Effects of cytochrome P450 3A modulators ketoconazole and carbamazepine on quetiapine pharmacokinetics

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Aims

To explore the potential for drug interactions on quetiapine pharmacokinetics using *in vitro* and *in vivo* assessments.

Methods

The CYP enzymes responsible for quetiapine metabolite formation were assessed using recombinant expressed CYPs and CYP-selective inhibitors. P-glycoprotein (Pgp) transport was tested in MDCK cells expressing the human MDR1 gene. The effects of CYP3A4 inhibition were evaluated clinically in 12 healthy volunteers that received 25 mg quetiapine before and after 4 days of treatment with ketoconazole 200 mg daily. To assess CYP3A4 induction *in vivo*, 18 patients with psychiatric disorders were titrated to steady-state quetiapine levels (300 mg twice daily), then titrated to 600 mg daily carbamazepine for 2 weeks.

Results

CYP3A4 was found to be responsible for formation of quetiapine sulfoxide and N- and O-desalkylquetiapine and not a Pgp substrate. In the clinical studies, ketoconazole increased mean quetiapine plasma $C_{\max}$ by 3.35-fold, from 45 to 150 ng ml⁻¹ (mean $C_{\max}$ ratio 90% CI 2.51, 4.47) and decreased its clearance (Cl/F) by 84%, from 138 to 22 l h⁻¹ (mean ratio 90% CI 0.13, 0.20). Carbamazepine decreased quetiapine plasma $C_{\max}$ by 80%, from 1042 to 205 ng ml⁻¹ (mean $C_{\max}$ ratio 90% CI 0.14, 0.28) and increased its clearance 7.5-fold, from 65 to 483 l h⁻¹ (mean ratio 90% CI 6.04, 9.28).

Conclusions

Cytochrome P450 3A4 is a primary enzyme responsible for the metabolic clearance of quetiapine. Quetiapine pharmacokinetics were affected by concomitant administration of ketoconazole and carbamazepine, and therefore other drugs and ingested natural products that strongly modulate the activity or expression of CYP3A4 would be predicted to change exposure to quetiapine.

Introduction

Quetiapine fumarate, a dibenzothiazepine psychotropic, is extensively metabolized *in vivo* via sulfoxidation, considered the major metabolic pathway, as well as oxidation to carboxylic acid, hydroxylation, and dealkylation (Figure 1) [1–3]. Quetiapine is often used in

combination with other drugs; thus, an understanding of its potential for clinically significant drug–drug interactions is essential to successful therapy [4–7].

A combination of commonly used *in vitro* approaches, including metabolism by recombinant human cytochrome P450 (CYP) and enzyme selective

Figure 1

Quetiapine and its principal metabolites in human liver microsomes. A carboxylic acid metabolite found *in vivo* was not detected in the microsomal incubates

inhibitors in human liver microsomes [8, 9], enabled identification of the CYP enzymes that catalyse the formation of the primary circulating metabolites of quetiapine. Based on these *in vitro* results, we assessed the effects of ketoconazole, a strong CYP3A4 inhibitor, and carbamazepine, a strong CYP3A4 inducer, on the pharmacokinetics of quetiapine in healthy men and psychiatric patients, respectively.

Methods

In vitro studies of quetiapine metabolism

Materials Unlabelled and ^{14}C -labelled quetiapine (specific activity 52.1 Ci mg^{-1}), all unlabelled quetiapine metabolites, dehydronifedipine and dextrorphan were synthesized by Zeneca Pharmaceuticals (now AstraZeneca Pharmaceuticals LP, Macclesfield, UK, and Wilmington, DE, USA). Phenacetin, acetaminophen, ketoconazole and nifedipine used *in vitro* were reference standards obtained from the US Pharmacopeial Convention, Inc. (Rockville, MD, USA). S-mephenytoin, 4-hydroxymephenytoin, hydroxytolbutamide, sulfaphenazole and furafylline were obtained from Ultrafine Ltd (Manchester, UK). Diethyldithiocarbamate (DDC) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). Tolbutamide, chlorpropamide, quinidine, nicotinamide adenine dinucleotide phosphate (NADPH) and all other reagents

were purchased from Sigma Chemical Co. (St Louis, MO, USA) or other standard sources. Fresh or snap-frozen human liver tissues were obtained from the International Institute for the Advancement of Medicine (Jessup, PA, USA).

