IN VITRO STUDIES ON QUETIAPINE METABOLISM USING THE SUBSTRATE DEPLETION APPROACH WITH FOCUS ON DRUG-DRUG INTERACTIONS

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SUMMARY

The metabolism of the atypical antipsychotic quetiapine was investigated by *in vitro* methods. Pharmacokinetic parameters were determined in human liver microsomes and recombinant cytochrome P450 measuring substrate depletion and product formation. The cytochrome P450 isozymes CYP3A4 and CYP2D6 displayed activity towards quetiapine. The isozyme CYP2D6 played a minor role in the metabolism of quetiapine as CYP3A4 contributed 89% to the overall metabolism. A K_m value of 18 μM was determined by substrate depletion, suggesting linear kinetics under therapeutic conditions. Drugs known to inhibit CYP3A4, such as ketoconazole and nefazodone, displayed almost complete inhibition at low concentrations, whereas inhibitors of CYP2D6 do not seem to have a clinically relevant effect.

KEY WORDS

quetiapine, metabolism, drug-drug interactions, substrate depletion

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INTRODUCTION

Quetiapine is one of the newer, atypical antipsychotics that has less severe motor side-effects than classic antipsychotics. The drug interacts with a wide range of neurotransmitter systems: dopamine D_1 and D_2, serotonergic 5-HT_{1A} and 5-HT_2, and α_1- and α_2-adrenergic receptor sites /1/. The most common adverse events of quetiapine are somnolence, dizziness, headache, dry mouth, and agitation /2,3/.

Quetiapine is extensively metabolized in the liver by the cytochrome P450 system (CYP), and 11 metabolites have been detected so far /4/. Recently, Lin *et al.* /5/ and Grimm *et al.* /6/ published *in vitro* investigations of the metabolism of quetiapine, finding that CYP3A4, CYP2D6 and to some extent CYP2C9 and CYP2C19 are involved in the metabolism of quetiapine. Both studies suggest CYP3A4 to be the isozyme responsible for the major part of quetiapine metabolism, but there are disagreements on the relative contribution of CYP2D6 to the metabolism of quetiapine.

The objective of this *in vitro* study was to evaluate the inhibitory effect of commonly co-prescribed drugs with quetiapine and to quantitatively assess the involvement of the cytochrome P450 3A4 and 2D6 isozymes. Medical treatment of psychiatric patients usually involves a combination of several psychoactive drugs, which obviously increases the risk for metabolism-based drug-drug interactions. This polypharmacy was observed in a previous study concerning serum concentrations of quetiapine in psychiatric patients /7/.

A major problem in studies on drug metabolism is the limited availability of metabolites as reference compounds. Usually drug metabolism using human liver microsomes or human recombinant cytochrome P450 isozymes (cDNA) is investigated by recordings of the production rates of metabolites. This approach, which is sensitive and specific from a measurement point of view, has the drawback that only specific metabolic routes are assessed. Therefore, when monitoring one or a few metabolites, the estimates of the total metabolic rate or the involvement of the different CYP-isozymes may be biased. Thus, it may be valuable to record the depletion of a compound rather than the production of metabolite(s). In recent years this approach has been adopted by several authors /8-16/. In this study, we applied this type of approach for the *in vitro* study of quetiapine metabolism.

MATERIALS AND METHODS

Chemicals

Quetiapine was obtained as a gift from Astra-Zeneca, Copenhagen, Denmark, and the internal standard (IS), fluphenazine, was a gift from Novo Nordisk, Bagsværd, Denmark. The following substances were obtained from Sigma-Aldrich (St. Louis, MO): ketoconazole, quinidine, nefazodone, erythromycin, perphenazine, haloperidol, clozapine, orphenadrine, methadone, magnesium chloride ($MgCl_2$), isocitric acid, isocitric dehydrogenase, β-nicotinamide adenine dinucleotide phosphate disodium (NADP), and Tris (tris(hydroxymethyl)aminomethane.HCl). The following were a gift from Merck Research Laboratories, Rahway, USA (simvastatin and lovastatin), Glaxo Wellcome Research and Development, UK (ziduvudine), H. Lundbeck A/S, Copenhagen, Denmark (citalopram) and Bristol-Myers Squibb Company, New York, USA (aripiprazole). All other chemicals and reagents were of analytical grade. Pooled human liver microsomes (HLM), individual human liver microsomes not expressing CYP2D6 (HLM PM), human cDNA CYP-isozymes Baculovirus-insect cell-expressed, pooled human liver cytosol, human lymphoblast control and insect control microsomes were purchased from BD Gentest™, Woburn, MA, USA.

Stock solutions of quetiapine were prepared in either methanol (5 mM quetiapine) or 20% dimethyl sulfoxide (20 mM quetiapine, dissolved in dimethyl sulfoxide and diluted with water 1:4). Stock solutions of inhibitors were prepared in methanol, ethanol or dimethyl sulfoxide. All stock solutions were prepared in concentrations resulting in an organic solvent concentration in the incubation assay equal to or below 1% (v/v).

Analytical assay/quantification

LC-MS/MS analysis of quetiapine and metabolites was performed on a Quattro Micro (Waters, Taunton, MA, USA) instrument operating in positive electrospray ionization (ESI) mode. The analytical column was a Luna C8(2) 100 A (100 x 2.0 mm I.D., 3 μm) with a C8 (4.0 x 2.0 mm I.D.) guard column from Phenomenex (Torrance, CA, USA).

The optimal settings for multiple reaction monitoring (MRM) quantification of the transitions quetiapine (m/z: 384.1→253.0), *O*-dealkylated quetiapine (M1) (m/z: 340.2→253.0), *N*-dealkylated quetiapine (M2) (m/z: 296.2→253.0), quetiapine sulfoxide (M3) (m/z: 400.2→352.2), and IS (m/z: 438.2→171.3) were: cone voltage: 35 V (quetiapine, M1, M2 and M3) and 32 V (IS), collision energy: 23 eV (quetiapine, M1, M2, M3) and 26 eV (IS), source and desolvation temperature: 130°C and 300°C, respectively, desolvation gas flow: 800 l/h, and collision gas pressure: $3.9 \cdot 10^{-3}$ mbar. Nitrogen was used as nebulizer and desolvation gas. Argon (purity: >99.996%) served as collision gas. The dwell time was 0.15 seconds. The LC-MS/MS system was controlled by MassLynx software version 4.0 and quantification was done using Quanlynx software version 4.0.

