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Identification of Cytochrome P450 Isoforms Involved in the Metabolism of Paroxetine and Estimation of Their Importance for Human Paroxetine Metabolism using a Population-Based Simulator

Jakob Jornil, Klaus Gjervig Jensen, Frank Larsen and Kristian Linnet

Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health Sciences, University of Copenhagen, Denmark (J.J., K.L.); Drug ADME Research, H. Lundbeck A/S, Denmark (K.G.J.); Clinical Pharmacology and Pharmacokinetics, H. Lundbeck A/S, Denmark (F.L.)
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CYTOCHROME P450 ISOFORMS INVOLVED IN PAROXETINE METABOLISM

Address correspondence to: Jakob Jornil, Forensic Chemistry, Frederiks V’s vej 11, 2100 Copenhagen O, Denmark. Tel.: +4528756259, Fax: +4535326085. E-mail: jakob.jornil@forensic.ku.dk

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List of nonstandard abbreviations used: CLpo, oral clearance; COMT, catechol-O-methyltransferase; CYP, Cytochrome P450; EM, extensive metabolizer; IVIVE, in vitro in vivo extrapolation; fu, fraction unbound; HLM, human liver microsomes; ICC, insect cell control microsomes; ICM, baculovirus-infected insect cell microsomes; IVIVE, in vitro in vivo extrapolation; LC, liquid chromatography; LOQ, limit of quantification; MAB, inhibitory monoclonal antibodies; MBI, mechanism-based inhibition; MM, Michaelis-Menten; MS/MS, tandem mass spectrometry; pHLM, pooled human liver microsomes; PM, poor metabolizer; RAF, relative activity factors; sdHLM, single donor human liver microsomes; SOD, superoxide dismutase; SS, steady state.
Abstract

We identify here for the first time the low-affinity CYP isoforms that metabolize paroxetine, using cDNA-expressed human CYPs measuring substrate depletion and paroxetine-catechol (product) formation by liquid chromatography tandem mass spectrometry. CYP1A2, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 were identified as paroxetine-catechol–forming CYP isoforms, and CYP2C19 and CYP2D6 were identified as metabolizing CYP isoforms by substrate depletion. Michaelis-Menten constants $K_m$ and $V_{\text{max}}$ were determined by product formation and substrate depletion. Using selective inhibitory studies and a relative activity factor approach for pooled and single-donor human liver microsomes, we confirmed involvement of the identified CYP isoforms for paroxetine-catechol formation at 1 and 20 µM paroxetine. In addition, we used the population based simulator Simcyp® to estimate the importance of the identified paroxetine metabolizing CYP isoforms for human metabolism taking mechanism based inhibition into account. The amount of active hepatic CYP2D6 and CYP3A4 (not inactivated by mechanism based inhibition) was also estimated by Simcyp®. For extensive and poor metabolizers of CYP2D6, Simcyp®-estimated pharmacokinetic profiles were in good agreement with those reported in published in vivo studies. Considering the kinetic parameters, inhibition results, relative activity factor calculations, and Simcyp® simulations, CYP2D6 (high affinity) and CYP3A4 (low affinity) are most likely to be the major contributors to paroxetine metabolism in humans. For some individuals CYP1A2 could be of importance for paroxetine metabolism, whereas the importance of CYP2C19 and CYP3A5 is probably limited.
Introduction

Paroxetine is a selective serotonin reuptake inhibitor used for the treatment of depression, generalized anxiety disorder, obsessive-compulsive disorder, panic disorder, post-traumatic stress disorder, and social anxiety disorder. It is extensively metabolized in humans and exhibits non-linear kinetics (Haddock et al., 1989; Kaye et al., 1989). After administration of a single dose of paroxetine, poor metabolizers (PMs) and extensive metabolizers (EMs) of CYP2D6 display a 7-fold difference in the median total clearance. Under steady-state (SS) conditions, this difference falls dramatically to 2-fold. Non-linear paroxetine kinetics is more prominent in EMs of CYP2D6 than PMs of CYP2D6 (Sindrup et al., 1992a; Sindrup et al., 1992b).

Fig. 1 shows the major reported CYP metabolic pathways. CYP2D6 catalyzes demethylation of the methylenedioxy group, presumably yielding paroxetine-catechol (Haddock et al., 1989) and formate (Bloomer et al., 1992). The paroxetine-catechol metabolite is described as an unstable intermediate (Kaye et al., 1989). Catechol-O-methyltransferase (COMT) enzymes methylate paroxetine-catechol (Maurer et al., 2000), yielding metabolites I and II. In humans, metabolites I and II are found as the conjugated glucuronide or sulfate conjugates in urine, with metabolite I as the main metabolite (Haddock et al., 1989). CYP2D6 has been identified as a high-affinity paroxetine-metabolizing enzyme, and because PMs of CYP2D6 also metabolize paroxetine, a low-affinity CYP isoform or CYP isoforms must exist that have yet to be identified (Bloomer et al., 1992; Sindrup et al., 1992a; Sindrup et al., 1992b).

Paroxetine is a mechanism-based inhibitor of CYP2D6 (Bertelsen et al., 2003) and CYP3A (Obach et al., 2007). The mechanism-based inhibition (MBI) is most likely caused by an irreversible binding of a paroxetine-reactive metabolite, one that does not leave the active site, to the heme complex in the CYP enzyme (Bertelsen et al., 2003; Zhao et al.,...
2007). The MBI kinetic constants ($K_{\text{act}}$, $K_i$) have been determined for CYP2D6 and CYP3A (Bertelsen et al., 2003; Obach et al., 2007). With the MBI kinetic constants for CYP2D6 and the EM data from Sindrup et al. (1992b), Venkatakrishnan and Obach (2005) used in vitro/in vivo extrapolation (IVIVE) to successfully predict the pharmacokinetic consequences of CYP2D6 inactivation by paroxetine. Sindrup et al. observed a 5-fold decrease in clearance under SS conditions compared to the single-dose situation for EMs of CYP2D6 (1992b). All of this points towards the MBI of CYP2D6 as an explanation, one that is further supported by the lack of decrease in clearance from single dose to SS conditions for the PMs of CYP2D6 (Sindrup et al., 1992b).

To our knowledge, the identity of the low-affinity paroxetine-metabolizing CYP isoform(s) remains unknown, but indirect evidence from Kuss and Hegerl (1998) points to involvement of CYP3A4; they found that patients co-medicated with carbamazepine (a CYP3A4 inducer) had about half the paroxetine concentration levels of patients not treated with carbamazepine. This is supported by results from van der Lee et al (2007) who state that combing phenytoin (a CYP3A4 inducer) with paroxetine decreased paroxetine levels. These inferences are also supported by the finding that paroxetine is a weak MBI of CYP3A (Obach et al., 2007), especially given that the reactive species most likely does not leave the active site before inactivation (Zhao et al., 2007), also suggesting involvement of CYP3A4 in paroxetine metabolism.