Liver microsomes were prepared by three-step differential centrifugation, as described previously [10], and stored at -70°C . Microsomal protein content was assayed using bicinchoninic acid reagent (Pierce Chemical Co., Rockford, IL, USA) with bovine serum albumin as the protein standard. Microsomes were pooled from several individual donors by combining an equivalent amount of microsomal protein from each sample. The complementary deoxyribonucleic acid-derived expressed human CYP isoforms were obtained from Gentest Corporation (Woburn, MA, USA).

Identification and kinetics of quetiapine metabolites formed by human liver microsomes

For *in vitro* identification of quetiapine metabolites, human liver microsomes ($1 \text{ mg protein ml}^{-1}$) were incubated for 60 min at 37°C with $50 \mu\text{M}$ ^{14}C -quetiapine in 2.0 ml of assay buffer [50 mM N-[2-hydroxyethyl]piperazine N-[2-ethanesulphonic acid] (HEPES), pH 7.6, containing 5 mM MgCl_2 and 1 mM NADPH].

Parent compound and metabolites were extracted with ethyl acetate after making the incubation mixture basic with NH_4OH . The organic layer was isolated and

evaporated under nitrogen. The extracted metabolites were redissolved in the high-pressure liquid chromatography (HPLC) mobile phase (see below) and subjected to liquid chromatography with mass spectrometric detection. All *in vitro* extracts (50 µl) were separated using a Zorbax SB-C8 4.6 × 25 mm column and a pre-column with the same packing. The HPLC mobile phase consisted of 0.1% aqueous trifluoroacetic acid (adjusted to pH 3.0 with NH₄OH) and 100% acetonitrile, with gradient elution between 80 : 20 (v/v) and 65 : 35 (v/v) at 1.5 ml min⁻¹ over 30 min. Authentic metabolite standards were analysed under the same conditions.

The kinetics of quetiapine metabolite formation were similarly evaluated. Duplicate samples of pooled microsomes (1 mg protein ml⁻¹) were incubated for 20 min at 37 °C with ¹⁴C-quetiapine (5–100 µM) in 0.25 ml of the same assay buffer. After incubation, the reaction was terminated by precipitation of the microsomal protein by addition of acetonitrile. Quetiapine metabolites formed in the mixture were separated by gradient reverse-phase HPLC (described above) and monitored using both solid-phase radiochemical and ultraviolet-photodiode array detection. Peak areas of each metabolite in the chromatograms were plotted against the initial concentration of quetiapine in the incubations. Enzyme kinetic parameters for formation of each quetiapine metabolite were calculated by using nonlinear regression (PCNonlin; SCI Software, Lexington, KY, USA).

Effect of specific CYP inhibitors on quetiapine metabolism in human liver microsomes

Quetiapine (15 µM) was coincubated with selective CYP inhibitors at 37 °C with human liver microsomes (1 mg protein ml⁻¹) in assay buffer as described. A concentration of 15 µM of quetiapine was used in these experiments because it was well below the apparent *K_m* values for metabolite formation in human liver microsomes but allowed for analytical detection of the metabolites formed, even though this concentration is approximately sevenfold greater than the steady-state plasma maximal drug concentration (*C_{max}*) following a clinically used 300-mg twice-daily dose [11].

The CYP inhibitors included furafylline, sulfaphenazole, quinidine, DDC and ketoconazole, which selectively inhibit CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A4, respectively. The amount of quetiapine metabolites formed in the presence of these specific inhibitors was compared with a control sample containing only quetiapine, microsomes, other reaction cofactors, and solvent vehicle assay buffer (no inhibitor).

Quetiapine metabolism by heterologously expressed human CYP enzymes

Quetiapine (15 µM) was incubated for 1 h at 37 °C in assay buffer (as described above) containing microsomal fractions isolated from human lymphoblastoid cell lines expressing CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. Exogenous CYP reductase (0.5 U ml⁻¹) was added to incubations containing CYP1A2 and CYP2C19 because reductase was not coexpressed with CYP in these cell lines. Control samples were prepared by coincubating quetiapine with vector-transfected microsomal fractions lacking expressed CYP protein.

Transport of quetiapine across MDR-1-MDCK cell monolayers

Madin-Darby canine kidney cells transfected with human multidrug resistance gene (MDR-1-MDCK cells) were obtained from the Netherlands Cancer Institute (Amsterdam, the Netherlands) and cultured in DMEM supplemented with 10% fetal bovine serum. Directional [basolateral to apical (B–A) and apical to basolateral (A–B)] assays were conducted 3 days after seeding MDR-1-MDCK cells onto polycarbonate Transwell membranes at a density of 1.5 × 10⁶ cm⁻². Transport assays were conducted with 1 µM quetiapine or the known P-glycoprotein (Pgp) substrate loperamide at 37 °C for 60 min. After incubation, samples from both the donor and receiver chambers were analysed for quetiapine or loperamide concentration using LC/MS/MS.