Gradient elution was used at a flow rate of 0.3 ml/min with the following mobile phase composition: 10 mM ammonium acetate adjusted to pH 3.5 with formic acid (A) and acetonitrile (B): 0.0-1.0 min (23% B), 1.0-3.0 min (23→95% B), 3.0-3.1 min (95→23% B) and 3.1-6.0 min (23% B). Calibration standards and quality controls were separately prepared in 100 mM potassium phosphate buffer containing 50 mM HCl and measured in duplicate. Quadratic calibration curves without weighting were applied. The upper limit of quantification was determined to be 10 µM quetiapine, which necessitated dilution of groups II and III (Table 1). Imprecision (CV%) was less than 13.5% calculated from 2 × 10 runs on different days for each group.

Ion suppression and other deleterious effects were investigated by simultaneous injection of matrix or inhibitors and post-column infusion of a mixture of quetiapine and the internal standard. The retention times of ion suppression effects were compared with the retention times of quetiapine and the internal standard, and ion suppression was only a problem for erythromycin. Correction for the ion suppression was done by adding erythromycin to control incubations after the incubation was terminated. Metabolites were pseudo-quantified using quetiapine as calibrator assuming equal ionization behavior.

General *in vitro* metabolism conditions

Incubation assays with human liver microsomes (HLM or HLM PM) or cDNA expressed CYPs were carried out in polypropylene

tubes placed at 37°C in a 96-well thermomixer. The total incubation volume of 200 µl consisted of substrate, 100 mM potassium phosphate buffer (pH 7.4), NADPH generating system and microsomes. The final composition of the NADPH generating system in each assay was 3 mM Tris, 6 mM isocitric acid, 6 mM magnesium chloride, 1 mM β-nicotinamide adenine dinucleotide phosphate disodium and 1 U iso-citrate dehydrogenase. Unless otherwise stated, inhibitors were pre-incubated with co-factors and substrate and the reaction was started by adding enzyme. The enzyme concentration was 0.33 mg/ml for HLM (pooled and PM), and 25 nM and 50 nM for cDNA CYP3A4 and cDNA CYP2D6, respectively, using substrate concentrations of group I (Table 1).

TABLE 1

Analytical parameters for groups I-III

Group	Substrate conc. (µM)	Dilution factor	Calibrator levels (µM QTP)	Quality control levels (µM QTP)
	1	0	0.5	1
I	5	0	2.5	5
	10	0	10	
	25	10	5	10
II	50	10	25	50
	100	10	100	
	300	100	50	100
III	600	100	250	500
	1000	100	1000	

QTP = quetiapine.

At the time points 20 sec, 10, 20, 30, 40 and 50 min, 20 µl were drawn from incubation assays and dispensed into a 96-well plate on ice containing 40 µl termination reagent consisting of 100% acetonitrile including IS. All samples were diluted by adding 190 µl 18% acetonitrile, resulting in a final concentration of 30% acetonitrile

(v/v). Further dilution was done according to the parameters listed in Table 1 depending on the substrate concentration. The diluted sample was centrifuged at 4,000 rpm for 10 min, and 10 µl of the supernatant was injected into the LC-MS/MS apparatus from the same 96-well plate.

All incubations were performed in triplicate, and for inhibition experiments, vehicle was added to a control incubation. Incubation assays were tested for linearity with regard to microsomal concentration and incubation time. In HLM, linearity was confirmed for up to 50 min incubation time and up to 1 mg HLM/ml, whereas cDNA CYP3A4 and CYP2D6 both demonstrated linearity up to 50 min incubation time and up to 100 nM enzyme concentration. Adjustments for unspecific microsomal binding were not performed.

Screening for active cytochrome P450 isozymes

The activity of the isozymes CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP3A4, CYP3A5 and CYP2E1 was screened at 5 µM quetiapine and using 50 nmol/l of each cDNA expressed isozyme. The selected substrate concentration of 5 µM was chosen as being representative of hepatic concentrations under therapeutic conditions /7,17/. Other incubation and analytical conditions were as described above. Control experiments were done by incubating quetiapine with pooled human liver cytosol, human lymphoblast control, and insect control microsomes not expressing CYP, and without any cytosolic or microsomal fraction added.

Saturation studies

Michaelis-Menten kinetics was studied by saturation experiments from 1 to 1,000 µM quetiapine in pooled HLM, HLM PM, CYP3A4, and CYP2D6. The general incubation and analytical conditions were followed. The enzyme concentration was multiplied by two and three in groups II and III, respectively (Table 1). The reaction velocities and velocity constants were corrected for the enzyme amounts used.

Relative contribution studies

Ketoconazole and quinidine were used as selective inhibitors of CYP3A4 and CYP2D6, respectively. The inhibitors were incubated in

different concentrations with recombinant systems expressing the target isozyme (25 nmol CYP3A4/1 and 50 nmol CYP2D6/1) and at 5 and 50 μM quetiapine. The relative contribution was assessed at therapeutic and toxic levels in the liver represented by the two concentrations of 5 and 50 μM, respectively. All other incubation conditions were as described above, and the samples were analyzed as above. The inhibitory effect on other isozymes was also investigated in recombinant systems expressing the non-target isozyme. Combining these two experiments, the optimum inhibitory concentrations were determined for ketoconazole and quinidine. At this concentration the inhibitors were incubated with 0.33 and 0.67 μg/μl HLM at 5 and 50 μM quetiapine, respectively, from which the relative contribution was calculated.