The objective of the present study was to identify additional CYP isoforms that metabolize paroxetine and estimate their kinetic parameters. We investigated 11 CYP isoforms for paroxetine metabolic capability in vitro by substrate depletion and paroxetine-catechol (product) formation. The kinetic parameters $K_m$ and $V_{\text{max}}$ of the metabolizing CYP isoforms were estimated by paroxetine depletion and product formation; such information is sparsely available in the literature and exists only for CYP2D6. Using selective inhibitor
studies in human liver microsomes (HLMs) and comparing the results to those obtained using a relative activity factor (RAF) approach, we investigated the validity of the obtained kinetic parameters. Finally, in order to estimate the importance of the CYP isoforms for human paroxetine metabolism an IVIVE of paroxetine metabolism was performed using the population-based simulator Simcyp® to generate pharmacokinetic simulations that incorporate aspects of MBI. These pharmacokinetic simulations were compared with existing in vivo pharmacokinetic data for paroxetine to validate the simulations in EM and PM CYP2D6 population groups. Comprehensive identification of the CYP isoforms involved in the metabolism of paroxetine, estimation of their kinetic parameters, and subsequent integration into a physiologically based pharmacokinetic model such as Simcyp® will provide a platform for a better fundamental understanding of paroxetine pharmacokinetics in vitro as well as in the clinic.
Materials and Methods

Chemicals and Reagents. Paroxetine was obtained from GlaxoSmithKline (Worthing, West Sussex, UK); 0.1 mg/ml paroxetine-D6 in methanol from Cerilliant (Round Rock, TX, USA); Z-10-OH-amitriptyline from H. Lundbeck A/S (Valby, Denmark); furafylline, quinidine, ketoconazole, NADP+, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and superoxide dismutase (SOD) from bovine erythrocytes from Sigma-Aldrich (St. Louis, MO, USA); paroxetine-catechol synthesized at H. Lundbeck A/S (to be published); pooled HLMs (pHLMs) (pool of 30 individuals), four single-donor HLMs (sdHLMs), human liver cytosol, baculovirus-infected insect cell microsomes (ICMs; Supersomes) containing the cDNA-expressed CYP-isoenzymes CYP1A2, 2A6*, 2B6*, 2C8*, 2C9*, 2C18, 2C19*, 2D6, 2E1*, 3A4*, and 3A5* (*with cytochrome b5), insect cell control microsomes (ICC; Supersomes), and inhibitory monoclonal antibodies (MABs) to CYP2C19 all from BD Biosciences (Woburn, MA, USA). The microsomal and cDNA preparations were stored at -80°C until use. Stock solutions of compounds were prepared in methanol and stored at 4°C. Paroxetine-catechol stock solution was made in acidic acetonitrile:water (1:1) (30 µl formic acid per 10 ml solution) and stored at -80°C. All other reagents were of analytical grade. The four sdHLMs (1–4) had the following characteristics: (1) PM CYP2D6, high activity of CYP3A4; (2) PM CYP2D6; (3) high activity of CYP2D6; (4) high activity of CYP2C19 and low CYP2D6 activity.

Microsomal Incubations for Paroxetine Depletion. A Tecan Genesis RMP200 (Tecan AG, Hombrechtikon, Switzerland) was used to perform in vitro experiments in polypropylene 96-well plates at 37°C. The reaction mixture consisted of a 1.3-mM NADPH-regenerating system, 3.3 mM glucose-6-phosphate, 0.4 U/ml glucose-6-phosphate dehydrogenase, 7 mM magnesium chloride, and 0.1 M phosphate buffer at pH 7.4. Incubations were done in a final volume of 100 µl. Microsomes were diluted in the reaction mixture prior to use. Background
depletion controls with the same protein concentration as the microsomes were made with ICC. Substrate was prepared by diluting stock solutions in the reaction mixture. Reactions were started by mixing 90 µl microsomes dissolved in reaction mixture at 37°C with 10 µl substrate. The final methanol concentration in the assay was below 0.5% (v/v), and assays in the same series had the same methanol concentration. Reactions were stopped by mixing the reaction solution (100 µl) with 100 µl acetonitrile internal standard, whereupon the solution was transferred to a sealed polypropylene 96-well plate. The well plate was centrifuged at 2000 ×g at 4°C for 10 min before analysis.

**Microsomal Incubations for Paroxetine-Catechol Formation.** In vitro assays were carried out in polypropylene 96-well plates at 37°C. The reaction mixture was as described above with the modifications that a concentration of 3.3 mM magnesium chloride was used and 200 U/ml SOD was added. Incubations were done in a final volume of 156 µL. Microsomes were diluted with 0.1 M phosphate buffer pH 7.4 prior to use. Substrate, inhibitors (quinidine or ketoconazole), SOD, and the NADPH-regenerating system were preincubated at 37°C for 5 min. Reactions were started by addition of 10 µl ice-cold microsomes, and the reaction time was 5 min. The final methanol concentration in the assay was below 0.5% (v/v), and assays used for comparison had the same methanol concentration. The reaction was stopped by transferring 30 µl reaction solution to 30 µl internal standard in a polypropylene 96-well plate. The well plate was sealed and centrifuged at 2000 ×g at 4°C for 10 min. Because of background formation of paroxetine-catechol, for cDNA-expressed microsomes, this background formation was estimated in an assay system without the NADPH-regenerating system. The HLM paroxetine-catechol background formation was estimated in an assay system without HLM.

**Liquid Chromatography/Tandem Mass Spectrometry Analysis for Paroxetine-Depletion Experiments.** Analysis was done on an Agilent 1100 (Agilent Technologies,
Waldbornn, Germany) liquid chromatography (LC) system with an HTC PAL autosampler (CTC Analytics, Zwingen, Switzerland) and an Applied Biosystems API 4000 (MDS Sciex, Concord, Ontario, Canada) tandem mass spectrometry (MS/MS) system using Analyst 1.4.2 software. The analytical column was a Waters (Waters, Millford, MA, USA) XTerra C18 (2.5 µm, 20 x 2.1 mm) operated at 60°C. The gradient consisted of 0.1% formic acid in a 95/5% water/acetonitrile solution (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) at a flow rate of 0.8 ml/min, 0.00–0.50 min (0→95% B), 0.50–1.00 min (95% B), 1.00–1.10 min (95→0% B), and 1.10–2.00 min (0% B). The injected sample volume was 10 µl. The mass spectrometer was operated in positive electrospray mode with a 5-kV ionization potential and an ion source temperature of 600°C. The analytes were quantified in multiple reaction-monitoring mode. The mass transitions used were m/z: paroxetine (330.10→70.20) and paroxetine-D6 (336.00→76.10). Dwell time was 30 ms. The internal standard was 0.22 µM paroxetine-D6 (internal standard to paroxetine) dissolved in acidic acetonitrile (30 µl formic acid per 10 ml acetonitrile). Calibration standards at six levels (0.01, 0.05, 0.24, 1.2, 6.1, and 30.4 µM) were prepared daily by addition of stock solution of paroxetine (3150 µM) to the acidic reaction mixture (30 µl formic acid per 10 ml reaction mixture) for serial dilutions. Calibration standards were analyzed in duplicate, and quality control samples at four levels were run daily (paroxetine concentrations of ~0.02, ~0.1, ~1, and ~5 µM) and checked to confirm that they were at the nominal value, as was the paroxetine concentration in the assay. Nonlinear regression and statistical analysis were done with GraphPad Prism 5.00 (La Jolla, CA, USA). Nonlinear regression was used to evaluate whether a straight line or a quadratic curve was the best calibration model using 1/x weighting.

