METABOLISM OF THE ANTIDEPRESSANT MIRTAZAPINE IN VITRO: CONTRIBUTION OF CYTOCHROMES P-450 1A2, 2D6, AND 3A4

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ABSTRACT:

The metabolism of the antidepressant mirtazapine (MIR) was investigated in vitro using human liver microsomes (HLM) and recombinant enzymes. Mean \( K_m \) values (± S.D., \( n = 4 \)) were 136 (±44) \( \mu M \) for MIR-hydroxylation, 242 (±34) \( \mu M \) for \( N \)-demethylation, and 570 (±281) \( \mu M \) for \( N \)-oxidation in HLM. Based on the \( K_m \) and \( V_{\text{max}} \) values, MIR-N-hydroxylation, \( N \)-demethylation, and \( N \)-oxidation contributed 55, 35, and 10\%, respectively, to MIR biotransformation in HLM at an anticipated in vivo liver MIR concentration of 2 \( \mu M \). Recombinant CYP predicted a 65\% contribution of CYP2D6 to MIR-hydroxylation at 2 \( \mu M \) MIR, decreasing to 20\% at 250 \( \mu M \). CYP1A2 contribution increased correspondingly from 30 to 50\%. In HLM, quinidine and \( \alpha \)-naphthoflavone reduced MIR-hydroxylation to 75 and 45\% of control, respectively, at 250 \( \mu M \) MIR. A >50\% contribution of CYP3A4 to MIR-\( N \)-demethylation at <1 \( \mu M \) MIR was indicated by recombinant enzymes. In HLM, ketoconazole (1 \( \mu M \)) reduced \( N \)-desmethylmirtazapine formation rates to 60\% of control at 250 \( \mu M \). Twenty percent of MIR-\( N \)-oxidation was accounted for by CYP3A4 at 2 \( \mu M \) MIR, increasing to 85\% at 250 \( \mu M \), while CYP1A2 contribution decreased from 80 to 15\%. Ketoconazole reduced MIR-\( N \)-oxidation to 50\% of control at 250 \( \mu M \). MIR did not substantially inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP1E2, and CYP3A4 activity in vitro. Induction/inhibition or genetic polymorphisms of CYP2D6, CYP1A2, and CYP3A4 may affect MIR metabolism, but involvement of several enzymes in different metabolic pathways may prevent large alterations in in vivo drug clearance.

Mirtazapine (MIR) is a recently introduced antidepressant that differs in structure and mode of action from other compounds of its class (Shader et al., 1997). MIR antagonizes presynaptic \( \alpha_2 \)-receptors and postsynaptic 5-HT\(_2\) receptors (de Boer, 1996), as well as postsynaptic 5-HT\(_1\) receptors resulting in increased noradrenergic and serotonergic (5-HT\(_1\)) activity. MIR was shown to be superior to placebo and at least equally effective but often better tolerated compared with tricyclic antidepressants, selective serotonin reuptake inhibitors, or trazodone (Smith et al., 1990; van Moffaert et al., 1995; Burrows and Kremer, 1997; Holm and Markham, 1999; Thompson, 1999). Preliminary data suggest that MIR may also be beneficial in various other indications including sleep disturbances, anxiety disorders, and other subtypes of depression (Falkai, 1999).

Effective MIR doses are 15 to 45 mg per day, and its elimination half-life of 20 to 40 h allows once daily dosing. The drug is 85\% bound to plasma proteins. Peak plasma levels of 30 to 40 ng/ml are reached after 2 h, following administration of a single 15-mg dose (Timmer et al., 1995; Stimmel et al., 1997; Delbressine et al., 1998). MIR is extensively metabolized. The primary oxidative metabolites are 8-hydroxymirtazapine (OHM), \( N \)-desmethymirtazapine (DMM), and mirtazapine-N-oxide (MNO) (Kelder et al., 1997; Delbressine et al., 1998). The major metabolite in vivo is OHM, accounting for about 40\% of the excreted dose. DMM accounts for approximately 25\% of excreted MIR and is the only pharmacologically active metabolite. It is 5 to 10 times less potent than the parent compound and contributes only 3 to 6\% to the net pharmacologic activity of MIR. MIR-\( N \)-oxidation contributes about 10\% to MIR clearance in vivo (Delbressine et al., 1998). MIR metabolism is enantioselective, and primary metabolites undergo secondary metabolism and glucuronidation (Dahl et al., 1997; Delbressine et al., 1998). An additional metabolic pathway found in humans but not in animals is the formation of the quaternary MIR-\( N \)\(^{2}\)-glucuronide (Kelder et al., 1997; Delbressine et al., 1998). A previous in vitro study showed that MIR-hydroxylation is significantly correlated with CYP2D6 activity, while MIR-\( N \)-demethylation and MIR-\( N \)-oxidation correlated well with CYP3A4 activity in human liver microsomes (HLM) (Dahl et al., 1997). MIR clearance in vivo was similar in poor and extensive debrisoquine metabolizers (Dahl et al., 1997), indicating involvement of multiple cytochrome P-450 (CYP) isoenforms in MIR metabolism.