Identification of metabolites

Screening for metabolites of quetiapine was performed by incubating 5 μM quetiapine with HLM, cDNA CYP3A4 and cDNA CYP2D6 for 2 hours. The assay was stopped with ice-cold acetonitrile, and the supernatant was injected into the LC-MS/MS system. The analytes were eluted by a gradient consisting of ammonium acetate (pH 3.5):acetonitrile: 0-10 min (90:10), 10-15 min (85:15), 15-18.5 min (70:30), 23-23.5 min (10:90), followed by equilibration resulting in a total run time of 28.5 min. The samples were subjected to different MS conditions and scans, which included molecular ion scans (MS1), in source collision induced dissociation (CID) in order to do MS-MS-MS measurements, daughter ion scans, neutral loss scan and multiple reaction monitoring (MRM). Isotope modeling was used to support the suggested fragmentation pattern/reactions. Positive and negative electrospray ionization (ESI) was carried out.

Inhibition studies

Inhibitors were diluted from stock solutions with solvent and added to the incubation medium and pre-incubated with substrate. The total organic content did not exceed 1%. The inhibitors were tested at three concentrations levels (1, 10 and 100 μM) with the exception of aripiprazole (1, 10 and 50 μM). All inhibitors were used competitively, except erythromycin, which is a mechanism-based inhibitor of CYP3A4 /18/. Erythromycin, enzyme and co-factors were pre-incubated for

5 minutes, and then quetiapine was added to the incubation. The inhibitors tested at 5 μM quetiapine were as follows: antipsychotics (haloperidol, clozapine, perphenazine, and aripiprazole), antidepressants (nefazodone and citalopram), statins (simvastatin and lovastatin), the macrolide antibiotic erythromycin, the anti-Parkinson drug orphenadrine, and the protease inhibitor zidovudine. The inhibition studies were conducted at a quetiapine concentration corresponding to therapeutic levels in the liver.

Data analysis

The Michaelis-Menten constant K_m was determined by the substrate depletion approach and on the basis of product formation. With regard to the substrate depletion approach, a sigmoid fit was used for the velocity constant k plotted against the logarithmic substrate concentration (S) (Equation 1) /11/. The antilogarithm of the substrate concentration at the inflection point represents the K_m value, and k_{max} represents the theoretical maximum velocity constant at infinitely low substrate concentrations.

$$k = k_{max} * \left(1 - \left(\frac{S}{S + K_m}\right)\right) \qquad \text{Equation 1}$$

For the substrate depletion studies, an exponential decreasing curve (Equation 2) was fitted to the measured concentrations of quetiapine (y) plotted against each time point (x). The velocity constant k (min^{-1}) was estimated, from which the reaction velocity (V) was derived ($V = k*S_0$). S_0 is the substrate concentration at $t = 0$.

$$S = S_0 * e^{-kt} \qquad \text{Equation 2}$$

For product formation data, K_m and apparent V_{max} were determined by non-linear regression of the reaction velocity plotted against the substrate concentration (Equation 3). The apparent velocity was calculated on the basis of metabolite concentrations at a given time point to which the metabolite concentration increased linearly.

$$V = \frac{V_{max} * S}{K_m + S} \qquad \text{Equation 3}$$

Intrinsic clearance CL_{int} was calculated in two ways. Equation 4 was used for substrate depletion, and Equation 5 was used for product formation data.

$$CL_{int} = v * k, \qquad \text{Equation 4}$$

where v is the incubation volume and k is the velocity constant measured at a substrate concentration below K_m.

$$CL_{int} = \frac{V_{max}}{K_m} \qquad \text{Equation 5}$$

The fits were done using GraphPad Prism software version 3.02.

RESULTS

Screening

Hepatic enzymes were studied *in vitro* for their ability to metabolize quetiapine. Of the phase I isozymes, CYP3A4, CYP3A5 and CYP2D6 were able to metabolize quetiapine with reaction velocities of 31.1 (SE [standard error of the mean]: 1.6), 0.8 (SE: 0.2) and 3.1 (SE: 0.4) pmol quetiapine/min/pmol CYP (Fig. 1). Phase II enzymes (pooled human liver cytosol) displayed no depletion of quetiapine. Quetiapine levels remained stable throughout the total incubation time, and none of the control experiments displayed any activity.

Metabolite identification

Fifteen metabolites were detected in positive ESI, when incubating quetiapine with HLM. Seven chromatographic peaks had molecular ions of m/z: 400, three m/z: 356, three m/z: 312, one m/z: 340, and one m/z: 296. No peaks were detected in negative ESI, and no carboxylated quetiapine was found.

The detected metabolites could be classified into three subgroups: hydroxylated or sulfoxidated quetiapine, dealkylated quetiapine, and a combination of the two subgroups. The molecular ions of m/z: 340 and m/z: 296 were suggested to be *O*- and *N*-dealkylated quetiapine (M1 and M2), respectively (Figs. 2 and 3). Molecular ions of m/z: 400

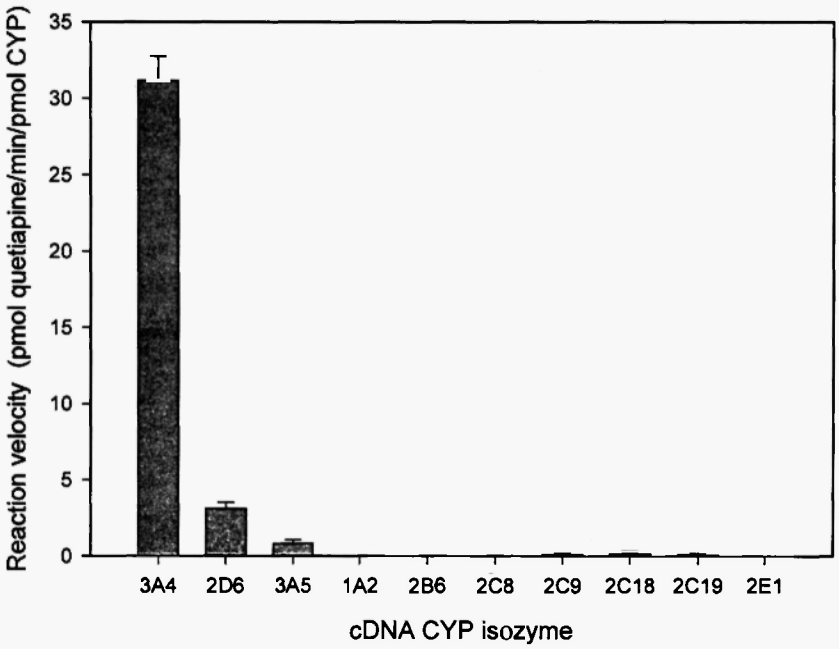


Fig. 1: Screening of ten different cytochrome P450 isozymes (cDNA CYP) using depletion of quetiapine.

