IN SILICO PREDICTION OF DRUG BINDING TO CYP2D6: IDENTIFICATION OF A NEW METABOLITE OF METOCLOPRAMIDE


Division of Cancer Medicine (J.Y., C.J.W., S.B., E.M.R.) and Molecular Pharmacology Unit (M.J.I.P., C.R.W.), Biomedical Research Centre, University of Dundee, Ninewells Hospital & Medical School, Dundee, United Kingdom; Manchester Interdisciplinary Biocentre, School of Chemical Engineering and Analytical Science, University of Manchester, the Mill, Manchester, United Kingdom (J.-D.M., M.J.S.); and Department of Biochemistry, University of Leicester, Leicester, United Kingdom (C.A.K., G.C.K.R.)

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ABSTRACT:

Patients with cancer often take many different classes of drugs to treat the effects of their malignancy and the side effects of treatment, as well as their comorbidities. The potential for drug-drug interactions that may affect the efficacy of anticancer treatment is high, and a major source of such interactions is competition for the drug-metabolizing enzymes, cytochromes P450 (P450s). We have examined a series of 20 drugs commonly prescribed to cancer patients to look for potential interactions via CYP2D6. We used a homology model of CYP2D6, together with molecular docking techniques, to perform an in silico screen for binding to CYP2D6. Experimental IC50 values were determined for these compounds and compared with the model predictions to reveal a correlation with a regression coefficient of \( r^2 = 0.61 \). Importantly, the docked conformation of the commonly prescribed antiemetic metoclopramide predicted a new site of metabolism that was further investigated through in vitro analysis with recombinant CYP2D6. An aromatic N-hydroxy metabolite of metoclopramide, consistent with predictions from our modeling studies, was identified by high-performance liquid chromatography/mass spectrometry. This metabolite was found to represent a major product of metabolism in human liver microsomes, and CYP2D6 was identified as the main P450 isoform responsible for catalyzing its formation. In view of the prevalence of interindividual variation in the CYP2D6 genotype and phenotype, we suggest that those experiencing adverse reactions with metoclopramide, e.g., extrapyramidal syndrome, are likely to have a particular CYP2D6 genotype/phenotype. This warrants further investigation.

Cytochromes P450 (P450s) are a superfamily of heme-containing enzymes that are responsible for the oxidation of a structurally diverse range of xenobiotic compounds. The human genome contains 55 P450s, but the vast majority (~90%) of therapeutic drugs are metabolized by five P450s: CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 (Wolf et al., 2000). Inhibition and/or induction of these isoforms is probably the most common mechanism underlying drug-drug interactions in vivo. Patients with cancer are potentially vulnerable to significant drug-drug interactions arising from drug metabolism by P450s because they are subject to polypharmacy. These patients often take drugs to relieve symptoms of their malignancy (e.g., analgesics, anticonvulsants for neuropathic pain), side effects of their treatment (e.g., antiemetics), as well as drugs to treat comorbidities (e.g., antihypertensives and lipid-lowering agents). Adverse drug interactions involving CYP2D6 are likely to be significant because up to 30% of drugs are substrates of this enzyme, including opioids, antidepressants, neuroleptics, and various cardiac medications.

Various methods have been developed to predict interactions between drugs and P450 isoforms, and these can be broadly divided into data modeling or molecular modeling approaches (van de Waterbeemd and Gifford, 2003). The former, involving statistical correlations between molecular and structural descriptors, is relatively fast and can provide a useful computational filter in early drug discovery (Ekins et al., 2003). We have used the latter molecular approach, combining experimental analysis of substrate binding with protein modeling, to study CYP2D6 active site-ligand interactions. This has helped us (Kirton et al., 2002; Paine et al., 2003; Flanagan et al., 2004; Kemp et al., 2004; McLaughlin et al., 2005) and others (Venhorst et al., 2003; Keizers et al., 2004, 2005) to identify residues that play a key role in metabolism, and to determine the binding orientation and affinity of ligands in the active site (Kirton et al., 2002; Kemp et al., 2004). The recently determined crystal structure of CYP2D6 (Rowland et al., 2006) has confirmed the correctness of many of the features of the active site of our model of the enzyme.