To anticipate metabolic drug interactions and to explore the relevance of polymorphisms of metabolic enzymes, the present study had the objective of identifying the CYP enzymes involved in MIR metabolism and estimating their contribution to the formation of OHM, DMM, and MNO using human liver microsomes and cDNA-expressed enzymes. The effect of MIR as a potential inhibitor of CYP...
isoform activity was assessed to evaluate the potential of MIR to interfere with the clearance of coadministered compounds.

Materials and Methods

**Chemicals.** MIR, DMM, OHM, and MNO were kindly provided by N.V. Organon (Oss, The Netherlands). Other drugs and chemicals were purchased from commercial sources or were kindly provided by their pharmaceutical manufacturers.

**Liver Samples and Microsome Preparation.** Healthy liver tissue was obtained from the International Institute for the Advancement of Medicine (Exton, PA) or the Liver Tissue Procurement and Distribution System (University of Minnesota, Minneapolis, MN). The tissue was kept at ~80°C until the time of microsome preparation. Microsomes were prepared and stored as described previously (von Moltke et al., 1993). Microsomal protein content was determined using the Bicinchoninic Acid Protein Assay (Pierce, Rockford, IL) and bovine serum albumin as a standard. The human liver samples were phenotyped for their CYP2D6 activity (dextromethorphan-O-demethylase at 10 μM), and no evidence for a poor metabolizer phenotype was found (mean activity, 177.5 nmol of dextrorphan/mg of protein/min; S.D., 67.1).

**cDNA-Expressed Enzymes.** Microsomes from cDNA-transfected human lymphoblastoid cells expressing CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 were obtained from Gentest Corp. (Woburn, MA).

**Incubations.** Human liver microsomes. Solutions of substrates and inhibitors were prepared in methanol and evaporated to dryness before addition of buffer and cofactors. Incubation mixtures contained 0.05 M KH_{2}PO_{4} buffer.
CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 were screened for their metabolic activity at 25 μM MIR. Those found to contribute at least 1% to one or more metabolic pathways were subjected to determination of enzyme kinetic parameters.

Incubations were done in duplicate, except for those using recombinant CYP. Identity of metabolites was verified by comparing the retention times to those of authentic standards. Solubility of MIR was evaluated for the concentration ranges used. Methanolic solutions of 50 to 2000 μM MIR were evaporated to dryness, reconstituted in incubation buffer, and analyzed by HPLC. Peak heights were linear up to 2000 μM MIR (r² = 0.9866). Metabolite formation was linear up to 40 min and 500 μg of protein/ml, respectively. Substrate consumption did not exceed 10% at any substrate concentration. Formation rates (v) were expressed in nanomoles per milligram of protein per minute in micromoles per picoMOL of CYP per minute where applicable.

**Calculation of Relative Contributions.** The relative contribution of each CYP isoform (i) to a particular metabolic pathway (p) was predicted as a function of substrate concentration (s), using the relative activity factor (RAF) (eq. 1) of each recombinant CYP and the reaction velocity [v_{max}] based on the enzyme kinetic parameters (K_{m}, V_{max}). Hill coefficient if applicable determined for each enzyme and pathway (n = number of isoforms catalyzing a pathway):

\[
f_{i}(%) = \frac{RAF_{i} \cdot v_{i}(s)}{\sum_{i=1}^{n} [RAF_{i} \cdot v_{i}(s)]} \times 100
\]

