Short Communication

Metabolism of Quetiapine by CYP3A4 and CYP3A5 in Presence or Absence of Cytochrome B$_5$

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ABSTRACT:

The antipsychotic drug quetiapine is extensively metabolized by CYP3A4, but little is known about the possible influence of the polymorphic enzyme CYP3A5. This in vitro study investigated the relative importance of CYP3A4 and CYP3A5 in the metabolism of quetiapine and compared the metabolic pattern by the two enzymes, in the presence or absence of cytochrome b$_5$. Intrinsic clearance (CL$_{int}$) of quetiapine was determined by the substrate depletion approach in CYP3A4 and CYP3A5 insect cell microsomes with or without coexpressed cytochrome b$_5$. Formation of the metabolites quetiapine sulfoxide, N-desalkylquetiapine, O-desalkylquetiapine, and 7-hydroxyquetiapine by CYP3A4 and CYP3A5 were compared in different microsomal preparations. CL$_{int}$ of quetiapine by CYP3A5 was less than 35% relative to CYP3A4. CL$_{int}$ was higher (3-fold) in CYP3A4 microsomes without cytochrome b$_5$ compared with CYP3A4 microsomes with coexpressed cytochrome b$_5$, whereas in CYP3A5 microsomes CL$_{int}$ was similar for both microsomal preparations. Metabolism of quetiapine by CYP3A5 revealed a different metabolic pattern compared with CYP3A4. The results indicated that O-desalkylquetiapine constituted a higher proportion of the formed metabolites by CYP3A5 compared with CYP3A4. In conclusion, the present study indicates that CYP3A5 is of minor importance for the overall metabolism of quetiapine, regardless of the presence of cytochrome b$_5$. However, a different metabolic pattern by CYP3A5 compared with CYP3A4 could possibly result in different pharmacological and/or toxico- logical effects of quetiapine in patients expressing CYP3A5.

Quetiapine, a dibenzothiazepine derivative, is an atypical antipsychotic drug used for the treatment of schizophrenia and acute episodes of mania. Recently, the U.S. Food and Drug Administration also approved quetiapine for the treatment of depressive episodes associated with bipolar disorder.

Quetiapine is extensively metabolized in the liver by the cytochrome P450 (P450) system, primarily by CYP3A (Grimm et al., 1997, 2006). The major metabolic pathways of quetiapine are through sulfoxidation, N- and O-desalkylation, and, to a lesser degree, through 7-hydroxylation (Grimm et al., 1997) (Fig. 1). The pharmacologically inactive metabolite quetiapine sulfoxide is considered to be the main metabolite of quetiapine (DeVane and Nemeroff, 2001), whereas the most important active metabolite seems to be N-desalkylquetiapine (McIntyre et al., 2007). Studies indicate that N-desalkylquetiapine, unlike the parent compound, is a potent inhibitor of the noradrenergic transporter and has partial agonist activity at the 5-hydroxytryptamine 1A receptor (Goldstein et al., 2007; Jensen et al., 2008). In addition, N-desalkylquetiapine was found to possess antidepressive-like activity in a mouse model (Jensen et al., 2008). This suggests that the antidepressive activity of quetiapine is, at least partly, mediated by N-desalkylquetiapine. The active metabolite 7-hydroxyquetiapine is formed by CYP2D6 in addition to by CYP3A (Grimm et al., 2006). Because of the low plasma concentration of this metabolite (Gefvert et al., 1998), genetic polymorphism in CYP2D6 is unlikely to be of importance for the in vivo metabolism of quetiapine.