Here we have used this approach to investigate interactions of CYP2D6 with a small set of 20 drugs that are commonly used by our patients to look for potential interactions via CYP2D6. We used a homology model of CYP2D6, together with molecular docking techniques, to perform an in silico screen for binding to CYP2D6. Experimental IC50 values were determined for these compounds and compared with the model predictions to reveal a correlation with a regression coefficient of \( r^2 = 0.61 \). Importantly, the docked conformation of the commonly prescribed antiemetic metoclopramide predicted a new site of metabolism that was further investigated through in vitro analysis with recombinant CYP2D6. An aromatic N-hydroxy metabolite of metoclopramide, consistent with predictions from our modeling studies, was identified by high-performance liquid chromatography/mass spectrometry. This metabolite was found to represent a major product of metabolism in human liver microsomes, and CYP2D6 was identified as the main P450 isoform responsible for catalyzing its formation. In view of the prevalence of interindividual variation in the CYP2D6 genotype and phenotype, we suggest that those experiencing adverse reactions with metoclopramide, e.g., extrapyramidal syndrome, are likely to have a particular CYP2D6 genotype/phenotype. This warrants further investigation.

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1 These authors contributed equally to this work.

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ABBREVIATIONS: P450, cytochrome P450; HPLC, high-performance liquid chromatography; AMMC, 3-[2-[(N,N-diethy-N-methylamino)ethyl]-7-methoxy-4-methylcoumarin; HLM, human liver microsome; MS, mass spectrometry; CID, collision-induced dissociation.
cancer patients. As well as showing a useful correlation between our in silico predictions and the corresponding experimental results, this has uncovered the correct metabolic route for metoclopramide, a drug frequently used to prevent the nausea and vomiting associated with cancer chemotherapy (Harrington et al., 1983).

Materials and Methods

Materials. Terrific Broth, chloramphenicol, δ-aminolevulinic acid, dithiothreitol, glucose 6-phosphate, NADP+, phenylmethylsulfonyl fluoride, sodium dithionite, cytochrome c, dextromethorphan, and all the coadministered drugs were purchased from Sigma (Poole, UK). Ampicillin was obtained from Beecham Research (Welwyn Garden City, UK), and isopropyl β-D-thioglucopyranoside was from Melford Laboratories (Ipswich, UK). Glucose 6-phosphate dehydrogenase (type VII) was purchased from Roche Molecular Biochemicals (Leeds, UK). Library efficient competent Escherichia coli JM109 was purchased from Promega (Madison, WI). Pooled human liver microsome was purchased from BD Gentest (Woburn, MA). High-performance liquid chromatography (HPLC) grade solvents were purchased from Rathburn Chemicals (Cheshire, UK). Dextrorphan was purchased from Ultra Fine Chemicals (Manchester, UK). All the other chemicals were from BDH (Poole, UK).

Coxpresssion of the P450 and P450 Reductase in E. coli. Expression was carried out essentially as described previously (Kemp et al., 2004). Briefly, pB81 plasmid was cotransfected with pJR7 into E. coli JM109 carried out essentially as described previously (Kemp et al., 2004). Briefly, pB81 plasmid was cotransfected with pJR7 into E. coli JM109 was purchased from Promega (Madison, WI). Pooled human liver microsome was purchased from BD Gentest (Woburn, MA). High-performance liquid chromatography (HPLC) grade solvents were purchased from Rathburn Chemicals (Cheshire, UK). Dextrorphan was purchased from Ultra Fine Chemicals (Manchester, UK). All the other chemicals were from BDH (Poole, UK).