Clinical studies of the effects of ketoconazole and carbamazepine on quetiapine pharmacokinetics

Two clinical studies were conducted to assess the effects of coadministration of drugs that strongly induce or inhibit CYP3A4 on quetiapine pharmacokinetics. In study 1, the effects of the CYP3A4 inhibitor ketoconazole were examined in healthy volunteers. In study 2, the effects of the CYP3A4 inducer carbamazepine were examined in patients. The patients were diagnosed by their treating physician based on Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text Revision (DSM-IV-TR) criteria [12]. In both studies, pharmacokinetic parameters obtained when quetiapine was used alone were compared with those obtained after coadministration with ketoconazole or carbamazepine.

Study participants

Healthy male volunteers aged 24–42 years were enrolled in study 1. Exclusion criteria included a positive test for hepatitis B surface antigen or human immunodeficiency virus (HIV) antibody; abnormalities in

baseline laboratory values or electrocardiographic findings; presence of an acute nonpsychiatric illness within 2 weeks before enrolment; and use of drugs that affect the CYP enzyme system within 6 weeks before study initiation. Study participants were asked to limit their caffeine intake and refrain from making major changes in their dietary habits throughout the study. Use of prescription and nonprescription medications was prohibited unless deemed appropriate by the investigator.

Patients in study 2 were men and women aged 29–63 years; met DSM-IV-TR criteria [12] for schizophrenia, schizoaffective disorder, or bipolar disorder; and were in remission from an acute exacerbation of their disorder for at least 3 months. All patients had been treated with antipsychotic medications during the year before enrolment and were in remission without psychotic symptoms at time of enrolment into the study. Some subjects had adverse events on their previous medications, and because of the lower incidence of dystonic movements on quetiapine, were considered to be eligible and good candidates to be switched to quetiapine. The subjects were withdrawn from any previous medication and started on quetiapine within 4 days of their last dose of previous antischizophrenic treatment, and were titrated to a high dose of quetiapine (300 mg twice per day). The high dose ensured that even with the enzyme induction secondary to carbamazepine, the subjects would be receiving effective exposures to quetiapine. The subjects all were inpatients during the study and were closely observed so that if there was any sign of relapse of the acute psychotic state, the carbamazepine would be terminated and the subject aggressively treated. During the study none of the subjects had an acute relapse of their psychosis (see clinical effect in the Results section).

Patients taking lithium for schizoaffective disorder or bipolar disorder were allowed to continue doing so if their dose had been stable for at least 1 month before enrollment. All other antipsychotic, psychotropic or mood-stabilizing medications except lithium were discontinued at enrolment. Only oral chloral hydrate and benztropine mesylate were permitted to treat agitation, insomnia or extrapyramidal symptoms. Acetaminophen was the only analgesic allowed throughout the study. Women of childbearing age were allowed to participate only if they were not pregnant and were using a reliable nonhormonal method of contraception. Exclusions included a DSM-IV-TR Axis I disorder other than schizophrenia, schizoaffective disorder, or bipolar disorder; a positive test for hepatitis B surface antigen or HIV antibody; presence of an acute nonpsychiatric illness during the 2 weeks before study entry; use of cloz-

apine within 2 months of enrolment; or use of CYP inducers or inhibitors within 6 weeks of enrolment.

The study in healthy volunteers was conducted at Christiana Care Research Institute in Newark, Delaware and was approved by the Institutional Review Board of Christiana Care Health Services. The patient study was conducted at two sites: Cincinnati VA Hospital and BHC Alhambra Hospital, Rosemead, California. The University of Cincinnati Medical Center Institutional Review Board and the Western Institutional Review Board approved the study, and the study protocol adhered to the ethical guidelines of the Declaration of Helsinki. Each subject gave informed consent. At the time of enrollment into the study, all of the patients were in remission, without active psychotic behaviour and were judged by the investigator to be capable of giving informed consent.

Study design

Study 1 was an 8-day, open-label, crossover trial in volunteers who resided at the clinical research centre during the study. After an 8-h fast, study participants were given a single oral dose of quetiapine (25 mg) at 08.00 h on days 1 and 6. Single oral doses of ketoconazole (200 mg day⁻¹) were administered at 06.00 h from day 3 through day 6. Ketoconazole was taken at least 1 h before or 2 h after meals (quetiapine was administered after fasting), with a 2-h interval between the doses of ketoconazole and quetiapine on day 6.