**LC/MS/MS Analysis for Paroxetine-Catechol Formation.** Analysis was done on an Agilent 1100 system with a microwell plate sampler (Agilent Technologies, Waldbronn, Germany) and a Quatro Micro (Waters, Millford, MA, USA) LC/MS/MS system using
MassLynx 4.1 software. The analytical column was a Waters Xbridge C18 2.5 µm 2.1 x 50 mm operated at 50°C. The solutions for the gradient were as described above; the gradient was 0.0–0.1 min (0→28% B), 0.1–1.0 (28→35% B), 1.0–2.0 min (35→90% B), 2.0–2.5 min (90% B), 2.5–2.6 (90→0 %B), and 2.6–5.0 (0% B). The flow was 0.4 ml/min, and the injection volume was 10 µl. The mass spectrometer was operated in positive electrospray mode with a 3-kV ionization potential and an ion source temperature of 500°C. The analytes were quantified in multiple reaction–monitoring mode. The mass transitions used were m/z: paroxetine-catechol (318.06→70.00), Z-10-OH-amitriptyline (294.30→57.80), paroxetine (330.06→70.00), and paroxetine-D6 (336.06→76.00). Dwell time was 150 ms. The internal standards were 0.34 µM Z-10-OH-amitriptyline (internal standard to paroxetine-catechol) and 0.22 µM paroxetine-D6 (internal standard to paroxetine) dissolved in acetonitrile containing 30 µl formic acid per 10 ml acetonitrile and 2 mM ascorbic acid. Calibration standards at six levels for catechol (0.0015, 0.0030, 0.015, 0.073, 0.37, 1.85 µM) and seven levels for paroxetine (0.004, 0.008, 0.043, 0.21, 1.06, 5.3, 26.6 µM) were prepared daily by serial dilution of stock solutions of paroxetine (3150 µM) and paroxetine-catechol (1019 µM) added to 0.1 mM phosphate buffer (as described above) containing 30 µl formic acid per 10 ml phosphate buffer and 1 mM ascorbic acid. All quantifications were done so that the samples for the calibration curve had the same matrix (except the NADPH-regenerating system) as the assay samples for quantification. Calibration standards were analyzed in duplicate, and quality control samples containing both paroxetine and paroxetine-catechol at four levels were run daily and checked for confirmation that they were at their nominal value, as was the concentration of paroxetine in the assay. Nonlinear regression was used to evaluate whether a straight line or a quadratic curve was the best calibration model using 1/x weighting.

Substrate Depletion Screening Studies. Incubations were performed with 0.2 and 1 µM paroxetine and 54 pmol/ml CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4,
and 3A5. The paroxetine concentration was measured after 0, 30, 60, 90, and 120 min incubation.

**Paroxetine-Catechol Formation Screening Studies.** Incubations were performed at 50 µM paroxetine and 50 pmol/ml CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, 3A5, pHLM, and human liver cytosol. Paroxetine-catechol formation was measured after 1, 6, 11, and 16 min incubation.

**Substrate Depletion Kinetic Studies.** Kinetic constants estimated by substrate depletion studies were derived at the following CYP concentrations, paroxetine concentrations, and incubation times, respectively: 2.25 pmol/ml CYP2D6, 0.03, 0.05, 0.1, and 0.2 µM, and 5, 10, and 15 min; 9 and 18 pmol/ml CYP2D6, 1 and 3 µM, and 5, 10, and 15 min; 36 pmol/ml CYP2C19, 0.03, 0.05, 0.1, 0.2, 0.5, 1, 3, and 10 µM, and 5, 15, 25, and 35 min. Experiments were done in triplicate. The first-order depletion constant $k$ was estimated at different paroxetine concentrations according to eq. 1 with the Excel trendline function.

$$[S]_t = [S]_0 e^{-kt} \quad (1)$$

To obtain the unbiased first-order depletion constant, the background depletion constant was subtracted from the ICM depletion constant. $k_{dep} \ [\mu l / min / pmol CYP]$ was obtained by dividing $k$ (corrected for background loss) with the concentration (pmol CYP/µl) of the microsome. $K_m$ and $V_{max}$ were estimated by nonlinear regression using eq. 2 proposed by Nath and Atkins (2006).

$$k_{dep} = \frac{V_{max}}{K_m} \left(1 - \frac{[S]}{[S] + K_m} \right) \quad (2)$$
**Paroxetine-Catechol Formation Kinetic Studies.** Incubations used to derive kinetic constants by paroxetine-catechol formation had the following CYP/HLM and paroxetine concentrations: CYP1A2 45 pmol/ml, CYP2C19 20 pmol/ml, CYP3A4 20 pmol/ml, CYP3A5 50 pmol/ml, and 0.61, 1.3, 2.2, 3.6, 6.1, 9, 12, 15, 20, 30, 51, 73, 103, and 152 µM paroxetine; CYP2D6 1.5 pmol/ml, and 0.0061, 0.010, 0.017, 0.028, 0.047, 0.079, 0.13, 0.22, 0.36, 0.61, 1.21, 2.19, 3.64, and 6.07 µM paroxetine; pHLM and sdHLM(1) (PM2D6 HLM) 0.20 mg protein/ml, and 0.010, 0.028, 0.079, 0.22, 0.61, 1.3, 3.6, 6.1, 15, 30, 51, 73, 103, and 152 µM paroxetine. Incubation time was 5 min, and all experiments were done with duplicates of microsomes and background formation samples. Background formation of paroxetine-catechol was subtracted from the formation in microsomes to obtain the amount of actively formed paroxetine-catechol. The paroxetine concentration was calculated by a geometric mean (eq. 3) if the depletion was above 20%:

\[
S = \frac{S_i - S_e}{\ln S_i - \ln S_e} \quad (3)
\]

where \(S_i\) is the initial concentration and \(S_e\) is the final paroxetine concentration.

Apparent \(K_m\) and \(V_{\text{max}}\) were estimated by nonlinear regression using Michaelis-Menten (MM) kinetics (eq. 4), a biphasic kinetic relation (two-site binding model, eq. 5), or a Michaelis-Menten model with uncompetitive inhibition by substrate (eq. 6. Copeland, 2000) where \(K_s\) is the inhibition constant for uncompetitive inhibition:

\[
v = \frac{V_{\text{max}} S}{K_m + S} \quad (4)
\]
Biphasic kinetics (eq. 5) was used if Eadie-Hofstee plots (Clarke, 1998) and a significance test (F-test, $p < 0.05$) indicated that this model was best to describe the paroxetine-catechol formation. Kinetics was described by a substrate inhibition model (eq. 6) if a significance test (F-test, $p < 0.05$) indicated this.

$K_m$ and $K_s$ values were corrected by $f_u$ (fraction unbound), estimated by extrapolation of paroxetine binding results for HLM by Hemeryck et al. (2001).

**Role of Individual CYP Isoforms in Paroxetine-Catechol Formation in HLMs.** The relative contributions of CYP1A2, CYP2C19, CYP2D6, and CYP3A4 to paroxetine-catechol formation at a total concentration of 1 and 20 µM paroxetine were assessed by specific chemical and MAB inhibition in pHLMs and sdHLMs. The protein concentration was 0.20 protein mg/ml. Inhibitor concentrations were 10 µM furafylline (inhibitor of CYP1A2), 4 µl CYP2C19 MAB per 100 µg HLM protein (inhibitor of CYP2C19), 5 µM quinidine (inhibitor of CYP2D6), and 0.5 µM ketoconazole (inhibitor of CYP3A4). Inhibitor concentrations were selected based on previous experience (Jornil and Linnet, 2009) and tested to assure adequate inhibition (see below). Inhibition was calculated as the paroxetine-catechol formation rate in the inhibited ($v_{\text{inhibited}}$) samples ($n=3$) relative to the control ($v_{\text{control}}$) samples ($n=3$) (eq. 7). The incubation time was 5 min.

\[
\text{inhibition} = (1 - \frac{v_{\text{inhibited}}}{v_{\text{control}}}) \cdot 100\% \quad (7)
\]
The background paroxetine-catechol formation was subtracted from both the inhibited and the control samples. Quinidine was added to background formation samples for quinidine experiments because quinidine has an indirect antioxidant property (Chattopadhyay et al., 2003). Inhibitory antibodies to CYP2C19 were preincubated with the microsomes for 20 min in an ice bath; controls were preincubated with 4 µL Tris buffer per 100 µg HLM protein according to the instructions from BD Gentest. Furafylline was preincubated 15 min at 37°C with the microsomes before starting the assay by addition of paroxetine.

