identified in our study population (A-G; Table III). Similar to the previously observed results regarding individual genotype effects, none of the SNP combinations significantly influenced any of the quetiapine pharmacokinetic parameters (data not shown). These findings suggest that no potential exists for any additive effect of any of the CYP3A5 SNPs upon concurrent combination with any of the ABCB1 SNPs. However, further examination of the impact of these genotype combinations should be investigated in other populations because the numbers of subjects in each subgroup in our study were relatively small and unbalanced.

DISCUSSION

Quetiapine is an atypical antipsychotic drug that was approved by the FDA for the management of acute

and chronic psychotic disorders and the acute phase of mania.[1-3] However, few published studies have explored the factors contributing to its wide interindividual and interethnic pharmacokinetic variability in humans.[29] Furthermore, reports of pharmacogenetic population models are virtually non-existent. Collectively, previous in-vitro investigations have suggested that CYP3A and P-gp may participate in various components of drug absorption, metabolism, distribution (particularly over the brain), and excretion.[11,14,20,24,25,27,28] In the present study, we sought to explore the associations between these genetic polymorphisms and various individual pharmacokinetic parameters and exposure indices of quetiapine in healthy Jordanian volunteers. Consistent with previous single-dose, two-period, crossover bioequivalence studies of quetiapine generic formulations,[34,35] the two quetiapine oral products used in this study were bioequivalent with respect to the rate and extent of absorption. This conclusion was made because the 90% confidence interval of the ratio of the natural logarithmically transformed test/reference ratios for the AUC_{last}, AUC_{∞}, and C_{max} were within the acceptance interval (according to FDA guidelines) of 80-125%.[36] Consistent with previous studies in psychiatric[4-8] and healthy subjects,[34,35] the interindividual variability in the pharmacokinetic parameters (C_{max}, T_{max}, $t_{1/2,}$ K_e, CL/F, and V_d) were high (Table I), indicating a potential influence for individual variables such as genetic composition on absorption and metabolism. However, because this was a single-dose study in healthy volunteers, all of the individuals tolerated both quetiapine products and reported no adverse events other than those known and expected with this antipsychotic drug (transient hypotension and sedation). The frequency of the variant allele (non-expressor *3/*3 genotype) in the current Jordanian population (73.5%) was similar to those previously reported for Asian, Indian, and Central American populations (approximately 50% to 80%).[37,38] However, in the Caucasian population, the variant allele frequency was approximately 90%.[39] Therefore, the quetiapine dosage requirement may differ significantly between various ethnic groups and between countries. In the present study, the CYP3A5 *3*3 genotype carriers showed consistently higher values for exposure indices, which varied between 1.34- and 1.6-fold, as compared to the non-carriers. In addition, the elimination parameters ($t_{1/2}$, K_e, and CL/F) indicated slower elimination in this carrier group as compared to the other two genotypes. However, the statistical analysis did not reveal any significant differences in these parameters. These results are consistent with those of a previous clinical pilot study in schizophrenic patients, which demonstrated that four CYP3 isoforms (CYP3A5, CYP3A4, CYP3A7, and CYP2D6) did not influence quetiapine plasma and cerebrospinal fluid concentrations.[29] One possible explanation for the observed insignificant impact of the CYP polymorphism in both of these studies could be attributed to the small sample size. Additionally, the acute, single-dose effect employed in our study in healthy volunteers may be quite different from the effects of chronic administration in real patients. In addition, the previous clinical findings[29] were only based on the analysis of quetiapine peak concentrations after 4 weeks of treatment rather than on the full pharmacokinetic profile, which is known to be more conclusive regarding exposure. In addition, one previous in vitro study suggested the absence of any relationship between the CYP3A5 SNPs and quetiapine pharmacokinetics as a consequence of the minor role of CYP3A5, as compared to CYP3A4, in quetiapine metabolism.[11] Another previous study indicated that the difference in the quetiapine metabolic pattern for CYPA3A5 as compared to CYP3A4, which resulted in a higher proportion of the active metabolite, likely affected the clinical outcome observed in CYP3A5 expressors.[40] This hypothesis was confirmed for risperidone when schizophrenic patients were genotyped to assess the influence of CYP2D6 and CYP3A5 on the steady-state plasma levels of risperidone, 9-hydroxyrisperidone, and the active moiety.[33] Although a significant impact of both enzymes on the parent drug and metabolite concentrations was observed, only CYP3A5 was shown to influence the total active moiety levels. Therefore, further quetiapine studies are required to confirm these conclusions. With regard to the ABCB1 C3435T polymorphism, the distributions of the wild-type CC, heterozygote CT, and homozygous TT genotypes in this Jordanian sample (32.4, 50, and 17.6%, respectively) were similar to those previously reported in Caucasians (35.8, 42%, and 22.2%, respectively)[41] but different from those of Chinese (25, 43.8, and 31.3%, respectively) and Indian populations (18.4, 36.8, and 44.8%, respectively).[42] Previous functional studies have demonstrated that the existence of the 3435 T allele in the ABCB1 cDNA SNP can reduce the expression level of P-gp in the intestinal mucosa, placenta, and kidney.[15-18] Therefore, it is plausible that TT genotype carriers may exhibit increased exposure to P-gp substrates due to the higher intestinal absorption mediated by the lower efflux of the P-gp pump. Surprisingly, although consistent with a previous clinical study,[29] the TT genotype subjects in the present study displayed lower AUC_{last} (1.2-fold), AUC_{∞} (1.2-fold), C_{max} (1.3-fold), and C_{max}/AUC_{∞} (1.2-fold) values as