Study 2 was a 36-day, open-label, multicentre, multiple-dose, pharmacokinetic study. Quetiapine was initiated at 25 mg twice daily on day 1 and increased to 300 mg twice daily by day 5. Patients remained on this dose through day 33 and then discontinued treatment after a final 300-mg dose given on the morning of day 34. Carbamazepine was initiated with a 200-mg dose on the evening of day 9, continued at 200 mg twice daily on days 10 through 12, and increased to 200 mg three times daily on days 13 through 33, ending after a final 200-mg dose on the morning of day 34. To attain a reliable determination of steady-state trough plasma concentrations of quetiapine both before and after the addition of carbamazepine, efforts were made to maintain a precise 12-h interval between the morning and evening doses of quetiapine on days 7 through 9 and days 32–33.

Pharmacokinetic sampling

In study 1, blood samples were obtained on days 1 and 6 at baseline and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 5, 8, 12, 16, 20, 24 and 30 h after quetiapine administration to measure concentrations of quetiapine and its sulfoxide metabolite.

In study 2, blood samples were obtained for measurement of quetiapine exposure on days 9 and 34 at 15 min before and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 h after the morning dose of quetiapine. Additional blood samples were taken 15 min before carbamazepine administration on the evening of day 9 and the morning of day 34.

In both studies, blood samples were collected into heparinized Vacutainer® tubes (BD, Franklin Lakes, NJ, USA). The blood was centrifuged within 15–30 min after collection, and the resulting plasma samples were placed into polypropylene tubes and frozen at -20°C until analysed using HPLC with atmospheric pressure chemical ionization and tandem mass spectrometry.

Plasma samples were analysed for concentrations of quetiapine and its major metabolite, quetiapine sulfoxide, using a validated procedure (KeyStone Analytical Laboratories, Inc., North Wales, PA, USA). These analytes were extracted from alkalized plasma with ethyl acetate and evaporated, and the dried residues reconstituted in 50 : 50 methanol:acetonitrile. Chromatographic separation was carried out on a reverse-phase liquid chromatography system utilizing a 3.5- μm Zorbax™ SB-phenyl (4.6×75 mm) column, with a mobile phase composed of 0.088% ammonium formate (pH 3.0), methanol and acetonitrile at a flow rate of 1.5 ml min^{-1} . Detection was achieved on a PE Sciex API 300 tandem mass spectrometer with turbo ionspray ionization. The parent/daughter ions monitored were m/z 384.2/253.0 (quetiapine) and m/z 400.1/221.1 (sulfoxide metabolite). The method has a quantification range of 2.50–500 ng ml^{-1} with an applicable range to 5000 ng ml^{-1} by sample dilution with plasma.

Pharmacokinetic variables

In study 1, primary pharmacokinetic variables included the area under the plasma concentration–time curve from baseline to t hours after dosing (AUC_{0-t}), the total area under the plasma concentration–time curve from time 0 to infinity (AUC), and C_{max} . The terminal half-life ($t_{1/2}$) and apparent oral clearance (CL/F) of quetiapine were evaluated as secondary pharmacokinetic parameters. The pharmacokinetic profile of the sulfoxide metabolite of quetiapine was also examined.

In study 2, all primary pharmacokinetic parameters were assessed at steady state (ss), confirmed by analysis of minimum plasma concentrations (C_{min}). Parameters included $C_{\text{max-ss}}$ and $\text{AUC}\tau\text{-ss}$, where τ is the dosing interval. Secondary pharmacokinetic parameters included time to reach $C_{\text{max-ss}}$ ($t_{\text{max-ss}}$), $C_{\text{min-ss}}$ and CL/F.

In both studies, all pharmacokinetic parameters were determined using a noncompartmental model.

Statistical analyses

In study 1, AUC_{0-t} , AUC, C_{max} and CL/F were logarithmically transformed before analysis of variance (ANOVA). The 90% confidence intervals of the geometric mean ratio for day 6 to day 1 for these parameters were constructed using Schuirmann's two one-sided tests procedure. The apparent $t_{1/2}$ was analysed in a similar fashion but not log transformed. Descriptive statistics were given for all analyses of ketoconazole.