**Specific Inhibitor Efficacy.** To assure adequate inhibition of the target CYP isoenzymes, the selected inhibitor concentrations were checked with cDNA-expressed CYP isoenzymes at the aforementioned concentrations and a total paroxetine concentration of 1 and 20 µM with an incubation time of 5 min.

**Prediction of Relative Paroxetine-Catechol Formation Contributions in HLMs.** The relative contribution of the identified paroxetine-catechol–forming CYP isoforms in HLMs were predicted using RAFs (Crespi and Miller, 1999; Venkatakrishnan et al., 2000a):

\[
f_i(\%) = \frac{RAF_i v_i(s)}{\sum_{i=1}^{n} RAF_i v_i(s)} \times 100
\]

, where \( f_i \) is the relative contribution of a specific CYP isoform, \( RAF_i \) is the relative activity factor, and \( v_i \) is the reaction velocity of the CYP isoform at a specific paroxetine concentration calculated by eq. 4 or 6 using the estimated \( V_{max} \) and unbound \( K_m \) and \( K_s \) using the free concentration of paroxetine. RAFs were calculated by eq. 9, using the supplier’s info sheets of probe substrate turnover rates in cDNA-expressed CYPs and HLMs. The probe substrates were: phenacetin deethylase activity (CYP1A2), (S)-mephenytoin 4′-hydroxylase.
(CYP2C19), (+/-)-bufuralol 1'-hydroxylase (CYP2D6), and testosterone 6β-hydroxylase (CYP3A4).

\[
RAF_i = \frac{TR_{HLM} \text{ [pmol product/min/mg protein]}}{TR_{cDNA} \text{ [pmol product/min/pmol CYP]}} \tag{9}
\]

**Simcyp® Simulations.** Using an IVIVE approach, populations-based pharmacokinetic simulations were carried out using the software Simcyp® ver. 9.10 (Simcyp® Limited, Sheffield, UK). Table 1 lists the parameter values used with Simcyp®, and Table 2 shows the \(V_{\text{max}}\), unbound \(K_m\) and \(K_s\) for the CYPs. All other parameters values used for simulations was set to default Simcyp® values. The virtual population consisted of the built-in Sim-Healthy Volunteers (18 to 65 years, 34% female). Simulations were done on an exclusive EM CYP2D6 population and an exclusive PM CYP2D6 population, with all other parameters equal (n=100 in each population).

Oral clearance (CL\(_{po}\)) was calculated by dose / area under the curve. The area under the simulated plasma concentration versus time curve after a single dose was calculated from time 0 to 336 h. The area under the curve for SS was calculated from time 0 to 24 h (dosing interval) after the last dose on day 14. The dose was 30 mg paroxetine daily. For each individual the time averaged relative contribution of the paroxetine metabolizing CYP isoforms to the hepatic metabolism and the percentage active hepatic CYP2D6 and CYP3A4 (the part not inactivated by MBI) were calculated at 30 mg dosing and in the aforementioned time intervals. Likewise was the individual mean percentage active hepatic CYP2D6 and CYP3A4 at SS calculated for EM of CYP2D6 at 10, 20, 30, 40, and 50 mg daily dosing in the aforementioned time interval.
Results

**LC/MS/MS Procedures.** The LC/MS/MS analytical method used for measurement of paroxetine-catechol formation provided baseline separation for the analytes. A quadratic calibration model was the best fit for paroxetine, and a quadratic calibration model through zero was the best fit for paroxetine-catechol for both LC/MS/MS procedures. No ion suppression effects were found for either LC/MS/MS method when checked by post-column infusion of analytes, analyzing samples containing the different matrices, or inhibitors used for the experiments.

**Substrate Depletion Screening Studies.** Of the 11 CYP isoforms tested, paroxetine depletion was seen for CYP2D6 and CYP2C19, as was a low depletion capability for CYP3A4.

**Paroxetine-Catechol Formation Screening Studies.** There was a significant formation of paroxetine-catechol by CYP1A2, CYP2C19, CYP2D6, CYP3A4, CYP3A5, and pHLM. Other CYP isoforms and human liver cytosol formed paroxetine-catechol comparable to background formation in ICCs.

**Substrate Depletion Kinetic Studies.** $K_m$ and $V_{max}$ by the substrate depletion method (eq. 2) could be estimated only for CYP2D6 and CYP2C19. There was an approximately 20–40% background depletion of paroxetine in the ICC control. Fig. 2 shows $K_{dep}$ plots corrected for this background depletion, and Table 2 shows the best-fit values of $K_m$ and $V_{max}$ by nonlinear regression to eq. 2.

**Paroxetine-Catechol Formation Kinetic Studies.** CYP2D6 and CYP3A5 exhibited MM kinetics. At high concentrations of paroxetine, there was substrate inhibition for CYP1A2, CYP2C19, and CYP3A4. The kinetics was best described by a MM model with uncompetitive inhibition (eq. 4 vs. eq. 6, $p < 0.0001$). Investigations showed a linear product formation relative to time and HLM protein amount or CYP concentration at the selected in
vitro conditions (data not shown). During the 5-min reaction time, 0.1% of the paroxetine was spontaneously converted into paroxetine-catechol. At the lowest paroxetine concentration for the CYP2D6 incubations, 94% of paroxetine was depleted, and the depletion decreased at higher paroxetine concentrations. Thus, the paroxetine concentrations for CYP2D6 were calculated by eq. 3. Because of experimental limitations [limit of quantification (LOQ) of the LC/MS/MS method], the estimation of CYP2D6 $K_m$ could not be performed under conditions in which less paroxetine was depleted. The paroxetine depletion for the other CYPs/HLMs was < 20%. Fig. 3 shows the MM plots, with and without substrate inhibition, for the CYPs, and Table 2 shows the best-fit values of $K_m$, $K_s$, and $V_{max}$ by nonlinear regression according to eq. 4 or eq. 6. For pHLM, biphasic kinetics (eq. 5) was the best model ($p < 0.01$), and MM kinetics (eq. 4) was the best model for PM CYP2D6 sdHLM(1). Fig. 3 shows the corresponding two-site binding model plot for pHLM and the MM plot for sdHLM(1), and Table 2 shows the best-fit values.

**Specific Inhibitor Efficacy.** Specific inhibition results (inhibitor, CYP inhibited, % inhibition at 1 or 20 µM paroxetine, respectively) are as follows: 5 µM quinidine, CYP2D6, 86%, 98%; 0.5 µM ketoconazole, CYP3A4, 87%, 91%; 10 µM furafylline, CYP1A2, 75%, 93%; and MAB 2C19, CYP2C19, 95%, 95%.

