Major Role of Human Liver Microsomal Cytochrome P450 2C9 (CYP2C9) in the Oxidative Metabolism of Celecoxib, a Novel Cyclooxygenase-II Inhibitor

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Accepted for publication January 17, 2000 This paper is available online at http://www.jpet.org

ABSTRACT

In vitro studies were conducted to identify the cytochromes P450 (CYP) involved in the oxidative metabolism of celecoxib. The hydroxylation of celecoxib conforms to monophasic Michaelis-Menten kinetics (mean ± S.D., n = 4 livers, $K_m = 3.8 ± 0.95 \mu M, V_{max} = 0.70 ± 0.45 \text{nmol/min/mg protein}$) in the presence of human liver microsomes, although substrate inhibition was significant at higher celecoxib concentrations. The treatment of a panel of human liver microsomal samples (n = 16 subjects) with antibodies against CYP2C9 and CYP3A4 inhibited the formation of hydroxy celecoxib by 72 to 92% and 0 to 27%, respectively. The presence of both antibodies in the incubation suppressed the activity by 90 to 94%. In addition, the formation of hydroxy celecoxib significantly correlated with CYP2C9-selective tolbutamide methyl hydroxylation ($r = 0.92, P < .001$) and CYP3A-selective testosterone 6β-hydroxylation ($r = 0.55, P < .02$). In contrast, correlation with activities selective for other forms of CYP was weak ($r \leq 0.46$). Chemical inhibition studies showed that ketoconazole (selective for CYP3A4) and sulfaphenazole (selective for CYP2C9) inhibited the formation of hydroxy celecoxib in a concentration-dependent manner, whereas potent inhibitors selective for other forms of CYP did not show any significant effect over a range of 1 to 10 \mu M. In agreement, cDNA-expressed CYP2C9 catalyzed the formation of hydroxy celecoxib with an apparent $K_m$ value (\mu M) and a $V_{max}$ value (pmol/min/pmol recombinant CYP) of 5.9 and 21.7, whereas a higher $K_m$ value (18.2) and a lower $V_{max}$ value (1.42) were obtained with rCYP3A4. It is concluded that methyl hydroxylation of celecoxib is primarily catalyzed by human liver microsomal CYP2C9, although CYP3A4 also plays a role.

Cyclooxygenase (COX) is an enzyme that catalyzes the first two steps in the biosynthesis of prostaglandins from arachidonic acid (Riendeau et al., 1997; Vane et al., 1998). Although it was long held that COX was a single enzyme present in most cells (Graul et al., 1997), more recent data have pointed to the existence of two forms (Vane et al., 1998). COX-I is the major form located in healthy tissues, and it plays a role in thrombogenesis and homeostasis of the gastrointestinal tract and kidneys (Smith and DeWitt, 1996). In contrast, COX-II is normally undetectable in most tissues and is inducible by cytokines, endotoxins, and mitogens. It has been associated with the elevated production of prostaglandins observed during inflammation, pain, and pyretic responses (Donnelly and Hawkey, 1997; Jouzeau et al., 1997; Lane, 1997; Lipsky and Isakson, 1997). Both forms of COX metabolize arachidonic acid via a similar mechanism, but they have different substrate specificities. COX-II accepts a wider range of substrates than COX-I (Battistini et al., 1994).

Most nonsteroidal anti-inflammatory drugs (NSAIDs) currently in use, such as indomethacin, ibuprofen, and diclofenac, inhibit both COX-I and COX-II with little or no selectivity for either form of the enzyme (Battistini et al., 1994; O’Neill et al., 1994). It is believed that NSAID-induced gastrointestinal damage and platelet and renal dysfunction result from the inhibition of COX-I, whereas the therapeutic benefit results from the inhibition of COX-II expressed at the sites of inflammation (Simon et al., 1998). Therefore, the identification and characterization of two isoforms of cyclooxygenase have stimulated tremendous efforts to develop potent and selective COX-II inhibitors (Penning et al., 1997; Simon et al., 1998). Celecoxib [Celebrex; SC-58635; 4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonylimide] is one example of a COX-II-selective agent (Fig. 1), and it has been approved by the Food and Drug Administration for the treatment of osteoarthritis and rheumatoid arthritis (Penning et al., 1997; Simon et al., 1998).

Celecoxib is well absorbed and is extensively metabolized in humans, with less than 3% of the dose excreted unchanged.