are metabolites formed by addition of oxygen, i.e. hydroxylation or sulfoxidation. A major metabolite with an m/z value of 400 showed a loss of 48 dalton. This was interpreted as the loss of the sulfoxide group, which was confirmed by modeling the isotopic pattern (M3). These three metabolites (M1-3) were monitored in all of the pharmacokinetic studies by multireaction monitoring (MRM) using the following channels: m/z: 400→352 (quetiapine sulfoxide), m/z: 340→253 (*O*-dealkylated quetiapine) and m/z: 296→253 (*N*-dealkylated quetiapine).

The metabolite pattern of cDNA CYP3A4 is very similar to that of HLM. The isozyme CYP2D6 produced small amounts of M1, M3 and probably a hydroxylated quetiapine metabolite with a mass spectrum different from M3 (Fig. 3). The hydroxylated metabolite (Fig. 3) was not seen when incubating quetiapine with CYP3A4.

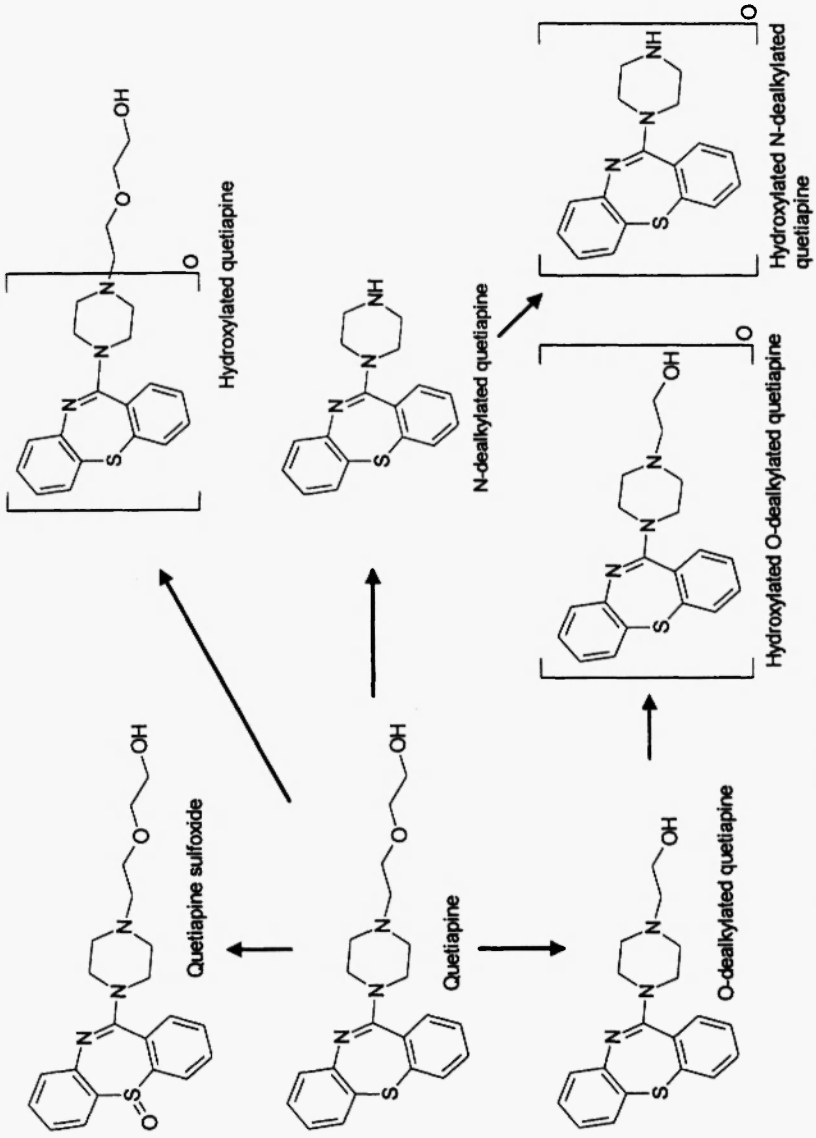
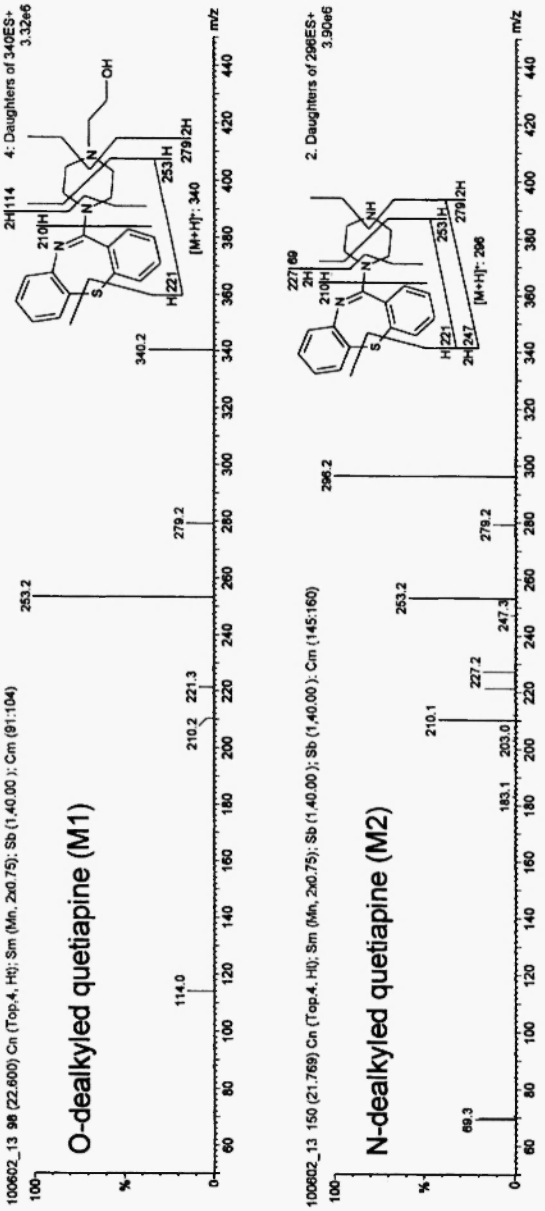