compared to the CC genotype carriers, although these differences were not statistically significant. Moreover, the elimination parameters of the TT carriers suggested a trend toward the more rapid disposition of quetiapine (2.2-fold higher CL/F) in this group as compared to the other genotypes. This observation may be explained by the more extensive distribution of a P-gp substrate in this genotype to other body cells containing similar P-gp expression, leading to a lower plasma concentration. In the current study, this result was clearly observed because more trends toward higher quetiapine V_d values were observed in the TT genotype carriers as compared to individuals with other genotypes (Table V). Interestingly, a previous clinical analysis[29] reported that CC genotype schizophrenic patients who had achieved higher quetiapine plasma and CSF peak concentrations showed significant improvement based on their clinical scores, whereas all TT genotype carriers were found to be non-responders. However, previous results regarding other atypical antipsychotic drugs have been contradictory. One study evaluating bromperidol suggested that although no difference in the total improvement scores between the C3435T genotypes were observed, the patients with a TT genotype generally demonstrated poor cognitive improvement, possibly due to the negative impact of the higher drug concentrations in the brain.[32] In contrast, another study of similar patients who received olanzapine reported a significant positive relationship between the olanzapine plasma levels and the reduction of schizophrenic symptoms in subjects with a T allele at the C3435T polymorphism as compared to subjects in the CC genotype group.[31] Although preliminary, the authors[31] attributed their findings to the reduced efflux of olanzapine by the mutant T allele, leading to improved efficacy associated with higher CSF levels in the former group. Finally, a study of risperidone implied that ABCB1 polymorphisms in general do not largely affect the treatment response or the incidence or severity of side effects during short-term therapy in patients with schizophrenia.[43] Therefore, these clinical findings must be confirmed in future studies involving Jordanian patients. It is important to note that P-gp substrates have been found to act as competitive inhibitors of the pump itself, thereby potentially reducing the bioavailability of these drugs; however, this inhibition was shown to occur at variable extents and potencies.[20-24] In addition, a previous in vitro study specifically demonstrated that all antipsychotic drugs display various degrees of inhibitory effects on P-gp activity, with quetiapine considered a good P-gp substrate and a potent inhibitor.[25] To our knowledge, this was the first bioequivalence study to examine the influence of ABCB1 SNPs on the bioavailability of a P-gp substrate with a potential inhibitory effect on P-gp activity (such as quetiapine) on its own bioavailability. We clearly demonstrated that subjects with a natural distribution of the CYP3A5 and ABCB1 genotypes did not exhibit altered levels of bioequivalence according to FDA criteria. In fact, the current observation of the nonsignificant difference in quetiapine pharmacokinetics among different ABCB1 genotypes may be attributed to the ability of quetiapine to inhibit its P-gp-mediated absorption transport in wild-type expressors, leading to a modified bioavailability similar to that obtained by non-expressors. However, this inhibitory potential of P-gp-mediated efflux of quetiapine has been described in experimental studies[24,25] at higher concentrations of quetiapine than those normally achieved in this study as well as in the clinical setting. The present study had several noteworthy limitations. In vitro, quetiapine has been found to be metabolized by CYP3A into numerous metabolites, including norquetiapine, which has been proposed as the primary mediator of its anti-depressive effect.[11,44] In this study, because this active metabolite was not measured, conclusions regarding the relative impact of the CYP3A5 polymorphism on its pharmacokinetic variability and bioequivalence could not be drawn. However, a previous retrospective analysis of routine TDM data from psychiatric patients revealed that the impact of CYP3A4 inducers was greater for quetiapine than norquetiapine.[5] In addition, we did not genotype individuals for other ABCB1 SNPs (C1236T and G2677TA), which may have enabled a more comprehensive appraisal of the function of the ABCB1 gene because these SNPs have been previously proposed to be associated with P-gp expression or function.[15,16,26] In fact, the insignificant association of ABCB1 C3435T with quetiapine pharmacokinetics observed in this study may be partially explained by the absence of linkage disequilibrium with other functional polymorphisms. Finally, the impact of additional nongenetic factors related to ethnic origin, which cannot be prohibited, was not examined in this study.