In study 2, the logarithmically transformed values of $\text{AUC}\tau\text{-ss}$, $C_{\text{max-ss}}$ and CL/F and the rank transformed values of $t_{\text{max-ss}}$ on day 9 (quetiapine alone) and day 34 (quetiapine plus carbamazepine) were analysed using a two-way ANOVA. The ANOVA results were then used to construct 90% confidence intervals for the geometric mean ratios of $\text{AUC}\tau\text{-ss}$, $C_{\text{max-ss}}$ and CL/F. The interaction of quetiapine and carbamazepine was assessed using the two one-sided tests procedure. If the 90% confidence interval for a given geometric mean ratio was between 0.8 and 1.25 (indicating a change of less than 20% between day 9 and day 34), no statistically significant interactions were recorded. To ensure achievement of steady state, a two-way ANOVA was used to compare C_{min} values for quetiapine and its metabolites on days 8 and 9 with values on days 33 and 34.

Results

In vitro studies

Quetiapine metabolites formed by human liver microsomes Four primary metabolites of quetiapine oxidation – quetiapine sulfoxide, 7-hydroxyquetiapine, and the N- and O-dealkylated products – were formed after quetiapine incubation with human liver microsomes (Figure 2). Structures of metabolites were verified by using mass spectrometry and by their retention times on HPLC in comparison with authentic metabolite standards. The apparent K_m values for the microsomal formation of quetiapine sulfoxide and the 7-hydroxy, N-desalkyl and O-desalkyl metabolites were estimated at 110, 160, 100, and 170 μM (no assessment of nonspecific microsomal binding as a correction factor), respectively, although the maximum velocity of metabolite formation (V_{max}) was not achieved for the four reactions at quetiapine concentrations up to 100 μM (the maximum concentration tested).

Inhibition of quetiapine metabolism by specific CYP isoenzyme inhibitors Decreases in quetiapine metabolite formation were observed after coincubation with CYP2C9, CYP2D6 and CYP3A4 inhibitors (Table 1). Ketoconazole, a CYP3A4 inhibitor, decreased the microsomal formation of the sulfoxide metabolite in a

Table 1

Effect of specific CYP inhibitors on quetiapine metabolism in human liver microsomes

Inhibitor	CYP specificity	C (μM)	7-hydroxy	Metabolite formation (% control activity)		
				Sulfoxide	N-dealkyl	O-dealkyl
Furafylline	CYP1A2	1	103	97	93	95
		5	87	97	96	98
		25	111	90	93	88
Sulfaphenazole	CYP2C9	5	80	86	89	86
		25	76	68	71	94
		100	41	19	28	46
Quinidine	CYP2D6	0.2	74	88	93	87
		1	42	79	84	86
		5	44	82	77	82
DDC	CYP2E1	5	90	85	87	95
		25	78	76	84	84
		100	82	80	88	105
Ketoconazole	CYP3A4	0.02	90	40	72	81
		0.1	107	18	49	65
		1	91	2	10	28

C, Concentration; CYP, cytochrome P450; DDC, diethyldithiocarbamate. Values represent percentage of metabolite formed during inhibitor incubations vs. control incubations with no inhibitor present, averages of duplicate determinations.

concentration-dependent manner, with greater than 50% inhibition achieved at 0.02 μM ketoconazole. Ketoconazole also decreased the formation of N- and O-desalkyl metabolites in a concentration-dependent manner, whereas the 7-hydroxylation pathway was not changed by more than 10% in a concentration-independent fashion. The substantial inhibition of sulfoxidation and O- and N-dealkylation pathways by ketoconazole indicated that CYP3A4 enzymes are primarily responsible for three of the four metabolic pathways for quetiapine measured in human liver microsomes.

Quinidine, a potent inhibitor of CYP2D6, had no effect on the formation of the sulfoxide or dealkylated metabolites of quetiapine. However, 0.2–5 μM quinidine decreased formation of 7-hydroxyquetiapine by more than 50%, suggesting that CYP2D6 may be involved in 7-hydroxyquetiapine formation [8].

Sulfaphenazole, a selective CYP2C9 inhibitor, reduced formation of all four quetiapine metabolites when incubated in human liver microsomes at a high concentration (100 μM). However, at 5.0 μM , a concentration that substantially decreases the metabolism of known CYP2C9 substrates [8], sulfaphenazole had little

effect on quetiapine metabolism. Microsomal incubation with furafylline (up to 25 μM) and DDC (up to 100 μM), inhibitors of CYP1A2 and CYP2E1, had little effect on quetiapine metabolism.

Quetiapine metabolism by heterologously expressed human CYP isoenzymes Quetiapine metabolites were not detected after 1-h incubations of quetiapine with microsomes from vector-control lymphoblastoid cell lines or those that expressed CYP1A2, CYP2C9, CYP2C19 or CYP2E1. In contrast, metabolite profiles produced when quetiapine was incubated in human liver microsomes (Figure 2A) were similar to those produced by expressed CYP3A4 (Figure 2B). Quetiapine sulfoxide was the major metabolite formed during incubations with expressed CYP3A4. The O- and N-desalkyl metabolites and detectable amounts of the 7-hydroxy metabolite also were formed by expressed CYP3A4. Small amounts of a secondary O-desalkylsulfoxide metabolite were identified in both human liver microsomes and expressed CYP3A4. These findings clearly implicate CYP3A4 as the major CYP involved in quetiapine metabolism.