Fig. 2: Chemical structures of quetiapine and metabolites of quetiapine.



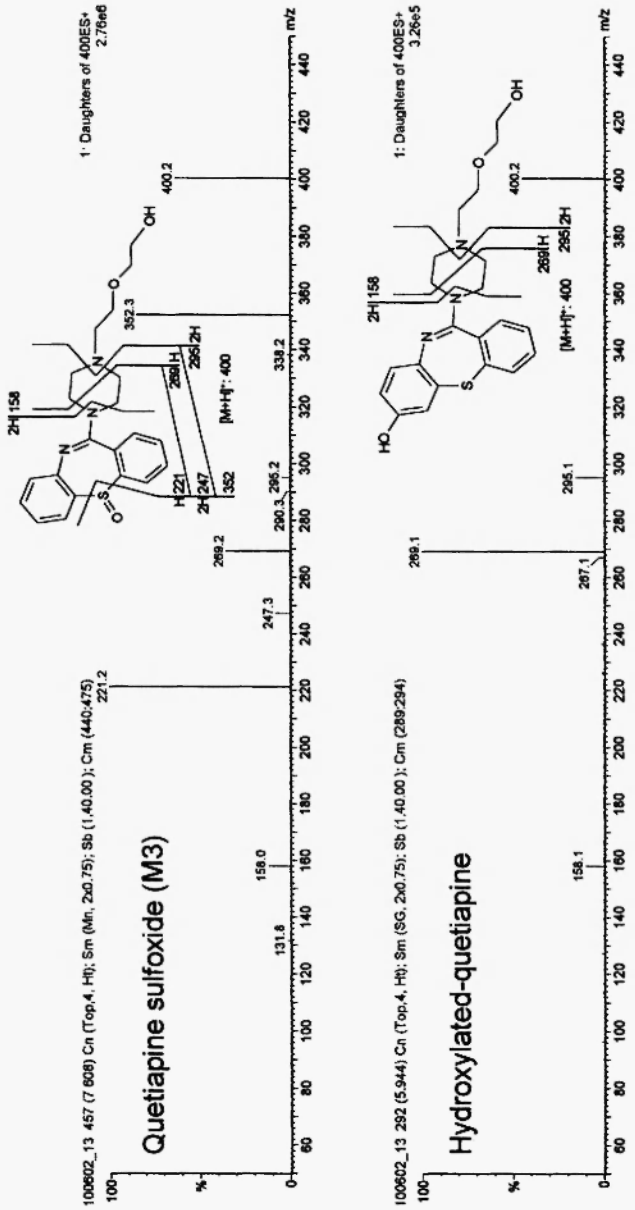


Fig. 3: Daughter ion mass spectra of four metabolites.

Determination of K_m and V_{max}

The pharmacokinetics of quetiapine was investigated by incubation of quetiapine (range: 1-1,000 μM) with HLM, HLM PM, cDNA CYP3A4 and cDNA CYP2D6. For the HLM, HLM PM, cDNA CYP3A4 and cDNA CYP2D6, K_m was determined by substrate depletion to 18 μM (confidence interval [CI]: 12.0-26.2), 17 μM (CI: 12.0-24.1), 51 μM (CI: 35.9-72.9) and 21 μM (CI: 15.6-28.6), respectively (Table 2 and Fig. 4). Concerning HLM product formation, all three metabolites obeyed saturation kinetics (Fig. 5). K_m values for the three metabolites M1-3 were 118 μM (SE: 26), 186 μM (SE: 43) and 3.0 μM (SE: 1), respectively. HLM PM displayed K_m values of 129 μM (SE: 9), 152 μM (SE: 14) and 10 μM (SE: 2) for the metabolites

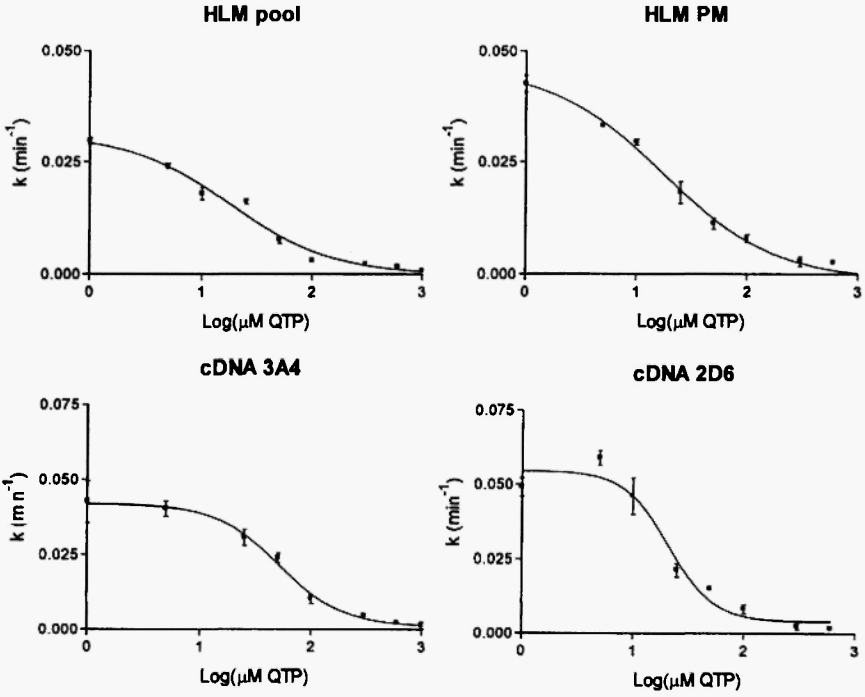


Fig. 4: Sigmoidal curves of the velocity constant k as a function of the logarithm of quetiapine (QTP) concentration for HLM pool (pooled human liver microsomes), HLM PM (single donor human liver microsomes not expressing 2D6), cDNA 3A4, and cDNA 2D6. Each point is a mean of three replicates. Standard error of the mean is indicated by error bars.