The relative contribution of each CYP isoform (i) to net human liver MIR biotransformation (f_{net,i}) was predicted as a function of substrate concentration (s), using the relative activity factor (RAF_{i}) (eq. 1) of each recombinant CYP and the reaction velocity [v_{max,i}] based on the enzyme kinetic parameters (K_{m,i}, V_{max,i}). Hill coefficient if applicable determined for each enzyme and pathway (n = number of isoforms catalyzing MIR biotransformation):

\[
f_{net,i}(%) = \frac{RAF_{i} \cdot v_{OHM}(s) + RAF_{i} \cdot v_{DMM}(s) + RAF_{i} \cdot v_{MNO}(s)}{\sum_{i=1}^{n} [RAF_{i} \cdot v_{OHM}(s) + RAF_{i} \cdot v_{DMM}(s) + RAF_{i} \cdot v_{MNO}(s)]} \times 100
\]

HPLC. MIR and its metabolites were separated using a 3.9 × 300-mm μBondapak (C18) 10-μm column (Waters Associates, Milford, MA) at 45°C. A fluorimetric detector (Perkin-Elmer 650-10S; Perkin-Elmer, Norwalk, CT) was set at 295 nm (excitation) and 365 nm (emission). The mobile phase consisted of 17% acetonitrile and 83% 0.05 M KH₂PO₄ (pH 3.5) and was delivered at a flow rate of 2 ml/min. Retention times were: OHM, 5.2 min; dextromethyl (internal standard), 6.1 min; DMM, 8.8 min; MIR, 10.5 min; and MNO, 13.5 min. Chromatograms were analyzed using the internal standard method and peak height ratios. The detection limits for OHM, DMM, and MNO were 0.25, 0.5, and 1 ng, respectively, injected directly onto the column. The intra-assay coefficient of variation for six identical samples was 5.5% for OHM (1.6 ng), 5.4% for DMM (6.6 ng), and 9.0% for MNO (2.3 ng). Samples were stable at room temperature for 3 days (c.v. < 10% for all metabolites). The mean coefficient of variation for duplicate MIR incubations with HLM was 5.4%.

**Inhibition of CYP Isoforms by MIR.** Index reactions used to study inhibition of distinct CYP isoforms by MIR were carried out and analyzed as described previously (Schmider et al., 1996b; von Molke et al., 1998a, 1999). Index compounds (dextromethorphan, phenacetin, tolbutamide, S-mephentoin, chlorzoxazone, triazolam) were incubated with HLM with increasing

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**Fig. 2. Effect of CYP-specific inhibitors on MIR metabolism at 25 and 250 μM MIR.**

Data represent formation rates relative to control activity (without inhibitor); mean ± standard deviation of four human livers. □, MIR-N-oxidation; □, MIR-N-demethylation; □, MIR-8-hydroxylation (pH 7.4 at 25°C), 0.5 mM NADP, 3.75 mM L-isoctric acid, 1 U/ml isocitrate dehydrogenase, and 5 mM Mg²⁺. Final volumes were 250 μl with a microsomal protein concentration of 250 μg/ml. MIR concentrations of 25 μM [possible in vivo concentrations following intentional (suicidal) or accidental MIR overdose (Gerritsen, 1997; Holzbach et al., 1998; Retz et al., 1998)] and 250 μM (approximate K_{m} for MIR biotransformation in HLM) were used for inhibition studies, and ranged from 2.5 μM [anticipated in vivo liver concentration based on data reported from autopsy studies (Anderson et al., 1999; Moore et al., 1999)] to 1500 μM for determination of enzyme kinetic parameters. MIR, competitive inhibitors, buffer, and cofactors were preincubated at 37°C for 5 min, and reactions were initiated by addition of microsomes. Diethylthiocarbamate was preincubated with buffer, cofactors, and microsomes for 20 min at 37°C, and the reaction was initiated by adding substrate. Incubation time was 30 min, and reactions were terminated by addition of 100 μl of acetonitrile and cooling on ice. Internal standard (dextrorphan) was added, samples were spun at 16,000g for 5 min, and supernatants were subjected to high performance liquid chromatography (HPLC).

**Heat inactivation.** To investigate the possible contribution of flavin-containing monoxygenases to metabolite formation, duplicate sets of samples containing buffer and microsomes were prepared. One set was supplemented with the NADPH-generating system, and both sets were incubated at 40°C for 5 min and then placed on ice (Grothausen et al., 1996).