CONCLUSIONS

In summary, the impact of the CYP3A5 and ABCB1 polymorphisms on interindividual variability of quetiapine was not statistically confirmed in the present study. However, the positive trends of increased exposure and slower elimination in the CYP3A5 *1*1 and ABCB1 CC carriers, which were consistent with previous in vitro experiments and one clinical study, warrant further verification in future

large-scale, in vivo metabolic evaluations. These types of studies are potentially useful for providing clinicians with additional data for optimizing the quetiapine dose administered to psychotic patients.

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Table 1: Pharmacokinetic parameters of quetiapine following a single oral dose of 25 mg tablets of Quetiapine® or Seroquel® in 34 healthy volunteers

Parameters	**Quetiapine®**			**Seroquel®**		
	Mean	SD	CV%	Mean	SD	CV%
$AUC_{last\ (ng\ h^{-1}\ ml^{-1})}$	280.2	1.23	0.44	264.7	1.19	0.45
$AUC_{\infty\ (ng\ h^{-1}\ ml^{-1})}$	296.2	1.32	0.45	276.6	1.23	0.45
Residual area (%)	5.26	3.1	58	4.56	2.25	49.35
$MRT_{0\rightarrow\infty}$ (h)	5.68	1.6	28.4	5.54	1.47	26.5
$C_{max\ (ng\ ml^{-1})}$	68.3	24.8	36.4	65.4	25	38.3
$C_{max}/\ AUC_{\infty\ (h^{-1})}$	0.24	0.06	24.5	0.248	0.073	29.2
$t_{1/2}$ (h)	4.1	1.58	38.6	3.85	1.29	33.6
	Median	Range	CV%	Median	Range	CV%
t_{max} (h)	0.88	0.5-4	62.3	1	0.25-3.5	48.9
K_e (h^{-1})	0.2	0.098-0.35	37	0.21	0.09-0.35	30.5
CL/F ($l\ h^{-1}$)	3.2	0.36-38.7	127.4	4.1	0.53-29.1	101.6
V_d (l)	17.3	3.6-109	91.8	18.2	4.76-82.4	73.2

CV: coefficient of variation; AUC: area under the serum concentration curve; C_{max}: maximum serum concentration; T_{max}: time of the peak concentration; $MRT_{0\rightarrow\infty}$: mean residence time from zero to infinity.

Table 2: Demographic data of the participants (n = 34) stratified according to the CYP3A5 and ABCB1 genotypes

Demographic Criterion	**Genotype Identification**					
	CYP3A5 genotypes			**ABCB1 genotypes (3435C>T)**		
	*1/*1	*1/*3	*3/*3	C/C	C/T	T/T
Patient No. (%)	3 (8.8)	6 (17.6)	25 (73.5)	11 (32.4)	17 (50)	6 (17.6)
Age (years)	26.7 ± 6.03	31 ± 7.7	27.6 ± 7.74	30.64 ± 7.5	27.8 ± 8.3	25.2 ± 4.8
Weight (kg)	69 ± 10.15	72 ± 6.1	72.8 ± 11.8	72.9 ± 8.93	71.8 ± 12.2	72.8 ± 10.7
Height (cm)	1.8 ± 7.5	1.72 ± 4.9	1.73 ± 7.34	1.76 ± 8.3	1.73 ± 6.8	1.7 ± 5.3
Body mass index (kg/m^2)	21.3 ± 1.53	24.2 ± 2.8	24.24 ± 2.9	23.64 ± 2	23.94 ± 3.4	24.7 ± 3
Smoker (%)	3 (8.8)	4 (11.8)	16 (47.1)	9 (26.5)	11 (32.4)	3 (8.8)

Values are presented as the mean ± SD.