TABLE 2

Pharmacokinetic parameters

	K_m (μM)				V_{max} [a]	CL_{int} [b]
	cDNA 3A4	**cDNA 2D6**	**HLM PM**	**HLM pool**	**HLM pool**	**HLM pool**
Substrate depletion [c]						
Quetiapine	51 (35.9-72.9)	21 (15.6-28.6)	17 (12.0-24.1)	18 (12.0-26.2)		0.0771
Product formation [d]						
M1	96 (19)	n.d.	129 (9)	118 (26)	0.30 (0.020)	0.0025
M2	62 (17)	n.d.	152 (14)	186 (43)	0.51 (0.029)	0.0027
M3	8.5 (1.5)	n.d.	10.1 (1.8)	3.0 (1.4)	0.05 (0.003)	0.0167

cDNA = recombinant expressed CYP isozymes; HLM pool = human liver microsomes; HLM PM = poor metabolizer not expressing 2D6; M1 = *O* dealkylated quetiapine; M2 = *N*-dealkylated quetiapine; M3 = quetiapine sulfoxide; n.d. = not determinable.

[a] Apparent V_{max} (pmol/min/μg protein); [b] intrinsic clearance (μl/min/μg protein); [c] 95% confidence interval shown in parentheses; [d] standard error shown in parentheses.

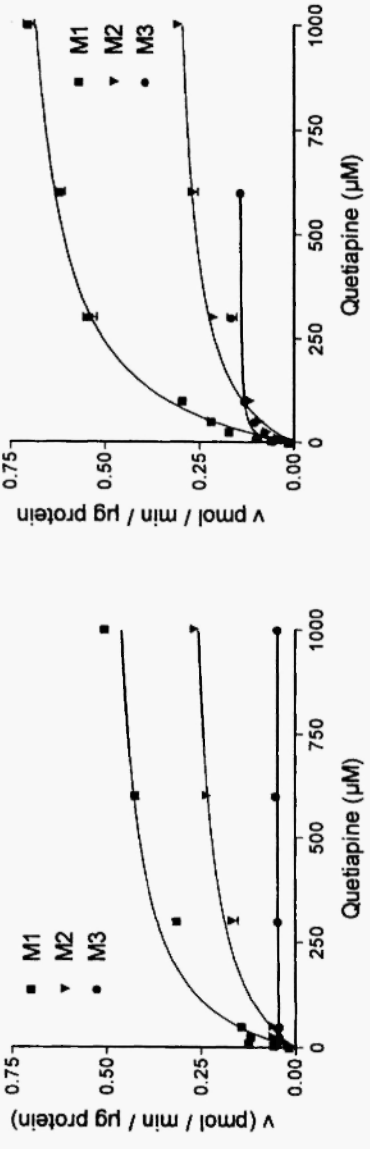


Fig. 5: Michaelis-Menten plots of three metabolites of quetiapine. HLM pool (pooled human liver microsomes), HLM PM (single donor human liver microsomes not expressing 2D6). M1 (*O*-dealkylated quetiapine), M2 (*N*-dealkylated quetiapine), and M3 (quetiapine sulfoxide). Each point is the mean of three replicates and error bars indicate standard error of the mean.

M1-3, respectively. The K_m values resulting from incubation with CYP3A4 were 96 (SE: 19), 62 (SE: 17) and 8.5 (SE: 1.5) (Table 2). Metabolites of CYP2D6 were not detectable.

Apparent maximum velocities V_{max} in HLM pool were estimated for M1-3 as 0.30 pmol/min/μg (SE: 0.020), 0.51 pmol/min/μg (SE: 0.029) and 0.05 pmol/min/μg (SE: 0.003). HLM PM displayed V_{max} values of 0.77 pmol/min/μg (SE: 0.016), 0.34 pmol/min/μg (SE: 0.010) and 0.14 pmol/min/μg (SE: 0.006) for the metabolites M1-3, respectively (Table 2).

The apparent CL_{int} values in HLM were determined to be 0.0025, 0.0027 and 0.0167 μl/min/μg protein for M1-3, respectively, while the CL_{int} determined by depletion was 0.0771 μl/min/μg protein (Table 2). The CL_{int} value measured by depletion was based on incubation of 1 μM quetiapine.

Relative contribution

In order to determine the relative contribution of CYP3A4 and CYP2D6, preliminary studies were performed. Different concentrations of ketoconazole and quinidine were tested on cDNA CYP3A4 and cDNA CYP2D6 to find the optimum concentration (Fig. 6), meaning a degree of inhibition above 75% with the target isozyme and below 10% with the other isozyme. All experiments were carried out at 5 and 50 μM quetiapine. The optimum concentrations of inhibitors were found to be 0.25 μM ketoconazole at both quetiapine concentrations and 2 and 5 μM quinidine at 5 and 50 μM quetiapine, respectively. At these inhibitor concentrations, ketoconazole showed no inhibition of CYP2D6, whereas quinidine displayed up to 10% inhibition of CYP3A4 (Fig. 6).