Figure 2

(A) Chromatographic profile of quetiapine metabolites formed during incubation with pooled human liver microsomes. (B) Chromatographic profile of quetiapine metabolites formed during incubation with recombinant expressed CYP3A4. AU, Absorbance units

Table 2

Transport of quetiapine (1 μM) across MDR-1-MDCK cell monolayers

	$P_{\text{app A} \rightarrow \text{B}}$ ($10^{-6} \text{ cm s}^{-1}$)	$P_{\text{app B} \rightarrow \text{A}}$ ($10^{-6} \text{ cm s}^{-1}$)	Flux ratio
Quetiapine	47.4 ± 1.3	56.2 ± 4.7	1.2
Loperamide	2.13 ± 0.3	48.9 ± 3.7	23.0

A $\rightarrow$ B, Apical to basolateral; B $\rightarrow$ A, basolateral to apical; MDR-1-MDCK, Madin-Darby canine kidney cells transfected with human multidrug resistance gene; P_{app} , apparent permeability. Values are the mean standard deviation of triplicates in a single experiment.

Expressed CYP2D6 formed detectable amounts of 7-hydroxyquetiapine but no other quetiapine metabolites. This result further corroborated the inhibition of this pathway in liver microsomes by the CYP2D6 inhibitor quinidine and confirmed that CYP2D6 is at least partially responsible for quetiapine 7-hydroxylation.

Table 3

Demographics and baseline characteristics

Characteristic	Ketoconazole study ($n = 12$)	Carbamazepine study ($n = 18$)
Age, years		
Mean (range)	33 (24–42)	44 (29–63)
Sex, n		
Men	12	15
Women	0	3
Race/ethnicity, n		
White	3	7
Black	9	8
Hispanic	0	3
Mental status, n		
Healthy volunteers	12	0
Patients with underlying psychotic disorder	0	18

Transport of quetiapine across MDR-1-MDCK cell monolayers The results of the experiments on transport of quetiapine across MDR-1-MDCK cell monolayers expressing human Pgp are shown in Table 2. Quetiapine was highly permeable in MDR-1-MDCK cell monolayers when incubated in either the apical-to-basolateral or basolateral-to-apical direction. There was little difference in flux in either direction (flux ratio = 1.2), indicating that quetiapine is not an efflux substrate of Pgp.

Clinical studies

Demographics In study 1, 12 healthy men (mean age 33 years) were enrolled. All study participants completed the trial and were included in the pharmacokinetic analysis. In study 2, 18 patients (men and women; mean age 44 years) were enrolled. Patients had diagnoses of paranoid schizophrenia ($n = 4$) or schizoaffective ($n = 6$) or bipolar disorder ($n = 8$). Fourteen patients had complete pharmacokinetic data. Baseline demographics for both studies are summarized in Table 3.

Pharmacokinetic evaluations In study 1, concomitant use of ketoconazole resulted in substantial increases in plasma concentrations of quetiapine (Figure 3A). The mean C_{max} and AUC of quetiapine were increased by 235% and 522%, respectively. Conversely, the geometric mean AUC and C_{max} of the sulfoxide metabolite were decreased by 46% and 87%, respectively (Figure 3B). Mean CL/F of quetiapine was decreased by 84%, and mean $t_{1/2}$ was increased from 2.61 to 6.76 h. Data for all pharmacokinetic variables are summarized in Table 4.

Table 4

Geometric means of the pharmacokinetic parameters for a single dose of quetiapine administered alone and concurrently with ketoconazole

Parameter	Quetiapine 25 mg (day 1)	Quetiapine 25 mg + ketoconazole 200 mg (day 6)	Ratio of means (day 6/day 1)	90% CI
AUC (ng h ⁻¹ ml ⁻¹)	181	1123	6.22	4.93, 7.83
AUC _(0-t) (ng h ⁻¹ ml ⁻¹)	165	1074	6.49	5.07, 8.31
C _{max} (ng ml ⁻¹)	45	150	3.35	2.51, 4.47
t _{max} (h)*	1.25	1.25	—	—
CL/F (l h ⁻¹)	138	22	0.16	0.13, 0.20
t _{1/2} (h)†	2.61	6.76	2.59	—

AUC, Area under the plasma concentration–time curve from time 0 to infinity; AUC_{0-t}, area under the plasma concentration–time curve from baseline to *t* hours after dosing; CL/F, apparent oral clearance; C_{max}, maximal drug concentration; t_{1/2}, terminal half-life. *Median. †Arithmetic mean.