**Role of Individual CYP Isoforms in HLM.** Table 3 shows the inhibitions by the different inhibitors in HLMs, as well as the paroxetine-catechol formation speed in the control samples and the sum of inhibitions. If CYP2D6 or CYP3A4 to a large degree were inactivated in the in vitro experiments, the results would suffer a systematic error. It was calculated using the literature MBI constants and assuming 1.order inactivation that on average 69% of CYP2D6 and 98% of CYP3A4 were active during the 5-min incubation at 20 µM total paroxetine concentration. This indicates that the error from MBI for the in vitro experiments is moderate.
Prediction of Relative Paroxetine-Catechol Formation of CYPs in HLMs. Table 4 gives the predicted relative contributions of the paroxetine-catechol–forming CYP isoforms (CYP1A2, CYP2C19, CYP2D6, and CYP3A4) using the RAF approach (eq. 8 and eq. 9) for the different HLMs. The RAF predictions were made at 0.4 and 8 µM unbound paroxetine concentrations (corresponding to total concentrations of 1 and 20 µM). CYP3A5 was not included for the RAF calculations, but considering the large $K_m$ of CYP3A5, the contribution would be minimal.

Simcyp® Simulations. Table 5 presents the oral clearances calculated by Simcyp® using kinetic constants estimated from paroxetine-catechol formation, and literature values. Table 6 shows the contribution of the CYP isoforms to hepatic metabolism. Table 7 gives the active percentage hepatic CYP2D6 and CYP3A4 (the part not inactive by MBI) at single dose and SS conditions. Table 8 shows the active percentage hepatic CYP2D6 and CYP3A4 at SS for EM of CYP2D6 at different paroxetine daily doses.
Discussion

The objective of this work was to identify the CYP isoforms that have paroxetine metabolic capability. According to the literature (Kaye et al., 1989), paroxetine-catechol is an unstable metabolite. In the present study, the metabolite was stabilized by addition of SOD to the in vitro assay, lowering the pH when the assay is stopped (catechols are more stable at low pH), and by addition of ascorbic acid. Using paroxetine-catechol formation, we identified the previously reported CYP isoform CYP2D6 and thus far unreported CYP isoforms CYP1A2, CYP2C19, CYP3A4, and CYP3A5 as paroxetine-metabolizing CYP isoforms. This study therefore confirmed that formation of paroxetine-catechol is an important metabolic pathway for paroxetine.

The estimated $Cl_{int}$ ($V_{max}/K_m$) for paroxetine-catechol formation ranked as CYP2D6>>CYP3A4>CYP1A2>CYP2C19>CYP3A5. The formation of paroxetine-catechol exhibited biphasic kinetics for pHLM, whereas sdHLM(1), which lacked CYP2D6 activity, exhibited monophasic kinetics. The biphasic kinetics for pHLM and the ranking of the estimated $Cl_{int}$ of the CYP isoforms is in good agreement with a high-affinity and at least one low-affinity site of paroxetine metabolism (Bloomer et al., 1992; Sindrup et al., 1992a; Sindrup et al., 1992b). The unbound (applying $f_u$) $K_m$ of ICM CYP2D6 is in the range of the high-affinity site $K_m$ of pHLM. It is therefore reasonable to assume that the high-affinity site of pHLM is CYP2D6. Considering the large standard error of the low-affinity site unbound $K_m$ pHLM(2), it could be close to $K_m$ for CYP3A4, CYP1A2, or CYP2C19 or an average $K_m$ of any combination of these three CYP isoforms.

Inhibition studies were performed at 1 and 20 µM total paroxetine concentration. At 1 µM paroxetine, CYP2D6 should be the major metabolizing CYP isoform because $K_m$>1 µM for other CYP isoforms. At 20 µM paroxetine, CYP1A2, CYP2C19, and CYP3A4 should contribute to the metabolism. The specific inhibitors all inhibited a
satisfactory 75% or better. Inhibition of CYP3A by ketoconazole co-segregates with CYP3A5 inhibition (Liu et al., 2007), but considering the high \( K_m \) of CYP3A5, this CYP isoform should have a low contribution to the paroxetine-catechol formation at the selected concentrations. The sum of inhibitions was \( \geq 70\% \) (excluding sdHLM(2) at 1 µM paroxetine, where paroxetine-catechol formation was close to LOQ). This finding suggests that the majority of paroxetine-catechol forming CYP isoforms have been included in the inhibition experiments. For pHLM, the major paroxetine-catechol–forming CYP isoform is CYP2D6 at the 1- and 20-µM levels. There is a CYP3A4 contribution at the 20-µM level, but the contribution of other CYP isoforms is minimal at 20 µM. The major contributor in the PM CYP2D6 sdHLM(1+2) is CYP3A4, indicating the importance of CYP3A4 for paroxetine-catechol formation in the absence of CYP2D6 (or when CYP2D6 is inactivated, which would be the case under chronic dosing).

Using the RAF approach, CYP2D6 and CYP3A4 are predicted as major metabolizing enzymes in pHLM at the 1-µM and 20-µM levels. CYP1A2 is predicted by RAF to have moderate importance for the different HLMs, and CYP2C19 is only predicted to have a minor contribution for sdHLM(4). In a comparison of the inhibition results and the RAF results, there seems to be an over-prediction of the importance of CYP3A4 and an under-prediction of the importance of CYP2D6. According to the inhibition experiments, the importance of CYP1A2 is moderate and the influence of CYP2C19 is minor, findings in line with the RAF results. Considering the nature of the inhibition experiments and the RAF calculations, the RAF outcomes give a satisfactory prediction of the roles of CYP isoforms for paroxetine-catechol formation when compared to the inhibition results, further validating the estimated values of \( K_m \), \( K_s \), and \( V_{\text{max}} \) for ICM.

We acknowledge that the kinetic parameters estimated with substrate depletion in this study are far from perfect, but we find it important to show these results because
substrate depletion is the only option if no metabolites are available. Using paroxetine depletion, we found that CYP2D6 and CYP2C19 emerged as definite metabolizing CYP isoforms. There were different obscuring factors in the substrate depletion experiments, including background depletion in the control ICC samples (approx. 20–40%). This background depletion is probably the result of a protein adsorption phenomenon, adsorption to experimental equipment, or a combination of these. For CYP2D6, up to 80% depletion was observed in vitro at the lowest paroxetine concentrations, contributing to the uncertainty of the estimated kinetic parameters. Nath and Atkins (2006) have calculated that 80% depletion would overestimate $V_{\text{max}}$ and $K_m$ by 30%. Because of the LOQ limitations of the LC/MS/MS method, we could not use sufficiently low paroxetine concentrations to obtain a data set for CYP2D6 that would also cover the entire concentration range needed for a good fit to eq. 2. Nevertheless, comparing kinetic parameters found for CYP2D6 by these methods (substrate depletion and paroxetine-catechol formation) gives some confirmation that $K_m$ and $V_{\text{max}}$ (CYP2D6) are relatively in the same range, although CYP2C19 $K_m$ and $V_{\text{max}}$ estimates differed clearly between the two methods. $V_{\text{max}}$ was estimated as higher for CYP2C19 and CYP2D6 by paroxetine-catechol formation than by substrate depletion. Thus, there is no indication of an important alternative metabolic CYP2D6 or CYP2C19 pathway.

Bloomer et al. (1992) give the only value of $K_m$ for the high-affinity site of HLM in the literature, estimating $K_m$ to be about 3 µM (corresponding to $K_m$ of 0.75 when corrected by $f_m$, as described previously). But considering that they had unfavorable experimental conditions (5–50 µM paroxetine used for kinetic experiments) to estimate the low $K_m$ of the high-affinity site, an overestimation of $K_m$ is expected.