**Recombinant enzymes.** Incubation mixtures were prepared as those for HLM with a final protein concentration of 500 μg/ml. Incubation time was 30 min except for CYP2D6, which was incubated for 5 min only to assure substrate consumption of <10%. MIR concentrations ranged from 2.5 to 1000 μM except for CYP2D6 (0.25–250 μM). Following the manufacturer’s instructions, phosphate buffer was replaced by Tris (0.05 M, pH 7.4) for CYP2A6 and CYP2C9. Formation rates obtained with recombinant CYP were normalized to human liver activity by calculating the relative activity factor (RAF) (Crespi and Penman, 1997; Venkatakrishnan et al., 1998):

\[
RAF_{isoform} = \frac{V_{max, HLM}[nmol/mg of protein/min]}{V_{max, recombinant CYP}[pmol/pmol of CYP/min]} (1)
\]

V_{max} values for the recombinant enzymes and for a pool of human liver microsomes were provided by Gentest Corp. Recombinant CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 were screened for their metabolic activity at 25 μM MIR. Those found to contribute at least 1% to one or more metabolic pathways were subjected to determination of enzyme kinetic parameters.

Incubations were done in duplicate, except for those using recombinant CYP. Identity of metabolites was verified by comparing the retention times to those of authentic standards. Solubility of MIR was evaluated for the concentration ranges used. Methanolic solutions of 50 to 2000 μM MIR were evaporated to dryness, reconstituted in incubation buffer, and analyzed by HPLC. Peak heights were linear up to 2000 μM MIR (r² = 0.9866). Metabolite formation was linear up to 40 min and 500 μg of protein/ml, respectively. Substrate consumption did not exceed 10% at any substrate concentration. Formation rates (v) were expressed in nanomoles per milligram of protein per minute in micromoles per picoMOL of CYP per minute where applicable.

**Calculation of Relative Contributions.** The relative contribution of each CYP isoform (i) to a particular metabolic pathway (p) was predicted as a function of substrate concentration (s), using the relative activity factor (RAF_{i}) (eq. 1) of each recombinant CYP and the reaction velocity [v_{max,i}] based on the enzyme kinetic parameters (K_{m,i}, V_{max,i}). Hill coefficient if applicable determined for each enzyme and pathway (n = number of isoforms catalyzing a pathway):

\[
f_{i}(%) = \frac{RAF_{i} \cdot v_{i}(s)}{\sum_{i=1}^{n} [RAF_{i} \cdot v_{i}(s)]} \times 100
\]

The relative contribution of each CYP isoform (i) to net human liver MIR biotransformation (f_{net,i}) was predicted as a function of substrate concentration (s), using the relative activity factor (RAF_{i}) (eq. 1) of each recombinant CYP and the reaction velocity [v_{max,i}] based on the enzyme kinetic parameters (K_{m,i}, V_{max,i}). Hill coefficient if applicable determined for each enzyme and pathway (n = number of isoforms catalyzing MIR biotransformation):

\[
f_{net,i}(%) = \frac{RAF_{i} \cdot v_{OHM}(s) + RAF_{i} \cdot v_{DMM}(s) + RAF_{i} \cdot v_{MNO}(s)}{\sum_{i=1}^{n} [RAF_{i} \cdot v_{OHM}(s) + RAF_{i} \cdot v_{DMM}(s) + RAF_{i} \cdot v_{MNO}(s)]} \times 100
\]
MIR-n-demethylation was consistent with the Hill equation (Fig. 1c) or the Michaelis-Menten equation with mean $K_m$ and $V_{max}$ values of 570 ($\pm 281$) $\mu$M and 0.64 ($\pm 0.27$) nmol/mg of protein/min (Table 1). Ketoconazole reduced the reaction velocity to 50% of control at 25 $\mu$M MIR (Fig. 2).

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At 2 $\mu$M MIR, a concentration consistent with anticipated in vivo liver MIR concentrations, MIR-8-hydroxylation, N-demethylation, and N-oxidation contributed 60, 30, and 10%, respectively, to MIR biotransformation in HLM (Fig. 3); values were 25, 60, and 15%, respectively, at 250 $\mu$M MIR.