Table 3: Frequencies of CYP3A5 and ABCB1 genotype combinations in our study population

		Gene Polymorphism	
Group	**No. of Patients**	**CYP3A5**	**ABCB1 C3435T**
A	1	*1*1	CC
B	2	*1*1	CT
C	1	*1*3	CC
D	5	*1*3	CT
E	6	*3*3	TT
F	10	*3*3	CT
G	9	*3*3	CC

Table 4: Impact of CYP3A5 genotypes on the quetiapine pharmacokinetic parameters after a single oral dose of 25 mg in healthy volunteers

Parameter	CYP3A5 genotype			*p*
	*1/*1 (3)	*1/*3 (6)	*3/*3 (25)	
$AUC_{last\ (ng\ h^{-1}\ ml^{-1})}$	170.6 ± 49.4	247.1 ± 1.1	280.16 ± 1.3	0.311
$AUC_{\infty\ (ng\ h^{-1}\ ml^{-1})}$	176.67 ± 49.7	260.47 ± 1.1	292.46 ± 1.3	0.297
Residual area (%)	3.6 ± 1.012	5.42 ± 3.2	4.5 ± 2.1	0.497
$MRT_{0\rightarrow\infty}$ (h)	4.7 ± 0.68	6.4 ± 2.6	5.4 ± 1.1	0.182
$C_{max\ (ng\ ml^{-1})}$	51.29 ± 32.1	57.95 ± 23.4	68.83 ± 24.7	0.388
$C_{max}/\ AUC_{\infty\ (h^{-1})}$	0.276 ± 0.12	0.235 ± 0.1	0.248 ± 0.06	0.74
t_{max} (h)	2 ± 1.4†	1.3 ± 0.5	1.05 ± 0.36	0.017
K_e (h^{-1})	2.5 ± 0.008	1.69 ± 0.065	2 ± 0.06	0.171
$t_{1/2}$ (h)	2.8 ± 0.09	4.7 ± 1.9	3.8 ± 1.1	0.092
CL/F (l h^{-1})	9.24 ± 2.4	3.85 ± 3.75	5.56 ± 6.2	0.418
V_d (l)	37.34 ± 11.2	19.3 ± 12.9	23.4 ± 18.6	0.341

*Data are presented as the mean ± SD. ANOVA was used to compare differences in the pharmacokinetic parameters between different groups. AUC: area under the serum concentration curve; C_{max}: maximum serum concentration; T_{max}: time of the peak concentration; $MRT_{0\rightarrow\infty}$: mean residence time from zero to infinity; †, statistically significant compared to *3/*3 ($p < 0.05$).*

Table 5: The impact of ABCB1 genotypes on the quetiapine pharmacokinetic parameters after a single oral dose of 25 mg in healthy volunteers

Parameter	ABCB1 genotypes (3435C>T)			*p*
	C/C (11)	C/T (17)	T/T (6)	
$AUC_{last\ (ng\ h^{-1}\ ml^{-1})}$	297.1 ± 1.59	251.7 ± 99.6	242.1 ± 94.7	0.558
$AUC_{\infty\ (ng\ h^{-1}\ ml^{-1})}$	307.3 ± 1.65	264.91 ± 1.02	253.4 ± 98.4	0.61
Residual area (%)	3.55 ± 1.6	5.23 ± 2.6	4.5 ± 1.58,	0.158
$MRT_{0\rightarrow\infty}$ (h)	5.2 ± 1.32	5.87 ± 1.65	5.19 ± 1.17	0.452
$C_{max\ (ng\ ml^{-1})}$	74.3 ± 26.2	61.895 ± 23.9	58.82 ± 25.77	0.354
$C_{max}/\ AUC_{\infty\ (h^{-1})}$	0.267 ± 0.084	0.243 ± 0.078	0.227 ± 0.028	0.518
t_{max} (h)	1.2 ± 0.82	1.14 ± 0.49	1.21 ± 0.25	0.959
K_e (h^{-1})	0.197 ± 0.056	0.186 ± 0.05	0.239 ± 0.084	0.179
$t_{1/2}$ (h)	3.8 ± 1.2	4.1 ± 1.35	3.27 ± 1.3	0.446
CL/F (l h^{-1})	4.67 ± 1.41	3.64 ± 0.88	10.3 ± 4.2	0.218
V_d (l)	23.4 ± 17.8	21.5 ± 13.3	31.58 ± 26.9	0.49

Data are presented as the mean ± SD. ANOVA was used to compare differences in the pharmacokinetic parameters between different groups. AUC: area under the serum concentration curve; C_{max}: maximum serum concentration; T_{max}: time of the peak concentration; MRT_{∞}; mean residence time from zero to infinity.

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