Figure 3

(A) Mean ± SE plasma concentrations of quetiapine in the absence and presence of ketoconazole (days 1 and 6). (B) Mean ± SE plasma concentrations of quetiapine sulfoxide metabolite in the absence and presence of ketoconazole (days 1 and 6). SE, Standard error. Day 1 (quetiapine alone) (●); Day 6 (quetiapine + ketoconazole) (○)

Table 5

Geometric means of the pharmacokinetic parameters for quetiapine used alone and in combination with carbamazepine*

Parameter	Quetiapine 300 mg b.i.d. (day 9)	Quetiapine 300 mg b.i.d. + carbamazepine 200 mg b.i.d. (day 34)	Ratio of means	90% CI
AUC τ -ss (ng h ⁻¹ ml ⁻¹)	4650	621	0.13	0.11, 0.17
C _{max} -ss (ng ml ⁻¹)	1042	205	0.20	0.14, 0.28
t _{max} (h)†	1.5	1.3	—	—
CL/F (l h ⁻¹)	65	483	7.49	6.04, 9.28

AUC τ -ss, Area under the concentration–time curve between τ (dosing interval) and steady-state (ss); b.i.d., twice daily; CL/F, apparent oral clearance; C_{max}-ss, maximum concentration at ss; t_{max}, time to C_{max}. *Data are from one 12-h period following the morning dose. †Median.

Figure 4

Mean $\pm$ SE plasma concentrations of quetiapine in the absence and presence of carbamazepine (days 9 and 34). SE, Standard error. Day 9 (quetiapine alone) (●); Day 34 (quetiapine + carbamazepine) (○)

In study 2, concomitant use of carbamazepine resulted in substantial decreases in plasma concentrations of quetiapine (Figure 4). The geometric mean AUC τ -ss and C_{max}-ss of quetiapine were decreased by 87% and 80%, respectively. Geometric mean CL/F of quetiapine was increased approximately sevenfold. Although the median t_{max}-ss of quetiapine was slightly decreased in the presence of carbamazepine, this difference was not considered statistically significant. Data for all pharmacokinetic variables are summarized in Table 5.

Clinical effect In study 1, evaluation of effect was not applicable because the participants were healthy men. In study 2, in order to ensure that the patients did not relapse into an acute psychotic reaction, in addition to clinical observations, serial recordings of scores on the Brief Psychiatric Rating Scale (BPRS) and the Clinical Global Impressions (CGI) scale were determined. Because of the small number of patients and the lack

of a comparative group, only descriptive statistics were determined and the effect of carbamazepine on clinical efficacy could not be judged. The mean total BPRS score improved by 6 points from baseline (before treatment) to discharge (mean baseline score 15.0; mean discharge score 9.1). No change was seen in the CGI-Severity of Illness score (mean baseline score 3.89; mean discharge score 3.69) and/or the CGI-Improvement score (mean discharge score 3.31).

Adverse events that were spontaneously reported by patients to staff members were recorded on the case report forms and analysed; all reported events were consistent with the known safety profile of quetiapine and were mild or moderate in intensity; none was serious. The most common adverse events were dizziness and somnolence (reported by five patients each). Other adverse events were asthenia, hypotension, nausea and rash.

Discussion

Four quetiapine metabolites were formed by pooled human liver microsomes: the 7-hydroxyl, sulfoxide and N- and O-desalkyl metabolites. *In vivo* studies, reviewed by DeVane and Nemeroff [13], have identified at least 10 measurable metabolites of quetiapine, although many of these are secondary oxidative or conjugated metabolites of the primary metabolite species seen in the *in vitro* microsomal experiments.

Taken together, our *in vitro* results with specific CYP inhibitors and the heterologously expressed CYP forms demonstrated that CYP3A4 is a major enzyme responsible for quetiapine metabolism, and that 7-hydroxylation, a minor metabolic pathway *in vivo*, is also catalysed by CYP2D6. Other CYP enzymes probably do not contribute substantially to quetiapine clearance. However, many substrates for CYP3A4 are also CYP3A5 substrates [14]. CYP3A5 may metabolize quetiapine similarly to CYP3A4, although we did not specifically evaluate this or other minor CYPs in this study.