The relative contribution of the two isozymes in HLM were measured with the above-mentioned concentrations and corrected for incomplete inhibition indicated in the recombinant systems. For example, 0.25 μM ketoconazole inhibited quetiapine metabolism by 81% in HLM and 91% in cDNA CYP3A4, which resulted in a corrected inhibition of 81/0.91% = 89% (Table 3). Thus, the HLM studies showed that CYP3A4 contributed 89% at the low and 88% at the high concentration. CYP2D6 was responsible for a minor part of the metabolism, corresponding to 9% at 5 μM, and 12% at 50 μM.

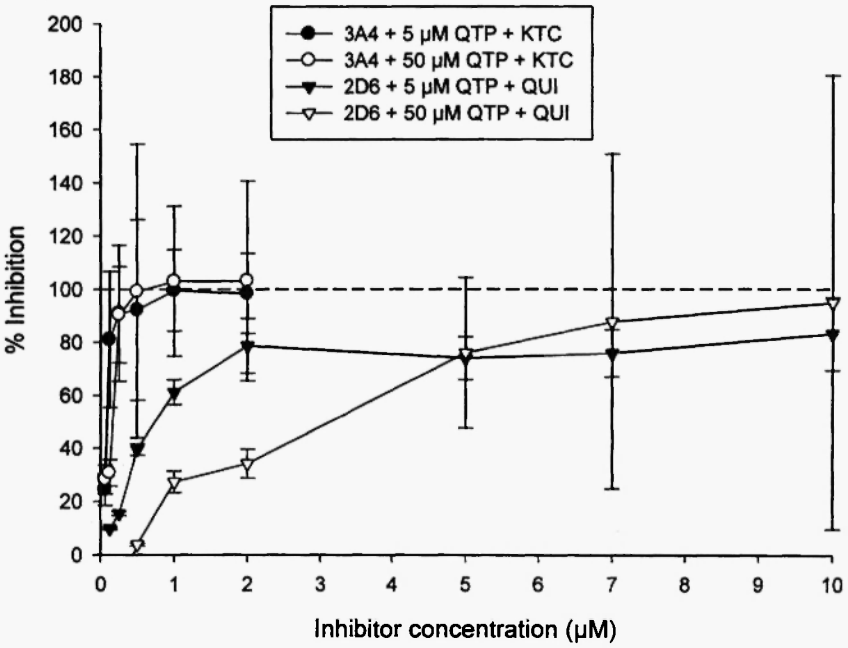


Fig. 6: Effect of the inhibitors ketoconazole (KTC) and quinidine (QUI) on cDNA 3A4 and cDNA 2D6 quetiapine (QTP) metabolism, respectively, at 5 and 50 µM quetiapine.

TABLE 3

Relative contribution of cytochrome P450 isozymes to the depletion of quetiapine (QTP) in pooled human liver microsomes

	CYP3A4	CYP2D6
5 µM QTP	89%	9%
50 µM QTP	88%	12%

Interaction studies

The effect of other drugs on the metabolism of quetiapine was studied *in vitro* by simultaneous incubation with HLM. The drugs were selected on the basis of knowledge of their metabolism, either substrates or inhibitors of CYP3A4 or CYP2D6, and/or from a previous study, which indicated the types of drugs commonly co-prescribed with quetiapine /7/. The inhibitory effects at the three concentration levels are shown in Figure 7. The drugs known to inhibit CYP3A4, such as ketoconazole and nefazodone, had the expected effect on quetiapine metabolism, with high degrees of inhibition at 1 μM, 78% and 65%, respectively. Erythromycin is also known to inhibit CYP3A4, but it only exerted notable inhibition at high concentration levels. Inhibitors of CYP2D6 influenced quetiapine metabolism only to a minor extent.

DISCUSSION

The finding of CYP3A4 being the major CYP isozyme responsible for quetiapine metabolism at both substrate concentrations, and CYP2D6 the smaller participant, agrees with the findings of Grimm *et al.* /6/. Concerning the study by Lin *et al.* /5/, however, the estimated contribution of CYP2D6 amounting to 60% was probably too high due to the relatively high concentrations of quinidine used (10 μM). We investigated the inhibitory effect of quinidine concentrations up to 10 μM on the depletion of quetiapine in the cDNA CYP3A4 experiment and found an inhibitory effect of up to 33% (data not shown). A comparison of the inhibitory effects of ketoconazole and quinidine in HLM and HLM PM, which amounted to a difference of 7%, supports that CYP2D6's contribution is limited to around 10% (data not shown). When using specific chemical inhibitors, this study shows the importance of properly investigating the inhibitory effect on other isozymes believed to have activity. Otherwise the relative importance of the specific isozyme may be overestimated.

Concerning contributions from other isozymes, Grimm *et al.* /6/ ascribed activity to CYP2C9 on the basis of inhibition by sulfaphenazole applied in a relatively high concentration. However, it does not appear from the study whether the inhibitory effect on other isozymes was investigated. Lin *et al.* /5/ found activity of CYP2C19 on the basis