CYP3A4 and CYP3A5 comprise the two main CYP3A isoforms in adults. The individual variability in phenotype is substantial for both enzymes, but this variability is linked to genetic polymorphism only for CYP3A5 (Westlind-Johnsson et al., 2003). CYP3A5 is expressed in approximately 10 to 30% of whites, 30% of Japanese, and 60% of African Americans (Kuehl et al., 2001; Burk and Wojnowski, 2004). The relative contribution of CYP3A5 to total hepatic CYP3A protein differs, but in some individuals CYP3A5 can constitute more than 50% of total CYP3A (Kuehl et al., 2001; Lin et al., 2002; Westlind-Johnsson et al., 2003). Individuals expressing CYP3A5 may have higher clearance and lower bioavailability of CYP3A substrates compared with individuals not expressing CYP3A5 (Kuehl et al., 2001). This has been demonstrated for both the immunosuppressive drug tacrolimus and for several HMG-CoA reductase inhibitors (statins) where patients expressing CYP3A5 need higher drug dosages to reach target serum concentration or to achieve sufficient lipid-lowering response, respectively (Hesselin et al., 2003; Zheng et al., 2003; Goto et al., 2004; Kivisto et al., 2004; Ferraresso et al., 2007).

CYP3A4 and CYP3A5 overlap in substrate specificity, but the relative importance of CYP3A4 and CYP3A5 in overall CYP3A-mediated metabolism differs between substrates (Lamba et al., 2002). The role of CYP3A5 in the metabolism of quetiapine is sparsely known. Therefore, the objective of this in vitro study was to investigate the relative importance of CYP3A4 and CYP3A5 in the metabolism of quetiapine and to compare the metabolic pattern by the two enzymes. Because the presence of cytochrome b$_5$ (cyt b$_5$) in the incubation assay has been shown to affect the metabolism of many drugs via CYP3A4 and CYP3A5 in vitro (Yamaori et al., 2003), the effect of cytochrome b$_5$ on quetiapine metabolism was also examined.

ABBREVIATIONS: P450, cytochrome P450; cyt b$_5$, cytochrome b$_5$; CL$_{int}$, intrinsic clearance.
Materials and Methods

Materials. Quetiapine and N-desalkyquetiapine were purchased from Toronto Research Chemicals Inc. (North York, ON, Canada), quetiapine sulfoxide was obtained from Synthetica AS (Oslo, Norway), and promazine was from Sigma-Aldrich (St. Louis, MO). Baculovirus insect cell microsomes expressing human CYP3A enzymes, cytochrome P450 reductase, and cytochrome b$_5$ (Supersomes) were purchased from BD Gentest (Woburn, MA), whereas similar CYP3A microsomes without coexpressed cytochrome b$_5$ (Xenosomes) were purchased from Larodan Fine Chemicals AB (Malmö, Sweden).

Experimental Conditions. Preliminary metabolism studies of quetiapine using the metabolite formation method revealed a solubility problem of quetiapine, because the concentration of quetiapine in the buffer solutions was only approximately 30% of the nominal concentration. Organic solvents (dimethyl sulfoxide and methanol) were added to increase the solubility of quetiapine. The effect of 10% methanol on quetiapine metabolism was investigated, and an extensive inhibition (>40%) in the formation of the four metabolites by CYP3A4 and CYP3A5 was observed. Therefore, quetiapine metabolism was instead examined by the substrate depletion approach, which requires low substrate concentrations (Jones and Houston, 2004). Quetiapine concentrations of 1 gM in CYP3A4 microsomes and 5 gM in CYP3A5 microsomes were well below $K_{\text{m}}$ for all metabolites (data not shown). These concentrations resulted in linear formation of the metabolites and provided quantifiable metabolite amounts.

Incubation assays contained either CYP3A4 or CYP3A5, with or without coexpressed cytochrome b$_5$. Quetiapine (1 gM for CYP3A4 and 5 gM for CYP3A5) was incubated at 37°C in 118 mM Tris-H$_2$SO$_4$ (pH 7.5), 0.5 mM MgSO$_4$, and 1.6 mM NADPH, incubation conditions that were optimized by Hermann et al. (2006). Microsomes were diluted in a solution (pH 7.4) consisting of 0.25 M sucrose, 10 mM Hepes, and 2 mM EDTA. The reaction was initiated by adding preheated microsomes, and final enzyme concentration was 4 mM in the total incubation volume of 220 l. Reactions were terminated after 0 to 60 min with 150 l of ice-cold acetonitrile including internal standard (promazine). The concentration of internal standard was 4 gM in each sample. After at least 30 min on ice, the samples were centrifuged at 1600g for 5 min at 4°C (Universal 32R; Hettich Zentrifugen, Tuttingen, Germany), and 5 l of the supernatant was injected into the liquid chromatography-mass spectrometry/mass spectrometry instrument. For each of the four microsomal preparations studies, three separate experiments were performed. All of the incubations in each experiment were performed in duplicate.