Based on the moderate inhibition by sulfaphenazole at relatively high concentrations, a contribution to all quetiapine metabolic pathways by CYP2C9 cannot be ruled out. However, no inhibition of quetiapine metabolism was observed when coincubated in human liver microsomes at concentrations that substantially decrease the metabolism of known CYP2C9 substrates [8], and incubations with recombinant CYP2C9 did not form detectable metabolites.

Our *in vitro* findings suggested that concurrent administration of quetiapine with drugs that induce or inhibit CYP3A4-mediated metabolism would be more likely to alter quetiapine pharmacokinetics than drugs affecting other CYP enzymes. Therefore, the two clinical studies were undertaken to determine if the pharmacokinetics of quetiapine would be altered by concomitant administration of strong CYP3A4 inhibitors or inducers. These studies demonstrated that coadministration with ketoconazole or carbamazepine substantially altered the pharmacokinetic profile of quetiapine.

Concurrent administration of quetiapine with the CYP3A4 inhibitor ketoconazole *in vivo* increased plasma concentrations of quetiapine, with increases in the mean $C_{\max}$ and AUC exceeding twofold and fivefold, respectively. The finding of a substantially increased AUC and $C_{\max}$ is consistent with significant first-pass metabolism and hepatic clearance of quetiapine by CYP3A4. In this study, an 84% reduction in quetiapine clearance paralleled the increases in both $C_{\max}$ and AUC. Because quetiapine was given as a single low dose (25 mg), no adverse events were expected in the study

participants despite exposure to increased plasma concentrations of quetiapine.

Results of both clinical studies are consistent with the *in vitro* data predicting CYP3A4 as the primary CYP enzyme for quetiapine and with an earlier pharmacokinetic interaction study in humans treated with phenytoin, another inducer of CYP3A4 [15].

In the quetiapine–ketoconazole interaction study (study 1), a low dose of quetiapine was used because the study was performed in healthy volunteers. A low dose was believed to be scientifically appropriate because quetiapine is known to have linear pharmacokinetics at doses approved for use in the clinic. High doses of quetiapine in healthy volunteers are associated with considerable sedation, with a risk of developing symptomatic hypotension.

However, in clinical practice in patients with psychiatric illness, quetiapine is administered at substantially higher dosages (up to 750 mg day⁻¹) [16, 17]. Ketoconazole has been shown to decrease the clearance and thus potentially increase adverse effects associated with many drugs that are CYP3A substrates [9, 18, 19], including midazolam [20], triazolam [21, 22], zolpidem [23] and tacrolimus [24]. Ketoconazole has also been shown to inhibit Pgp [25] and may cause interactions with drugs that are Pgp substrates. In addition, Boulton *et al.* [26] suggested that quetiapine was a substrate for Pgp in an *in vitro* study that assessed the stimulation of adenosine triphosphatase activity in membranes with expressed Pgp. We showed here using monolayer assays in cells expressing Pgp that quetiapine is not a substrate of this transporter and therefore the effects of ketoconazole on quetiapine pharmacokinetics are likely to be due to inhibition of its metabolic elimination. Therefore, plasma concentrations of quetiapine, when administered in usual clinical doses concurrently with ketoconazole or another strong CYP3A4 inhibitor, would be expected to increase substantially compared with those of quetiapine given alone. Likewise, drinking grapefruit juice during treatment with quetiapine may be expected to increase exposure to the drug owing to inactivation of intestinal CYP3A4 [27].

Coadministration of carbamazepine led to a significant decrease in the steady-state plasma concentrations of quetiapine. These results demonstrate that concurrent administration of quetiapine with a strong CYP3A4 inducer can lead to a significant increase in quetiapine metabolism and, potentially, a loss of clinical efficacy. Possibly because of the short duration of this study and the fact that patients were in clinical remission before enrolment, no loss of quetiapine efficacy was observed.

Conclusions

The *in vitro* and clinical studies described here demonstrate that clinically important pharmacokinetic changes may occur when drugs that potently modulate the expression or activity of CYP3A4 enzymes are administered concurrently with quetiapine. Patients with severe mental illness typically require long-term treatment with antipsychotic medication, often in conjunction with other psychotropic drugs [6] or with nonpsychiatric medications. In addition to ketoconazole and carbamazepine, other drugs that induce or inhibit CYP3A4 (e.g. rifampin, ritonavir) could affect quetiapine exposure, efficacy and adverse event profile. If concomitant use of drugs that potently change CYP3A4 activity is necessary in patients treated with quetiapine, clinicians should monitor their patients for signs of adverse effects or decreased efficacy and titrate dosages accordingly.

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