Recombinant Enzymes. Screening of recombinant CYP for their metabolic activity at 250 $\mu$M MIR indicated that CYP1A2, CYP2C8, CYP2C9, CYP2D6, and CYP3A4 contributed at least 1% to one or more metabolic pathways. Enzyme kinetic parameters were determined (Table 2), and data were normalized using the relative activity factor approach (Crespi and Penman, 1997; Venkatakrishnan et al., 1998) (eq. 1). Formation rates from cDNA-expressed enzymes were multiplied by the RAF for the respective isoform (CYP1A2, 5.13; CYP2C8, 1.15; CYP2C9, 0.71; CYP2D6, 0.28; and CYP3A4, 3.11) and normalized to 100% over a concentration range of 0.1 to 250 $\mu$M MIR.

OHM was formed by recombinant CYP2D6, CYP1A2, CYP3A4, and CYP2C9 (Fig. 4). The RAF approach predicted a 65% contribution of CYP2D6 to MIR-hydroxylation at 2 $\mu$M MIR, a concentration consistent with anticipated in vivo liver drug concentrations, decreasing to 20% at 250 $\mu$M MIR. Contribution of CYP1A2 increased correspondingly from 30 to 50%. CYP3A4 contributed 10 to 20% to the reaction, and CYP2C9 was involved at high MIR concentrations only (10% at 250 $\mu$M MIR) (Fig. 5a).

MIR-N-demethylation was mediated by recombinant CYP3A4, CYP2C8, and CYP1A2 (Fig. 4). A 50 to 70% contribution of CYP3A4 over a concentration range of 1 to 250 $\mu$M MIR was predicted. The contribution of CYP1A2 decreased from 50% at 2 $\mu$M MIR to 5% at 250 $\mu$M. CYP2C8 and CYP2C9 appeared to be of minor importance, accounting for <20 and <5%, respectively, to the reaction (Fig. 5b).

MIR-N-oxidation was catalyzed by recombinant CYP1A2 and CYP3A4 (Fig. 4). At 2 $\mu$M MIR, the predicted CYP1A2 contribution is 80%, decreasing to 15% at 250 $\mu$M MIR. Correspondingly, the contribution of CYP3A4 increased from 20 to 85% (Fig. 5c).
These results indicate that CYP3A4, CYP1A2, and CYP2D6 contribute 25 to 45% each to net clearance of MIR through all three metabolic pathways at anticipated in vivo liver MIR concentrations (2 μM), while CYP2C8 and CYP2C9 account for less than 10% of MIR biotransformation. With increasing MIR concentrations, CYP3A4 contribution increases to 70% at 250 μM MIR, while CYP2D6, CYP2C8, CYP2C9, and CYP1A2 account for less than 15% each (Fig. 5d).

Inhibition of Cytochromes P-450 by Mirtazapine. MIR concentrations equimolar to the concentration of index substrate (250 μM) reduced triazolam-4-hydroxylation (reflecting CYP3A activity) in HLM to 19% of control (±5% S.D., n = 4). However, the IC50 value of MIR (37.1 ± 36.9 μM) versus CYP3A4 was 3 orders of magnitude higher than that for ketoconazole (0.07 ± 0.02 μM), indicating only a modest inhibitory capacity of MIR. Index reactions reflecting CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP2E1 activity were not substantially affected (Table 3).

Discussion

In vitro incubations of MIR with human liver microsomes and recombinant CYP led to formation of OHM, DMM, and MNO (Fig. 1); these represent the main MIR metabolites also found in vivo (Delbressine and Vos, 1997; Delbressine et al., 1998). Application of the relative activity factor approach (Crespi and Penman, 1997; Venkataraman et al., 1998) (eq. 1) allowed quantitative predictions of the contribution of each enzyme to a particular pathway of MIR biotransformation. To apply these in vitro data to the situation in vivo, an estimation of intrahepatic drug concentrations in vivo is critical. Multiplication of plasma drug concentrations with a scaling factor reflecting partitioning of the drug from plasma into liver tissue (Obach et al., 1997; von Moltke et al., 1998b; Schmider et al., 1999) provides an estimate of liver concentration. Two autopsy studies (total number of 21 cases) reported post-mortem liver MIR concentrations of 5- to 30-fold (mean 10-fold) higher than the concentration in peripheral blood (Anderson et al., 1999; Moore et al., 1999). Steady-state peak plasma MIR concentrations in a regimen of 15 to 45 mg of MIR/day range from 39 to 115 μg/l, equivalent to 0.1 to 0.4 μmol/l (Timmer et al., 1995). Based on the above-mentioned 10-fold difference between blood and liver concentration, we estimated liver MIR concentrations of 1 to 4 μmol/l, which is reflected by the lowest MIR concentration of 2.5 μM used in HLM in this study.