In HLMs prepared from one liver with EM CYP2D6 status, Bloomer et al. (1992) estimated the formate formation velocity to be 102 pmol/mg/min at a free paroxetine concentration of 1.25 µM (free concentration calculated by extrapolation as described earlier).
and 167 pmol/mg/min at 7.5 µM paroxetine. For the pHLM in the current study, the corresponding paroxetine-catechol formation rates at these free concentrations were 60 and 114 pmol/mg/min. This result is in the same range as that of Bloomer et al., considering the potential differences between the pHLM and the use of one EM CYP2D6 liver.

We note the Clint for CYP2D6, which is quite large. For a perfect enzyme, an enzyme in which every collision of substrate and enzyme would result in the formation of an enzyme–substrate complex, Clint is larger by a factor of approximately 20–200 (Mathews and Van Holde, 1996). From this, we obtain an indication of how effective CYP2D6 is in metabolizing paroxetine and an explanation for why paroxetine is such a potent MBI of CYP2D6: A larger number of molecules are turned over per time compared to the other paroxetine-metabolizing CYPs, offering a greater chance of inactivation by the reactive catechol metabolite. Obach et al. (2007) identified paroxetine as an MBI of CYP2D6 and CYP3A, whereas paroxetine showed no MBI potential towards CYP1A2 and CYP2C19. If the estimated Clint for the CYPs is understood as a measure of MBI potential, the results are in line with those of Obach et al. because paroxetine exhibits MBI for the CYPs with largest Clint (CYP2D6 and CYP3A4).

The fu-corrected Km for the CYP enzymes could be over- or underestimated because binding to the ICM could be different than binding to HLM; e.g., the binding of amitriptyline to human b-lymphoblastoid cells is lower than to HLM (Venkatakrishnan et al., 2000b). For the in vitro assay of paroxetine-catechol formation, there was background paroxetine-catechol formation (0.1% of total paroxetine concentration). This could be an in vitro artefact or in fact a metabolic contribution, and it cannot be excluded that there is a spontaneous element of paroxetine metabolism in vivo, but we did not attempt such an estimation here.
For the SS situation, with CYP2D6 to a large degree and CYP3A4 to a lesser degree inactivated because of MBI, CYP3A4, CYP1A2, and CYP2C19 will become more important for the metabolism. Using Simcyp® for IVIVE, we incorporated the aspect of MBI into the assessment of the importance of the CYP isoforms for human metabolism of paroxetine. With the estimated kinetic constants by paroxetine-catechol formation for the CYP isoforms the Simcyp® estimated oral clearances were generally within a 2–3-fold error margin compared to the literature values (Table 5). There was a good match in the trend between estimated and literature clearance values for the single-dose and SS situations for both EM/PM population groups. The ranges of the estimated clearances were well in agreement with the literature values, considering that the clinical studies had only 9 and 15 participants. This supports the Simcyp® simulations of the single-dose and SS situations, including the impact of MBI. If MBI was not incorporated into the Simcyp® simulations the oral clearances at SS for EM of CYP2D6 was over predicted by a 12 fold (data not shown), this points towards MBI and not saturation of CYP2D6 being the reason for the non linear kinetics of paroxetine. Table 6 shows the relative importance of the CYP isoforms for hepatic paroxetine metabolism. For EM of CYP2D6 in the single dose situation CYP2D6 is the major metabolizing isoform (median 98% contribution), but at SS conditions the importance is reduced to 44%. For comparison the importance of CYP3A4 increases from 2 to 35%. For PM of CYP2D6 CYP3A4 is the major metabolizing CYP isoform for single dose and SS situation. CYP2C19 and CYP3A5 are not of any major importance for the metabolism, whereas CYP1A2 might be of importance in certain individuals as seen from the ranges in Table 6. These findings clearly show how the incorporation of MBI into the IVIVE model is of high importance to estimate the relative importance of the CYPs for paroxetine metabolism. In Table 7 the Simcyp® predicted effect of MBI on the active hepatic CYP2D6 and CYP3A4 is given. The active amount of CYP2D6 is reduced from a median 87% to 2%
comparing single dose to SS conditions. However the 2% active CYP2D6 is still enough for CYP2D6 to be a major metabolizing enzyme at SS. For CYP3A4 the effect of MBI is most profound for PM of CYP2D6 where the median active percentage is reduced from 97% to 60% at SS conditions. There is solid evidence in the literature of the reduced CYP2D6 activity in chronic dosing with paroxetine e.g. Laine et al. (2001) and Solai et al. (2002).

Table 8 shows the Simcyp® predicted median active percentage hepatic CYP2D6 and CYP3A4 at different dosing levels for EM of CYP2D6. For CYP3A4 it is predicted that the activity is reduced by 40% at 50 mg paroxetine daily dosing. Paroxetine interaction studies with alprazolam (Calvo et al., 2004) and terfenadine (Martin et al., 1997) showed no interaction with paroxetine at chronic dosing. This is expected at the used dosing of 20 mg paroxetine daily which would give a very limited inhibition of CYP3A4 of 5% according to the prediction in Table 8. If the predictions in Table 8 are correct, paroxetine interactions with CYP3A4 are only observable at higher dosing of paroxetine. The estimated percentage active hepatic enzyme in Table 7 and 8 is highly dependent on many parameter values e.g. the half life of the hepatic CYP isoform (in this study CYP2D6 half life=70 h, CYP3A4 half life = 90 h, Simcyp® predefined values) and the estimates for of the kinetic parameter values for MBI. The results in Table 7 and 8 should be interpreted bearing this in mind.

Considering the kinetic parameters, inhibition results, RAF calculations, and Simcyp® simulations, CYP2D6 and CYP3A4 are most likely to be the major contributors to paroxetine metabolism in humans. CYP1A2 could be of importance for paroxetine metabolism in some individuals, whereas the importance of CYP2C19 and CYP3A5 is probably limited.

This report is the first to identify the low-affinity CYP isoforms metabolizing paroxetine. These data from studies on ICM and HLM and Simcyp® IVIVE simulations add new fundamental information to our understanding of the metabolism of paroxetine. For both
EM and PM of CYP2D6, CYP3A4 is most likely of major importance for the metabolism of paroxetine under steady-state conditions. Simcyp® has proved to be a useful tool for estimation of the relative importance of CYP isoforms taking the MBI aspect into consideration, and for the prediction of the active amounts of hepatic CYP isoforms in different scenarios.
Acknowledgments

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References


Kuss HJ and Hegerl U (1998) Serum concentrations of paroxetine are influenced by CYP3A. *Naunyn Schmiedebergs Arch Pharmacol* 358: R782-R782.


Venkatakrishnan K, von Moltke LL, Court M, Harmatz JS, Crespi CL, and Greenblatt DJ (2000a) Comparison between cytochrome P450 (CYP) content and relative activity approaches to scaling from cDNA-expressed CYPs to human liver microsomes: Ratios of accessory proteins as sources of discrepancies between the approaches. *Drug Metab Dispos* **28**: 1493-1504.


Footnotes

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Parts of this work have been presented as a poster at “Danish Pharmacology 2009” and at “Simcyp Consortium Meeting 2009”.
Legends for figures

Fig. 1. Paroxetine metabolism as proposed by Haddock et al. (1989) and reported catalytic enzymes (Bloomer et al., 1992; Maurer et al., 2000; Sindrup et al., 1992b).

*Reported in this study.