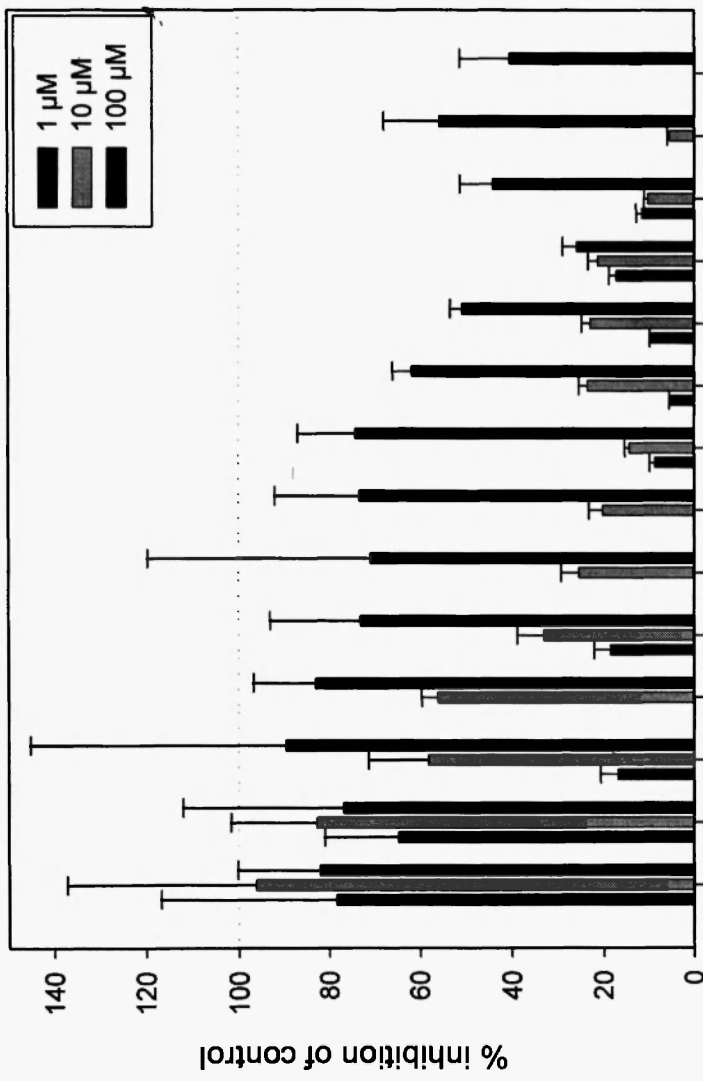


Fig. 7: Percent inhibition of control for 14 drugs tested in **three concentrations (1, 10 and 100 µM** [aripiprazol: 1, 10 and 50 µM]) at 5 µM quetiapine. Error bars represent standard error of the mean.

of quetiapine depletion in a cDNA based experiment, but the activity only accounted for 4.5% of the activity of CYP3A4.

In this study a substrate concentration of 5 μM quetiapine was applied, which is judged to be of the order of magnitude expected in the liver under therapeutic conditions /7,17/. The K_m values found here are comparable to those of Grimm *et al.* /6/ except for quetiapine sulfoxide. This questions the identification of quetiapine sulfoxide. The mass spectrum shown in Figure 3 is, however, similar to the spectrum of Li *et al.* /19/, even though the spectrum displayed there only ranges from 230 to 410 m/z values. The low K_m value of quetiapine sulfoxide corresponds well to observations of M3 being the major metabolite formed under therapeutic concentrations /20/.

The two approaches, drug depletion and product formation, can be compared by calculating the intrinsic clearance (CL_{int}) /11,21/. The sum of apparent CL_{int} for the recorded metabolites accounts for only 28% of the total Cl_{int} measured by the depletion approach, which supports that quetiapine is metabolized to a large number of metabolites, of which none can be considered dominant /4/.

In vivo interaction studies were performed with quetiapine and commonly co-administrated drugs, such as haloperidol and risperidone, which did not show any interactions /22,23/. Recently, *in vivo* results concerning co-medication of quetiapine with ketoconazole, erythromycin or carbamazepine showed a clinically relevant effect on quetiapine metabolism /6,20,24/, which is in accordance with the *in vitro* results in this study and previous *in vivo* results /7/.

Not unexpectedly, ketoconazole and nefazodone inhibited quetiapine metabolism at clinically relevant concentrations /25,26/. These findings support the results of the relative contribution experiments, suggesting that CYP3A4 is the major enzyme involved in quetiapine metabolism. However, quinidine does show an inhibitory effect at 10 μM, which is lower than the estimated hepatic concentration (32 μM) at therapeutic levels /27/. This effect may result from competitive inhibition of CYP3A4 by quinidine, which is metabolized by CYP3A4.

Surprisingly, erythromycin displayed a relatively minor inhibitory effect, even though erythromycin is a known CYP3A4 inhibitor. An *in vivo* study by Li *et al.* /24/ showed 52% decrease in quetiapine clearance during co-medication with erythromycin. In this study, ion

suppression at high erythromycin concentrations was detected, but correcting for this did not change the outcome of the inhibitory effect.

The anti-Parkinson agent orphenadrine is often used to treat side effects of antipsychotics, including those of quetiapine /7/. This drug does display some inhibition (23%) at 10 μM not far from therapeutic levels (8.4 μM in the liver) /27/. The same goes for the reverse transcriptase inhibitor ziduvudine that, even though it is mainly metabolized by glucuronidation, displays an inhibitory effect of 21% at 10 μM. Simvastatin and lovastatin are primarily transformed by CYP3A4 and do show an inhibitory effect, but not at therapeutic concentrations /28/. Therefore, it is unlikely that they will influence the metabolism of quetiapine in any clinically relevant way. The drugs clozapine, aripiprazole, methadone and citalopram displayed only a minor inhibitory effect.

In this study we applied the substrate depletion approach, partly due to lack of reference metabolites and partly due to the large number of metabolites produced. The substrate depletion approach, however, differs fundamentally from the product formation principle. Precise measurement of drug depletion is more difficult than measuring product formation due to the measurement of a small difference. Another issue is the measurement of initial velocities to avoid product inhibition, enzyme degradation, and change in substrate concentration over time /29/. The substrate depletion approach requires, in order to reduce variation, considerable substrate consumption, which again requires longer incubation times or larger enzyme amounts /11,30/. The latter should be kept at a minimum to reduce unspecific microsomal binding, and log-linearity must be confirmed within the incubation period as a control for product inhibition and enzyme degradation.

CONCLUSION

The present *in vitro* study verified that CYP3A4 is the quantitatively most important enzyme for quetiapine metabolism, being responsible for 89% of the turnover rate. The remaining ~10% is metabolized by CYP2D6, which is thus of limited importance for the metabolism of the drug. Concerning drug-drug interactions, strong CYP3A4 inhibitors exhibited a considerable inhibition of quetiapine turnover, as largely expected. Other CYP3A4 substrates exhibited

moderate degrees of inhibition. CYP2D6 inhibiting drugs are not likely to exert any significant inhibition of quetiapine metabolism.

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