Determination of Quetiapine and Metabolites. Quetiapine and metabolites were analyzed on a 2795 liquid chromatograph and a Micromass Quattro Micro tandem mass spectrometer (both obtained from Waters, Milford, MA). The analytes were separated on an ACE 3AQ C18 analytical column (Advanced Chromatography Technologies, Aberdeen, Scotland) protected by an Atlantis dC18 guard column (Waters). The samples were eluted at a flow rate of 0.25 ml/min by a gradient consisting of 25 to 80% acetonitrile in ammonium acetate (10 mM, pH 4.5). Total run time was 15 min. The retention times were 2.8 min for 7-hydroxyquetiapine, 3.1 min for quetiapine sulfoxide, 5.6 min for N-desalkyquetiapine, 5.7 min for O-desalkyquetiapine, 6.0 min for quetiapine, and 6.9 min for promazine. Detection was performed in the multiple reaction monitoring mode at the following transitions: 400~269 for 7-hydroxyquetiapine; 400~221 for quetiapine sulfoxide; 296~210 for N-desalkyquetiapine; 340~253 for O-desalkyquetiapine; 384~253 for quetiapine; and 285~212 for promazine. Processing was performed with QuanLynx software version 4.0 (Waters). Quetiapine, quetiapine sulfoxide, and N-desalkyquetiapine were quantified using quadratic standard curves with a 1/Y weighting. For quetiapine, quetiapine sulfoxide, and N-desalkyquetiapine, the validation data for intra- and inter-run accuracy and precision were ±16% at the limit of quantification (0.1 gM for quetiapine, and 0.01 gM for quetiapine sulfoxide and N-desalkyquetiapine) and ≥9% above the limit of quantification. Reference substances of O-desalkyquetiapine and 7-hydroxyquetiapine were not available, and these metabolites were therefore quantified using peak area ratio (metabolite/internal standard). Any deviation from linearity in mass spectrometry response will not be revealed using peak area ratio, implying more uncertainty in these results compared with the results based on standard curves.

Fig. 1. Metabolism of quetiapine in vitro.
Data Analysis. Substrate depletion data were fitted to a monoexponential decay model (eq. 1), with a 1/Y weighting using GraphPad Prism version 4.03 (GraphPad Software Inc., San Diego, CA).

\[
C_t = C_0 e^{-kt}
\]  

(1)

In each experiment, the velocity constant \(k\) (min\(^{-1}\)) for the substrate depletion was estimated and the intrinsic clearance (CL\(_{\text{int}}\)) was calculated (eq. 2).

\[
\text{CL}_{\text{int}} = kV,
\]  

(2)

where \(V\) is the incubation volume.

The substrate depletion curves were log-transformed to visualize how well the monoexponential decay model described the substrate depletion data. The log-transformed substrate depletion curves were linear in the entire time range for all microsomal preparations, with the exception of CYP3A4 microsomes without cytochrome b\(_5\) after 40-min incubation (data not shown). Substrate depletion was therefore studied for 0 to 40 min in all four microsomal preparations. The metabolic pattern by CYP3A4 and CYP3A5 was compared by assessment of the relative formation of the four metabolites of quetiapine after 40-min incubation in the different microsomal preparations.

An unpaired t test was used to determine any statistical difference in CL\(_{\text{int}}\) of quetiapine by CYP3A4 in microsomes with or without coexpressed cytochrome b\(_5\), and correspondingly for CYP3A5. A value of \(p < 0.05\) was considered statistically significant.

Results

Determination of CL\(_{\text{int}}\). Substrate depletion studies with quetiapine by CYP3A4 and CYP3A5 showed monoeponential decay in microsomes with and without coexpressed cytochrome b\(_5\) (\(r^2 > 0.9\) for CYP3A4 and \(r^2 > 0.5\) for CYP3A5) (Fig. 2).