Fig. 2. Paroxetine $K_{dep}$ plots for CYP2D6 and CYP2C19. Mean $K_{dep}$ (solid points) values and S.E.M. of triplicates (error bars) are shown. Curves are the best fit of eq. 2. $R^2$ is 0.63 for CYP2C19 and 0.67 for CYP2D6.

Fig. 3. Paroxetine-catechol formation plots for cDNA expressed CYP1A2, CYP2C19, CYP2D6, CYP3A4, CYP3A5, pooled HLM and PM2D6 sdHLM(1). Mean $v$ (solid points) and error bars (S.E.M.) are shown. Michaelis-Menten plots are shown for CYP2D6, CYP3A5, PM2D6 sdHLM(1), Michaelis-Menten with uncompetitive substrate inhibition plots for CYP1A2, CYP2C19, CYP3A4, and a biphasic kinetic plot for pHLM.
Tables

Table 1. Parameter values used for Simcyp® simulations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>329.3 g/mol</td>
<td></td>
</tr>
<tr>
<td>Protein binding</td>
<td>95%</td>
<td>(Kaye et al., 1989)</td>
</tr>
<tr>
<td>$K_{\text{inact}}$ (CYP2D6)</td>
<td>0.17 min$^{-1}$</td>
<td>(Bertelsen et al., 2003)</td>
</tr>
<tr>
<td>$K_I$ (CYP2D6) unbound</td>
<td>0.315 µM</td>
<td>(Bertelsen et al., 2003;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Venkatakrishnan and Obach,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2005)</td>
</tr>
<tr>
<td>$K_{\text{inact}}$ (CYP3A4)$^a$</td>
<td>0.011 min$^{-1}$</td>
<td>(Obach et al., 2007)</td>
</tr>
<tr>
<td>$K_I$ (CYP3A4) unbound$^{a+b}$</td>
<td>4.03 µM</td>
<td>(Obach et al., 2007)</td>
</tr>
<tr>
<td>Renal clearance$^c$</td>
<td>0.7 l/h</td>
<td>(Sindrup et al., 1992b)</td>
</tr>
<tr>
<td>Volume of distribution at SS</td>
<td>7.5 l/kg</td>
<td>Simcyp® predicted value</td>
</tr>
<tr>
<td>Log P</td>
<td>3.55</td>
<td>(Martin et al., 2008)</td>
</tr>
<tr>
<td>pKa</td>
<td>9.66</td>
<td>(Martin et al., 2008)</td>
</tr>
<tr>
<td>Blood/plasma ratio</td>
<td>1.25</td>
<td>Lundbeck – data on file</td>
</tr>
<tr>
<td>CACO-2 permeability</td>
<td>$17*10^{-6}$ cm/s</td>
<td>Lundbeck – data on file</td>
</tr>
</tbody>
</table>

$^a$It is assumed that 3A4 is the major component of the MBI kinetic parameter values for 3A by Obach et al. (2007)

$^b$Unbound $K_I$ estimated by extrapolation of results by Hemeryck et al. (2001)

$^c$Estimated from Sindrup et al. (1992b)
Table 2. Kinetic parameters of paroxetine biotransformation by CYP isoforms and HLMs. Kinetic data for the CYP isoforms and PM2D6 sdHLM(1) using paroxetine-catechol formation are estimated by eq. 4 or 6. Kinetic data for pHLM are estimated by eq. 5, pHLM(1) and pHLM(2) refers to the two different terms in eq. 5. Kinetic data for paroxetine depletion estimated by eq. 2. Standard error estimated by non linear regression.

<table>
<thead>
<tr>
<th>Paroxetine-catechol formation</th>
<th>CYP1A2</th>
<th>CYP2C19</th>
<th>CYP2D6</th>
<th>CYP3A4</th>
<th>CYP3A5</th>
<th>pHLM(1)</th>
<th>pHLM(2)</th>
<th>PM2D6 sdHLM(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K_m) (best fit value ± standard error)</td>
<td>8.8 ± 1.9</td>
<td>26.0 ± 4.9</td>
<td>0.028 ± 0.002</td>
<td>13.3 ± 1.1</td>
<td>108 ± 10</td>
<td>0.24 ± 0.18</td>
<td>8 ± 6</td>
<td>31 ± 3</td>
</tr>
<tr>
<td>(V_{max}) (best fit value ± standard error)</td>
<td>0.63 ± 0.06</td>
<td>2.43 ± 0.29</td>
<td>9.7 ± 0.1</td>
<td>5.32 ± 0.22</td>
<td>1.6 ± 0.1</td>
<td>65 ± 23</td>
<td>81 ± 21</td>
<td>300 ± 8</td>
</tr>
<tr>
<td>(K_i) (best fit value ± standard error)</td>
<td>141 ± 38</td>
<td>131 ± 34</td>
<td>298 ± 48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assay protein concentration (mg/ml)</td>
<td>0.45</td>
<td>0.10</td>
<td>0.012</td>
<td>0.24</td>
<td>0.45</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>(K_m) corrected by (f_u)^a</td>
<td>2.0</td>
<td>14.8</td>
<td>0.026</td>
<td>4.7</td>
<td>25</td>
<td>0.096</td>
<td>3.2</td>
<td>12.4</td>
</tr>
<tr>
<td>(K_i) corrected by (f_u)^a</td>
<td>32</td>
<td>75</td>
<td>106</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Cl_{int}(V_{max}/K_m)) (free concentration)</td>
<td>320</td>
<td>164</td>
<td>373000</td>
<td>1130</td>
<td>64</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Paroxetine depletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K_m) (best fit value ± standard error)</td>
</tr>
<tr>
<td>(V_{max}) (best fit value ± standard error)</td>
</tr>
<tr>
<td>Assay protein concentration (mg/ml)</td>
</tr>
<tr>
<td>(K_m) corrected by (f_u)^a</td>
</tr>
<tr>
<td>(Cl_{int}(V_{max}/K_m)) (free concentration)</td>
</tr>
</tbody>
</table>

^aNot estimated.

\(K_m\) unit in [µM], \(V_{max}\) (CYP) unit in [pmol/min/pmol CYP], \(V_{max}\) (HLMs) unit [pmol/min/mg protein], and \(Cl_{int}\) unit in [nl/min/pmol CYP].

^b\(f_u\) calculated by extrapolation of results by Hemeryk et al. (2001).

^cThe protein concentration at 0.03-0.2 µM paroxetine.
Table 3. Uninhibited paroxetine-catechol formation velocity and inhibition [%] by inhibitors in different HLMs. Measurements were done in triplicates for HLM controls, inhibited HLM samples, and background catechol formation samples. S.E.M.s are given.

<table>
<thead>
<tr>
<th>HLM</th>
<th>Paroxetine Concentration [µM]</th>
<th>Control paroxetine-catechol formation velocity [pmol/min/mg protein] ± S.E.M.</th>
<th>Inhibition [%] ± S.E.M. [%]</th>
<th>10 µM Furafylline (CYP1A2)</th>
<th>Antibodies (CYP2C19)</th>
<th>5 µM quinidine (CYP2D6)</th>
<th>0.5 µM ketoconazole (CYP3A4)</th>
<th>Σ inhibition [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>pHLM</td>
<td>1 20</td>
<td>63 ± 1 137 ± 5 6 1 ± 4 2 ± 2 97 ± 0.3 -1 ± 2 104</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sdHLM(1)</td>
<td>1 20</td>
<td>6.7 ± 0.1 156 ± 8 21 ± 6 -6 ± 9 -1 ± 3 51 ± 5 72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sdHLM(2)</td>
<td>1 20</td>
<td>2.5 ± 0.1 45 ± 2 6 ± 6 5 ± 7 4 ± 5 37 ± 10 52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sdHLM(3)</td>
<td>1 20</td>
<td>71 ± 2 147 ± 2 7 ± 2 4 ± 1 98 ± 0.1 0 ± 3 109</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sdHLM(4)</td>
<td>1 20</td>
<td>12 ± 0.1 80 ± 2 13 ± 2 7 ± 2 78 ± 1 4 ± 1 102</td>
<td></td>
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<td></td>
<td></td>
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</tr>
</tbody>
</table>

*aNegave inhibitions were set to zero to for the sum of inhibitions.