HPLC/mass spectrometry assay. Metoclopramide (0–150 μM) was incubated in 50 μM potassium phosphate buffer (pH 7.4) with 20 pmol of CYP2D6 or 0.1 mg of human liver microsomes (HLMs) (protein concentration of 8.9 mg/ml) and an NADPH regenerating system (0.41 mM glucose 6-phosphate, 0.41 mM magnesium chloride, 0.4 U of glucose 6-phosphate dehydrogenase, and 8.2 μM NADP+) in a total volume of 200 μl. After 3-min preincubation at 37°C, reactions were initiated with the addition of the NADPH regenerating system and incubated for up to 30 min with recombinant CYP2D6 or for 60 min with HLMS before stopped with the addition of 200 μl of ice-cold methanol. All the reactions were carried out in duplicate. After incubation, samples were centrifuged at 16,000 g for 5 min to remove particulate material. Typically, 25 μl of the reaction supernatant was analyzed by HPLC/mass spectrometry (MS). The analytes were separated on a Hyperclone BDS C18 column (5 μm, 150 x 2.0 mm) at a flow rate of 0.15 ml/min for metabolite screening and 0.2 ml/min for enzymatic determinations. A linear gradient was applied using water (A) and acetonitrile (B), both containing 0.1% (v/v) formic acid. The gradient for metabolite screening ran from 10 to 100% B in 10 min and was held for 3 min before returning to the initial conditions. The run time was 20 min. The gradient for enzyme assays ran from 15 to 100% B over 6 min.

Table 1

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Drug Type</th>
<th>IC50 μM</th>
<th>ChemScore</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxetine</td>
<td>Antidepressant</td>
<td>0.4 ± 0.1</td>
<td>38.3</td>
</tr>
<tr>
<td>Loperamide</td>
<td>Antidiarrheal</td>
<td>0.7 ± 0.1</td>
<td>41.8</td>
</tr>
<tr>
<td>Domperidone</td>
<td>Antiepileptic</td>
<td>2.2 ± 0.3</td>
<td>42.2</td>
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<tr>
<td>Amitriptyline</td>
<td>Antidepressant</td>
<td>3.6 ± 0.3</td>
<td>44.6</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>Antiestrogen</td>
<td>6.8 ± 0.6</td>
<td>34.2</td>
</tr>
<tr>
<td>Metoclopramide</td>
<td>Antiepileptic</td>
<td>9.7 ± 1.4</td>
<td>30.5</td>
</tr>
<tr>
<td>Citalopram</td>
<td>Antidepressant</td>
<td>9.7 ± 0.6</td>
<td>36.1</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>Antiepileptic</td>
<td>11.6 ± 1.3</td>
<td>31.2</td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>Antacid</td>
<td>13.3 ± 0.6</td>
<td>31.1</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>Antihyperlipidemic</td>
<td>23.1 ± 3.0</td>
<td>27.5</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>Antihypertensive</td>
<td>27.4 ± 1.5</td>
<td>28.2</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>Antacid</td>
<td>53.1 ± 2.1</td>
<td>34.0</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>Anxiolytic</td>
<td>94.3 ± 9.9</td>
<td>30.5</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>Antacid</td>
<td>164.4 ± 26.1</td>
<td>22.0</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>Cytotoxic</td>
<td>230.1 ± 56.0</td>
<td>22.6</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>Anxiolytic</td>
<td>233.0 ± 56.0</td>
<td>31.8</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>Steroid</td>
<td>451.7 ± 59.3</td>
<td>22.9</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>Antacid</td>
<td>680.0 ± 28.9</td>
<td>27.9</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Steroid</td>
<td>707.8 ± 162.5</td>
<td>24.9</td>
</tr>
<tr>
<td>Atenolol</td>
<td>Antihypotensive</td>
<td>737.4 ± 72.5</td>
<td>30.2</td>
</tr>
</tbody>
</table>