The major in vitro metabolite at anticipated in vivo MIR concentrations was OHM, accounting for an average 60% of total MIR biotransformation (Fig. 3). DMM contributed about 30%, and a 10% contribution of MIR-N-oxidation was extrapolated from the kinetic parameters determined for the reaction, although MNO formation was below the detection limit at MIR concentrations <25 μM. These findings are in excellent agreement with previous in vivo data demonstrating that 40, 25, and 10% of a single MIR dose were eliminated in the urine of healthy volunteers as OHM, DMM, and MNO, respectively. The remaining 25% of the in vivo MIR clearance was mainly accounted for by direct MIR-N'-glucuronidation (Delbressine et al., 1998), a pathway not observed under the conditions of this in vitro study.

MIR-8-hydroxylation was mainly mediated by CYP2D6 at low MIR concentrations, while recombinant enzymes indicated an increasing contribution of CYP1A2 with increasing MIR concentrations (Fig. 5a). Chemical inhibition studies in HLM supported these findings. At 25 μM MIR, quinidine (5 μM) was a more potent inhibitor than α-naphthoflavone (0.5 μM), reducing formation rates to 60 and 80% of control, respectively. At 250 μM MIR, α-naphthoflavone reduced OHM formation rates to 50% of control compared with 70% for quinidine, thus confirming the concentration-dependent changes in CYP2D6 and CYP1A2 contribution to MIR-8-hydroxylation. Although at least two enzymes are involved in the formation of OHM, the major MIR metabolite, in CYP2D6 extensive HLM, the statistically favored mathematical model is the one-enzyme Michaelis-Menten equation. Presumably, the net contribution of CYP2D6 to OHM formation in vitro over the complete MIR concentration range necessary to attain Vmax (up to 1500 μM MIR) is too small to justify the introduction of two additional parameters into the model equation (Yamaoka et al., 1978; Schmider et al., 1996a). However, the Km value determined for recombinant CYP1A2 falls within the range determined for OHM formation in HLM. Despite its low contribution at in vivo MIR concentrations, CYP1A2 was the major MIR-hydroxylating enzyme at MIR concentrations above 50 μM, and therefore determines the Km of the pathway in vitro.

The major enzyme catalyzing MIR-N-demethylation was CYP3A4 with a >50% contribution at concentrations above 1 μM MIR. CYP1A2 contributed 45% to the reaction at in vivo concentrations, while CYP2C8 became partially involved at concentrations above 25 μM MIR only (Fig. 5b). This is consistent with the reduction in reaction velocity to approximately 60% of control observed with ketoconazole (1 μM) at both 25 and 250 μM MIR, while other CYP-specific inhibitors had no substantial effect (Fig. 2). The Km value determined for recombinant CYP3A4 falls within the range determined for DMM formation in HLM.

For MIR-N-oxidation, recombinant enzymes predicted a major
contribution of CYP1A2 at anticipated in vivo MIR concentrations and a major role of CYP3A4 for concentrations above 25 μM (Fig. 5c). MNO formation was not detectable at MIR concentrations below 25 μM in HLM, and inhibition experiments conducted at 250 μM showed a decrease in MNO formation rates by ketoconazole (1 μM) to 50% of control. The high $K_m$ value for MNO formation in HLM compared with the value determined for recombinant CYP3A4 could indicate involvement of another low-affinity enzyme. However, flavin-containing monoxygenases, catalyzing N- and S-oxidations of various compounds (Ziegler, 1988), were ruled out because heat treatment of HLM (Grothusen et al., 1996) did not affect MNO formation. CYP3A4 seems to be the major enzyme at high MIR concentrations with a main CYP1A2 contribution at low MIR concentrations. MIR-N-oxidation is a minor pathway of MIR biotransformation accounting for about 10% of MIR clearance in vitro and in vivo.