CL\(_{\text{int}}\) for CYP3A4 and CYP3A5 with coexpressed cytochrome b\(_5\) was 3.2 and 1.0 µl/min/pmol P450, respectively (Table 1). CL\(_{\text{int}}\) of quetiapine by CYP3A5 was 32% (S.E.M. 7%) relative to CYP3A4. The corresponding CL\(_{\text{int}}\) values for CYP3A4 and CYP3A5 without coexpressed cytochrome b\(_5\) were 8.8 and 1.07 µl/min/pmol P450, respectively (Table 1), and CL\(_{\text{int}}\) for CYP3A5 was 12% (S.E.M. 1%) compared with CYP3A4.

CL\(_{\text{int}}\) of quetiapine by CYP3A4 was significantly different \((p < 0.0001)\) in microsomes with coexpressed cytochrome b\(_5\) compared with microsomes without cytochrome b\(_5\) (Table 1), whereas for CYP3A5 no difference in CL\(_{\text{int}}\) was observed \((p = 0.99)\).代谢模式

Measurement of the four metabolites after 40-min incubation revealed differences in metabolic pattern by CYP3A4 and CYP3A5 (Fig. 3; Table 2). The main differences between CYP3A4 and CYP3A5 were observed for the formation of quetiapine sulfoxide and O-desalkylquetiapine. The formation of quetiapine sulfoxide was more than 8-fold higher by CYP3A4 compared with CYP3A5 both in microsomes with and without cytochrome b\(_5\) (Table 2). The opposite was observed for O-desalkylquetiapine: the formation was approximately 1.5-fold and 2.5-fold higher by CYP3A5 compared with CYP3A4 in microsomes with or without cytochrome b\(_5\), respectively (Table 2). The formation of N-desalkylquetiapine and 7-hydroxyquetiapine was higher by CYP3A4 compared with CYP3A5 both in the presence or absence of cytochrome b\(_5\) (Table 2).

Discussion

This study demonstrates that quetiapine is metabolized by CYP3A5, but to a lesser degree than by CYP3A4. CL\(_{\text{int}}\) for quetiapine

<table>
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<tr>
<th>Microsomal preparation</th>
<th>CL(_{\text{int}}) µl/min/pmol P450</th>
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<tbody>
<tr>
<td>CYP3A4 + cyt b(_5)</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>8.8 ± 0.4</td>
</tr>
<tr>
<td>CYP3A5 + cyt b(_5)</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>1.07 ± 0.02</td>
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* Significantly different CL\(_{\text{int}}\) \((p < 0.0001)\).
by CYP3A5 was less than 35% relative to CYP3A4 both in microsomes with and without coexpressed cyt b5. Because CYP3A5 might constitute approximately 50% of total hepatic CYP3A protein in some individuals expressing CYP3A5 (Kuehl et al., 2001; Lin et al., 2002), theoretical clearance of quetiapine by CYP3A5 in vivo could accordingly be less than 35% relative to CYP3A4 in these individuals. Hence, this study indicates that CYP3A5 is of minor importance for the overall metabolism of quetiapine in vivo. A previous in vitro study by Lin et al. (2004) also implied similar results, because they reported the metabolism of quetiapine by CYP3A5 to be approximately 10% compared with CYP3A4. However, this result is somewhat uncertain because Lin et al. (2004) compared quetiapine metabolism in CYP3A5 microsomes not expressing cytochrome b5 with CYP3A4 microsomes coexpressing cytochrome b5.