Note: a: For inhibition of AMMC oxidation. Errors are goodness in fit to concentration-effect curve. b: Best ranked of 10 docked orientations. c: Drugs that are substrates of CYP2D6. Details of metabolites, and kM data where available, are as follows—amitriptyline: 10-hydroxylation, kM 5–13 mM (Olesen and Linnert, 1997); citalopram: N-demethylation, kM 18–22 mM (Rochat et al., 1997); diltiazem: O-demethylation, kM 5 mM (Molden et al., 2000); domperidone: 5-hydroxylation (Ward et al., 2004); fluoxetine: N-demethylation (Margolis et al., 2000); loperamide: N-demethylation (Kim et al., 2004); metoclopramide: N-hydroxylation, 6 mM (this study); omeprazole: 7- and 8-hydroxylation (Dixon et al., 1995); tamoxifen: 4-hydroxylation (Desta et al., 2004). 

**Analytical Assays.** Fluorescence assay. The fluorogenic substrate AMMC was used, and assays were conducted in 96-well microtiter plates using a final volume of 200 μl. IC50 determination requires the serial dilution of test compounds across a concentration range that optimally covers 0 to 100% inhibition of CYP2D6 activity. The compounds tested were serially diluted in 3-fold steps in a final volume of 100 μl of 2× enzyme/substrate stock solution (0.1 pmol/μl P450 in 100 mM potassium phosphate buffer, pH 7.4, with 2 mM AMMC). A solvent control was included to correct for any solvent effects across the dilution range. Plates were then preincubated for 3 min at 37°C, and the enzyme reaction was initiated by the addition of a 100-μl aliquot of prewarmed 160× NADPH generating system (1.3 mM NADP+, 66 mM glucose 6-phosphate, 66 mM MgCl2, and 0.8 U/ml glucose 6-phosphate dehydrogenase) in 100 mM potassium phosphate buffer (pH 7.4). The reaction was maintained at 37°C, and the activities of test compounds were determined by Fluoroskan Ascent FL microtiter plate reader (λex = 390, λem = 460 nm). Activity was expressed as a percentage of the corresponding solvent-only control, and IC50 values were calculated using GraFit 5.0.4 (Erithacus Software, Horley, Surrey, UK).

**Materials.** Terrific Broth, chloramphenicol, δ-aminolevulinic acid, dithiothreitol, glucose 6-phosphate, NADP+, phenylmethylsulfonyl fluoride, sodium dithionite, cytochrome c, dextromethorphan, and all the coadministered drugs were purchased from Sigma (Poole, UK). Ampicillin was obtained from Beecham Research (Welwyn Garden City, UK), and isopropyl β-D-thioglucopyranoside was from Melford Laboratories (Ipswich, UK). Glucose 6-phosphate dehydrogenase (type VII) was purchased from Roche Molecular Biochemicals (Leeds, UK). Library efficient competent Escherichia coli JM109 was purchased from Promega (Madison, WI). Pooled human liver microsome was purchased from BD Gentest (Woburn, MA). High-performance liquid chromatography (HPLC) grade solvents were purchased from Rathburn Chemicals (Cheshire, UK). Dextrorphan was purchased from Ultra Fine Chemicals (Manchester, UK). All the other chemicals were from BDH (Poole, UK).
before returning to the initial conditions. The total run was 10 min. HPLC/MS analyses were carried out using a Waters 2695 HPLC coupled with a Micro-mass Q-time of flight Micro MS (Waters, Hertsfordshire, UK). The major MS parameters were capillary = 4.5 kV, sample cone = 15 V, extraction cone = 5.0 V, desolvation temperature = 350°C, source temperature = 120°C, and cone and desolvation gases were nitrogen at 80 and 500 l/h, respectively. For MS/MS experiments, the collision energy was 25 eV, and the collision gas was argon.