Formation rate patterns of OHM were consistent with Michaelis-Menten kinetics in HLM but with Hill kinetics ($\alpha = 1.9$) in recombinant CYP2C9 (Table 2). Substrate consumption may cause an apparent sigmoid relationship of substrate concentration and metabolite formation. However, substrate consumption did not exceed 10% and is therefore unlikely as a cause of this finding. Presumably, the complexity of the microsomal enzyme system (e.g., cytochrome $b_5$ or reductase concentration) can only be partly reconstituted by cDNA-expressed enzymes resulting in differences in formation rate patterns. Similar reasons may explain the differences associated with the formation of OHM (Michaelis-Menten), DMM (Hill, $\alpha = 1.35$), and MNO (Hill, $\alpha = 1.72$) observed with recombinant CYP3A4 (Table 2).

Our findings are consistent with a previous in vitro correlation study which demonstrated that MIR-hydroxylation is significantly associated with CYP2D6 activity, while MIR-N-demethylation and MIR-N-oxidation correlated well with CYP3A4 activity in HLM (Dahl et al., 1997).

Methods other than the RAF approach, such as immunologically determined abundances of CYP isoforms in human liver (Shimada et al., 1994), can be used to apply in vitro data to in vivo situations. Estimated relative contributions will vary depending on the method used, and in any case can only provide an approximation of the true situation. In this study, we applied RAFs because the results were more consistent with inhibition data from HLM.

Summarizing the results obtained with recombinant CYP, MIR biotransformation through all three metabolic pathways appears to be almost equally distributed between CYP3A4, CYP2D6, and CYP1A2, each contributing 25 to 45% to net MIR clearance (Fig. 5d). Alterations of the activity of these enzymes by coadministered compounds or by genetic polymorphisms of a particular CYP isoform may there-

![Fig. 4. Formation of OHM, DMM, and MNO by cDNA-expressed CYP.](image-url)
fore alter MIR biotransformation. However, due to the involvement of three different enzymes, even complete inhibition or deficiency of one isoform is unlikely to result in a clinically significant increase in MIR plasma concentrations, a situation similar to that previously described for sertraline, a selective serotonin reuptake inhibitor (Greenblatt et al., 1999; Kobayashi et al., 1999). This is supported by preliminary data indicating that CYP2D6 phenotype does not influence MIR clearance in vivo (Dahl et al., 1997). Although a therapeutic range of plasma MIR concentrations has not been defined yet, the drug was shown to be safe in overdose and in several degrees of renal failure (Bengtsson et al., 1998; Bremner et al., 1998).

MIR did not substantially inhibit index reactions reflecting CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP2E1 activity in HLM (Table 3). MIR did produce a modest degree of inhibition of CYP3A activity, with an IC₅₀ value of 37 μM versus triazolam hydroxylation. The clinical significance of this finding is not established. In any case, our findings are consistent with a previous study that reported Kᵢ values of MIR for inhibition of 7-ethoxyresorufin-O-dealkylation (CYP1A2), bufuralol-1′-hydroxylation (CYP2D6), and testosterone-6β-hydroxylation (CYP3A4) several orders of magnitude higher than those of known index inhibitors of the respective enzyme (Dahl et al., 1997).

In conclusion, the novel antidepressant MIR appears to carry a low risk for drug interactions with respect to both the susceptibility of its

**Fig. 5. Relative contribution of CYP isoforms to MIR-8-hydroxylation, MIR-N-demethylation, and MIR-N-oxidation.**

Left panel, 0 to 250 μM MIR; right panel, 0 to 5 μM MIR, range of anticipated in vivo liver MIR concentration. a, mirtazapine-8-hydroxylation (eq. 2); b, mirtazapine-N-demethylation (eq. 2); c, mirtazapine-N-oxidation (eq. 2); d, net clearance of MIR by pathways a, b, and c (eq. 3). Calculations were based on kinetic parameters displayed in Table 2.
own metabolism to enzyme inhibition or genetic deficiency as well as its potential to alter the clearance of other CYP metabolized compounds. However, no in vivo drug interaction data are available to date, and caution dictates that clinical trials are important to verify the conclusions drawn from in vitro experiments.

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References