CLint for quetiapine by the two enzymes varied depending on the microsomal system studied. The metabolism of quetiapine was significantly different in CYP3A4 microsomes with coexpressed cytochrome b5 compared with that in CYP3A4 microsomes without cytochrome b5, whereas in CYP3A5 microsomes quetiapine was equally well metabolized independent of the presence of cytochrome b5. It is interesting to note that the metabolism of quetiapine was more extensive in CYP3A4 microsomes without cytochrome b5; CLint was almost 3-fold higher in these microsomes. However, the opposite has been reported in several studies, where a considerably increased metabolism in microsomal systems including cytochrome b5 has been demonstrated. For instance, Klees et al. (2005) reported a 5- to 7-fold increase in CLint of alfentanil in CYP3A4 microsomes coexpressed with cytochrome b5. Furthermore, the formation of the major metabolite of vincristine through CYP3A5 was stimulated (3-fold) by the presence of coexpressed cytochrome b5 (Dennison et al., 2006). The conflicting results indicate a substrate-dependent impact of cytochrome b5, and this should be further evaluated for more substrates. In addition, it would also be relevant to study possible differences in composition of microsomal preparations, such as expression of cytochrome P450 reductase, which may contribute to the conflicting data on the metabolic impact of cytochrome b5 in vitro.

The metabolic pattern was different for metabolism of quetiapine by CYP3A5 compared with CYP3A4. The most important difference was observed for O-desalkylquetiapine, where the formation of O-desalkylquetiapine was higher by CYP3A5 compared with CYP3A4. Concurrently, CLint of quetiapine was lower by CYP3A5 than by CYP3A4. These results indicate that O-desalkylquetiapine constituted a higher proportion of the formed metabolites by CYP3A5 compared with CYP3A4 microsomes.
CYP3A4. Several studies of other substrates have also reported differences in the metabolic pattern by the two enzymes. For instance, Huang et al. (2004) found a higher formation ratio of major metabolite to minor metabolite of midazolam by CYP3A5 than by CYP3A4. A difference in the metabolic pattern of quetiapine by CYP3A4 and CYP3A5 may theoretically affect the clinical outcome associated to CYP3A5 genotype, despite a limited impact of CYP3A5 on overall metabolic clearance of quetiapine. The results of the present study indicate a possible higher serum concentration of O-desalkylquetiapine in patients expressing CYP3A5. However, the pharmacological and toxicological activity of O-desalkylquetiapine is not known, nor is the serum concentration range of this metabolite after therapeutic use of quetiapine.

The substrate depletion method was applied in this study because of solubility problems of quetiapine with the traditional metabolite formation method. Substrate depletion has some disadvantages compared with typical kinetic estimations based on metabolite formation with increasing substrate concentrations. At least 20% of the substrate should be metabolized within the incubation period to distinguish substrate depletion from baseline variability (Jones and Houston, 2004). Consequently, longer incubation times and higher enzyme concentrations are generally required (Jones and Houston, 2004). The depletion of quetiapine was less than 20% within the incubation period of 40 min in the CYP3A5 microsomes. However, longer incubation times (60 min) resulted in deviation from linearity of the log-transformed substrate depletion curves for CYP3A4 without cytochrome b5. In addition, the enzyme concentration was kept at a low level to reduce the risk of nonspecific binding.

The interindividual variability in serum concentration of quetiapine is substantial (Gerlach et al., 2007), but the results of the current study indicate that other factors besides genetic polymorphism in CYP3A5 are determinant for this variability. However, discrepancies between in vitro and in vivo data are well known for many substrates and could be caused by experimental conditions, interindividual variability, extrahepatic metabolism, or nonlinear kinetics (Tucker et al., 2001; Donato and Castell, 2003). For instance, polymorphism in CYP3A5 is shown to influence the systemic exposure and lipid-lowering response to simvastatin (Kivisto et al., 2004; Kim et al., 2007), although CYP3A4 possessed a 3- to 9-fold higher affinity than CYP3A5 for the formation of three major metabolites of simvastatin in vitro (Prueksaritanont et al., 1997). Consequently, the metabolic pattern of quetiapine in vivo is a factor involved in the interindividual variability in serum concentration of quetiapine.

In conclusion, this in vitro study indicates that CYP3A5 is of minor importance for the overall metabolism of quetiapine, independent of the presence of cytochrome b5 in the microsomal preparation. However, discrepancies between in vitro and in vivo data are common, and clinical studies are therefore necessary to exclude a possible impact of CYP3A5 on the metabolism of quetiapine in vivo. The metabolic pattern of quetiapine was different for metabolism by CYP3A4 compared with CYP3A5. This could possibly result in different pharmacological and/or toxicological effects of quetiapine in patients expressing CYP3A5.

References

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