Modeling and docking. The homology model of CYP2D6 was produced as described previously (Kirton et al., 2002) using the comparative modeling program Modeler (Sali and Blundell, 1993) with five structural templates: P450 cam (Poulos et al., 1986), terp (Hasemann et al., 1994), eryF (Cupp-Vickery et al., 2000), BM3 (Ravichandran et al., 1993), and 2C5 (Williams et al., 2000). Docking studies of the 20 drug compounds listed in Table 1 were performed as described previously (Kemp et al., 2004) using the program GOLD version 3.0 (Jones et al., 1997), a program consistently found to perform well (Kellenberger et al., 2004; Kontoyianni et al., 2004), with the ChemScore (Eldridge et al., 1997; Verdonk et al., 2003; Kirton et al., 2005) fitness function. Ten solutions were generated for each ligand and ranked according to the value of the ChemScore fitness function, and the best ranked orientation of each compound was analyzed further.

Results

In Silico Docking. The CYP2D6 structural model (Kirton et al., 2002; Kemp et al., 2004) was used to screen in silico 20 drugs often taken as medication by patients with cancer undergoing chemotherapy. Each drug was docked into the CYP2D6 model, and the value of the ChemScore fitness function for the best-ranked solution is given in Table 1. All 20 drugs were screened experimentally for inhibition of AMMC demethylase activity and compared with the in silico data (Table 1). We obtained a good correlation between the ChemScore values and the experimental log IC₅₀ values (Fig. 1), with a regression coefficient of $r^2 = 0.61$ ($q^2 = 0.59$). The three drugs predicted by in silico analysis to be the strongest binders (Table 1) are loperamide, domperidone, and amitriptyline, which are three of the four tightest binding compounds, with experimental IC₅₀ values of 0.7, 2.2, and 3.6 μM, respectively. Of the 13 drugs predicted to inhibit CYP2D6 (ChemScore values $< -30$ kJ/mol) (Kemp et al., 2004), 11 are found experimentally to be inhibitors with IC₅₀ $< 100$ μM. Of the seven drugs predicted not to inhibit CYP2D6 (ChemScore values $> -30$ kJ/mol) (Kemp et al., 2004), five have experimental IC₅₀ values $> 100$ μM.

Metoclopramide Metabolism. In examining the orientations of the compounds in the active site predicted by our docking procedure, metoclopramide appeared to adopt a position in the active site that was inconsistent with the N-deethylation reaction previously reported (Destá et al., 2002). When docked into the active site of our CYP2D6 model, metoclopramide adopts two main orientations. In one of these (ChemScore $= -30.5$ kJ/mol for the most energetically favorable docking), the aromatic amino group of the drug lies in close proximity to the heme (Fig. 2A). This orientation suggests possible oxidative reactions at C₃, C₄, or the aromatic amino group (Fig. 3). In most of the solutions occupying this orientation, a salt bridge is observed between the basic nitrogen of metoclopramide and the side chain carboxyl group of Glu-216 (Fig. 2A). In the second docked orientation (ChemScore $= -26.0$ kJ/mol for the most energetically favorable
docking), the basic nitrogen of metoclopramide is in close proximity to the iron, and the aromatic moiety forms a good π/π interaction with the side chain of Phe-120 (Fig. 2B). This orientation is consistent with oxidation of the tertiary amine concomitant with formation of the N-deethylated metabolite(s) described by Desta et al. (2002). The relatively small difference in predicted ChemScore values between these two orientations suggests that CYP2D6 can catalyze oxidation at both the basic nitrogen and the aromatic amino moieties of metoclopramide.