*bFormed paroxetine-catechol close to LOQ.

The four different sdHLMs (1-4) used had the following characteristics; (1) PM CYP2D6, high activity of CYP3A4, (2) PM CYP2D6, (3) High activity of CYP2D6, (4) High activity of CYP2C19 and low CYP2D6 activity.
Table 4. Predicted part of paroxetine-catechol formation in different HLMs using RAFs

<table>
<thead>
<tr>
<th>HLM</th>
<th>Total</th>
<th>Paroxetine Concentration[μM]</th>
<th>Predicted part of paroxetine-catechol formation [%] by RAF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CYP1A2</td>
</tr>
<tr>
<td>pHLM</td>
<td>1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>sdHLM(1)</td>
<td>1</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>sdHLM(2)</td>
<td>1</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>sdHLM(3)</td>
<td>1</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>sdHLM(4)</td>
<td>1</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

\(^a\)The total paroxetine was corrected by the free fraction for the calculations (f_w=0.4) corresponding to 0.4 and 8 μM free concentration.

\(^b\)The predicted part of paroxetine-catechol formation for CYP2D6 is set to zero because of the PM status (even if probe substrate turnover rate was not zero according to the manufacturer’s info sheet).
Table 5. Simcyp® simulated oral clearances (CL_{po}) and corresponding literature values for EM and PM of CYP2D6.

<table>
<thead>
<tr>
<th>Study</th>
<th>Phenotype</th>
<th>Individuals in the study</th>
<th>Single dose/SS</th>
<th>CL_{po} median (L/hr)</th>
<th>CL_{po} range (L/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Sindrup et al., 1992b)</td>
<td>EM</td>
<td>9</td>
<td>single dose</td>
<td>167</td>
<td>74–463</td>
</tr>
<tr>
<td>(Kaye et al., 1989)</td>
<td></td>
<td>a</td>
<td>single dose</td>
<td>157</td>
<td>57–3704</td>
</tr>
<tr>
<td>Simcyp – paroxetine-catechol formation parameters values</td>
<td>EM</td>
<td>100</td>
<td>single dose</td>
<td>340</td>
<td>30–2055</td>
</tr>
<tr>
<td>(Sindrup et al., 1992b)</td>
<td>EM</td>
<td>9</td>
<td>SS</td>
<td>36</td>
<td>24–67</td>
</tr>
<tr>
<td>(Kaye et al., 1989)</td>
<td></td>
<td>a</td>
<td>SS</td>
<td>29^b</td>
<td>16–346</td>
</tr>
<tr>
<td>Simcyp – paroxetine-catechol formation parameters values</td>
<td>EM</td>
<td>100</td>
<td>SS</td>
<td>14</td>
<td>3–1020</td>
</tr>
<tr>
<td>(Sindrup et al., 1992b)</td>
<td>PM</td>
<td>9</td>
<td>single dose</td>
<td>23</td>
<td>12–53</td>
</tr>
<tr>
<td>Simcyp – paroxetine-catechol formation parameters values</td>
<td>PM</td>
<td>100</td>
<td>single dose</td>
<td>8</td>
<td>3–50</td>
</tr>
<tr>
<td>(Sindrup et al., 1992b)</td>
<td>PM</td>
<td>9</td>
<td>SS</td>
<td>21</td>
<td>12–41</td>
</tr>
<tr>
<td>Simcyp – paroxetine-catechol formation parameters values</td>
<td>PM</td>
<td>100</td>
<td>SS</td>
<td>6</td>
<td>2–44</td>
</tr>
</tbody>
</table>

^aHealthy subjects, no information about phenotype of CYP2D6 is present.

^bMean CL_{po} calculated from dose / mean AUC_{24h}.
Table 6. The median relative contribution of CYP isoforms and range (min–max) to hepatic metabolism estimated by Simcyp® at single dose and steady state for EM and PM of CYP2D6 (n=100).

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>CYP2D6</th>
<th>Single dose / SS</th>
<th>Median percent contribution to hepatic metabolism (%) and range (min–max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>CYP2C19</td>
<td>CYP2D6</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>EM</td>
<td></td>
<td>Single dose</td>
<td>0.3 (0.05–2)</td>
</tr>
<tr>
<td>EM</td>
<td></td>
<td>SS</td>
<td>9 (0–53)</td>
</tr>
<tr>
<td>PM</td>
<td></td>
<td>Single dose</td>
<td>17 (2–70)</td>
</tr>
<tr>
<td>PM</td>
<td></td>
<td>SS</td>
<td>24 (2–74)</td>
</tr>
</tbody>
</table>

aNo active CYP2D6.
Table 7. Median active percentage hepatic CYP2D6 and CYP3A4 at single dose and steady state estimated by Simcyp® for EM and PM of CYP2D6 (n=100).

<table>
<thead>
<tr>
<th>CYP isoform</th>
<th>Phenotype CYP2D6</th>
<th>Single dose / SS</th>
<th>Median active percentage</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; percentile</th>
<th>95&lt;sup&gt;th&lt;/sup&gt; percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6</td>
<td>EM</td>
<td>Single dose</td>
<td>87</td>
<td>67</td>
<td>97</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>EM</td>
<td>SS</td>
<td>2</td>
<td>0.4</td>
<td>50</td>
</tr>
<tr>
<td>CYP2A4</td>
<td>EM</td>
<td>Single dose</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>CYP2A4</td>
<td>EM</td>
<td>SS</td>
<td>82</td>
<td>50</td>
<td>99</td>
</tr>
<tr>
<td>CYP2A4</td>
<td>PM</td>
<td>Single dose</td>
<td>97</td>
<td>93</td>
<td>99</td>
</tr>
<tr>
<td>CYP2A4</td>
<td>PM</td>
<td>SS</td>
<td>60</td>
<td>37</td>
<td>87</td>
</tr>
</tbody>
</table>
Table 8. Median percent active hepatic CYP2D6 and CYP3A4 for EM of CYP2D6 at steady state with different daily doses estimated with Simcyp® (n=100).

<table>
<thead>
<tr>
<th>CYP isoform</th>
<th>10 mg/day</th>
<th>20 mg/day</th>
<th>30 mg/day</th>
<th>40 mg/day</th>
<th>50 mg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6</td>
<td>48 (4–85)</td>
<td>8 (1–68)</td>
<td>2 (0.4–50)</td>
<td>1 (0.3–29)</td>
<td>0.8 (0.2–10)</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>99 (93–100)</td>
<td>95 (71–100)</td>
<td>82 (50–99)</td>
<td>70 (39–98)</td>
<td>60 (28–95)</td>
</tr>
</tbody>
</table>
Figure 2

CYP2D6

CYP2C19

K_{dep} [μL/min/pmol CYP]

Paroxetine [μM]

Paroxetine [μM]