To investigate the possibility of hydroxylation of the aromatic amino group of metoclopramide by CYP2D6, we analyzed the metabolism of metoclopramide by HLMs and recombinant CYP2D6 using HPLC/MS. Extracted ion chromatograms of a test sample (metoclopramide incubated with HLMs or CYP2D6), a control sample (without NADPH regenerating system), and a test sample in the presence of quinidine, a specific CYP2D6 inhibitor, are shown in Fig. 4. A metabolite peak (m/z 316) with a retention time of 8.7 min was formed after incubation with both HLMs and CYP2D6. Its appearance was dependent on the presence of NADPH and was inhibited by quinidine (5 μM), a diagnostic inhibitor of CYP2D6. The formation
of the m/z 316 metabolite followed Michaelis-Menten kinetics with a $K_{m}$ value of $6.41 \pm 0.42 \mu M$. The $V_{max}$ could not be calculated because of the absence of a standard. This low $K_{m}$ is consistent with the measured IC_{50} value of 9.2 $\mu M$ and the $K_{i}$ value of 4.7 $\mu M$ reported for the inhibition of CYP2D6-mediated dextromethorphan O-demethylation by metoclopramide (Desta et al., 2002).

We also located an m/z 272 ion with the same retention time as the m/z 316 ion, which is presumably the deethylated product described by Desta et al. (2002). However, the intensity of this ion was approximately 6 times lower than that of the m/z 316 ion. In addition, we found the formation of the m/z 272 ion was independent of NADPH, an essential cofactor for CYP2D6 catalysis (Fig. 5). Importantly, we found the m/z 316 ion to be relatively unstable compared with the m/z 272 ion (the m/z 316 ion peak disappeared from samples left at room temperature for ~32 h, whereas no significant change was found in the m/z 272 signal). Taken together, these data indicate that the m/z 316 ion is the primary metabolite produced after the metabolism of metoclopramide by CYP2D6.

Identification of the m/z 316 Metabolite. The m/z 316 metabolite represents a 16-mass unit increase over metoclopramide, indicative of the addition of an oxygen atom. To identify the location of the reaction, we compared the collision-induced dissociation (CID) mass spectra of metoclopramide and the metabolite. The CID of metoclopramide (m/z 300) produced four major fragments at m/z 227.03, 212.02, 184.00, and 156.01 (Fig. 6A). These correspond to the progressive loss of the following functional groups, as shown in Fig. 6A: m/z 227.03 ion, loss of the diethyloamino group (73 mass units); m/z 212.02, additional loss of a methyl group (15 mass units); m/z 184.00, additional loss of $\text{CHNH}$ (28 mass units); and m/z 156.01, additional loss of carbon monoxide (28 mass units).

The CID of the m/z 316 metabolite produced four major fragments at m/z 243.02, 226.01, 199.99, and 182.97 (Fig. 6B). The m/z 243.02 fragment corresponds to a 16-mass unit increase relative to the m/z 227.03 fragment seen in the CID spectrum of metoclopramide, corresponding to the addition of a hydroxyl group. The m/z 226.01 and 182.97 fragments appear to be derived from loss of 17 mass units from the m/z 243.02 and 199.99 fragments, respectively (Fig. 6B), corresponding to the facile loss of OH from a hydroxylamino group. Taken together, these data point to oxidation of the aromatic amino group to a hydroxylamino group by CYP2D6, consistent with the prediction from Fig. 2A.

**Discussion**

CYP2D6 metabolizes many drugs commonly taken by patients undergoing anticancer chemotherapy. We previously reported the validation of this CYP2D6 model by the successful prediction of relative affinities (Kemp et al., 2004) and sites of metabolism (Kirton et al., 2002; Kemp et al., 2004). Indeed, the recent publication of a CYP2D6 crystal structure (Rowland et al., 2006) shows good agreement with the global structure and the positioning of key active site residues predicted by our modeling data. We have now used the same method to screen a small but diverse range of concomitant medications used by patients with cancer; we recently performed a similar study of the interaction of comedication compounds with CYP3A4 (Marechal et al., 2006). The in silico screen successfully discriminated between compounds that bound to CYP2D6 and those that did not. Taking (as we have previously; Kemp et al., 2004) a ChemScore of $>30$ kJ/mol and an IC_{50} of 100 $\mu M$ as the dividing lines between “binders” and “nonbinders,” the docking calculations produced two false-positive and two false-negative results out of the 20 compounds examined. In terms of a quantitative comparison, the experimental data for these 20 compounds correlated reasonably well with the values predicted from the model, with a regression coefficient of $r^2 = 0.61$; this is significantly better than random.

In addition to predicting binding affinity, our modeling approach can also be used to predict the sites of metabolism of a wide range of drugs (Kirton et al., 2002; Kemp et al., 2004). In the present work, this has proved useful in predicting a novel metabolic pathway for metoclopramide, a commonly prescribed antiemetic drug. The aryldihydrazone metabolite of metoclopramide was identified from urine analysis (Maurich et al., 1994), and our study now identifies CYP2D6 as mediating the oxidative metabolism of metoclopramide to this hydroxylamino derivative rather than to the N-deethylated product previously reported (Desta et al., 2002). The discrepancy may be associated with the instability of the hydroxylamino metabolite, allied to differences in sample handling before MS analysis (the analysis of Desta et al. was based on off-line MS), which creates the potential for the degradation of the genuine metabolite.
With respect to the clinical significance of this new finding, it is unlikely that P450-mediated metabolism determines the overall clearance of metoclopramide (Teng et al., 1977; Bateman et al., 1980). However, minor metabolic pathways could be clinically relevant if linked to the generation of a toxic metabolite. In this respect, it is well documented that metoclopramide induces movement disorders, such as parkinsonism, dystonia, tardive dyskinesia, and akathisia (Jimenez-Jimenez et al., 1997), as well as arrhythmias (Malkoff et al., 1995; Baguley et al., 1997), hypertension (Harrington et al., 1983), and disorders involving hemoglobin oxidation (Grant et al., 1994; Langford and Sheikh, 1999; Kuehl et al., 2001). Thus, because toxic aromatic amines are known to be generated from related compounds such as procainamide (Radomski, 1979), further investigation into the potential toxicity of the metoclopramide metabolite is warranted. Furthermore, patients experiencing extrapyramidal or other side effects from metoclopramide should be screened to see whether the adverse effect is related to their CYP2D6 genotype. If this is the case, then there may be implications for the patients who may be particularly prone to adverse effects from other drugs using this as the major metabolic pathway.

In cancer treatment, drug-drug interactions involving CYP2D6 are probably most significant with tamoxifen, an estrogen receptor antagonist that is widely used to treat breast cancer. CYP2D6 has been probably most significant with tamoxifen, an estrogen receptor antagonist that is widely used to treat breast cancer. CYP2D6 has been shown to play an important role in the metabolism of this compound, increasing potency through conversion to the antiestrogen 4'-hydroxytamoxifen (Dehal and Kupfer, 1997; Crewe et al., 2002), and we measured a relatively low IC₅₀ for tamoxifen of 6.8 μM. Importantly, the efficacy of tamoxifen varies widely, with recent evidence linking the efficacy of tamoxifen varies widely, with recent evidence linking the molecular basis for drug-drug interactions with comedication commonly used by patients with cancer.


References


Jimenez-Jimenez FJ, Garcia-Ruiz DJ, and Molina JA (1997) Drug-induced movement disorders. In: Langford and Sheikh, 1999; Kuehl et al., 2001). Thus, because toxic aromatic amines are known to be generated from related compounds such as procainamide (Radomski, 1979), further investigation into the potential toxicity of the metoclopramide metabolite is warranted. Furthermore, patients experiencing extrapyramidal or other side effects from metoclopramide should be screened to see whether the adverse effect is related to their CYP2D6 genotype. If this is the case, then there may be implications for the patients who may be particularly prone to adverse effects from other drugs using this as the major metabolic pathway.

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Address correspondence to: C. R. Wolf, Biomedical Research Centre, University of Dundee, Ninewells Hospital & Medical School, Dundee, DD1 9SY, UK. E-mail: roland.wolf@cancer.org.uk