ABBREVIATIONS: COX, cyclooxygenase; rCYP, recombinant CYP; mAb, monoclonal antibody; SLF, sulfaphenazole; KTZ, ketoconazole; QND, quinidine; LC, liquid chromatography; MS, mass spectometry; $K_m$, apparent Michaelis constant; $V_{max}$, maximal initial reaction velocity; NSAID, nonsteroidal anti-inflammatory drug.
CYP proteins.

In vitro incubations were carried out at 37°C in a Fisher shaking water bath with 13 × 100-mm borosilicate glass disposable culture tubes. The incubation mixture (final volume of 0.5 mL) consisted of 0.1 M potassium phosphate buffer, pH 7.4, 10 mM MgCl2, 1 mM EDTA, 1.0 mM NADP+, 10 mM D-glucose-6-phosphate, 2.0 U/mL D-glucose-6-phosphate dehydrogenase (Sigma Type VII, from baker’s yeast), 0.10 to 0.20 mg/mL microsomal protein, and 0.5 to 50 μM celecoxib dissolved in acetonitrile (0.3% v/v final concentration). The reaction was started by the addition of the NADPH-generating system after a 5-min preincubation and was terminated with 2 mL of acetonitrile. The internal standard, diclofenac (50 μL of a 100 μM stock), was added to the sample before centrifugation. The supernatant was transferred to a clean tube and evaporated to dryness (SpeedVac; Savant Instruments, Inc., Holbrook, NY). In each case, the residue was reconstituted in 150 μL of aqueous solution of acetonitrile (30%) for HPLC analysis. Under these conditions, the rate of hydroxy celecoxib formation was linear with respect to protein concentration and time of incubation.

**Measurement of CYP Form-Selective Activities.** Microsomal samples (Xenotech, LLC) were prepared for incubation and analysis as described earlier. The rate of celecoxib (15 μM) hydroxylation was correlated with the various CYP monoxygenase activities (data provided by Xenotech, LLC), including 7-ethoxyresorufin O-deethylase (CYP1A2), coumarin 7-hydroxylase (CYP2A6), taxol 6-hydroxylase (CYP2C8), tolbutamide methyl-hydroxylase (CYP2C9), (S)-mephentoin 4’-hydroxylase (CYP2C19), chloroxazone 6-hydroxylase (CYP2E1), bufuralol 1’-hydroxylase (CYP2D6), and testosterone 6β-hydroxylase (CYP3A4/5). Correlation coefficients (r) were determined graphically and subjected to the Student’s t test (Rodrigues et al., 1996).

**CYP-Selective Inhibitors.** Inhibition studies with CYP form-selective chemical inhibitors were carried out at a final celecoxib concentration of 5 μM (Km). Microsomal preparations from the livers of three different subjects were used. SLF (0.1–10 μM, CYP2C9); QND (0.1–10 μM, CYP2D6), and KTZ (0.1–10 μM, CYP3A4) were dissolved in 50% (v/v) acetonitrile as stock solutions. The inhibitors were individually incubated with each microsomal sample (0.10 mg protein/mL) and substrate for 20 min. Control incubations contained the same concentration of acetonitrile but no inhibitor (≤1% v/v final concentration). Inhibitor concentrations were chosen on the basis of established Ki values (Bourrie et al., 1995; Newton et al., 1995; Rodrigues et al., 1996) to ensure maximal inhibition (>80%) of each CYP form ([I]/Ki > 10; [S] > Km).

**Incubations with cDNA-Expressed Human CYP Microsomes.** Incubations of celecoxib with various individual recombinant CYP (rCYP) proteins were carried out as described for liver microsomes, except that the enzyme concentration was 5 or 25 nM.

For all CYP proteins tested, the reaction rates (picomoles per minute per picomole of CYP) were normalized (picomoles per minute per picomole · picomoles of CYP per milligram) with respect to the corresponding nominal (mean) specific content of each CYP in native human liver microsomes (data provided by Gentest Corp.). The normalized rates (picomoles per minute per milligram) were then added, and the normalized rate for each CYP was expressed as the percentage of the total normalized rate (Rodrigues, 1998). The apparent Km and Vmax values were determined using in-house preparations of
rCYP2C9 and rCYP3A4 at final enzyme concentrations of 10 and 25 nM, respectively.

**Immunoinhibition Studies.** Mouse ascites, fluid containing the respective antibodies, were diluted with potassium phosphate buffer (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64 dilutions). An aliquot (20 μl) of each diluted ascites solution was incubated with 50 μg human liver microsomal protein (50 μl) at room temperature for 20 min before the addition of MgCl₂, EDTA, celecoxib, and phosphate buffer. The inhibitory potency of each antibody preparation was confirmed after coincubation with the appropriate rCYP. In a second experiment, a panel of human liver microsomes (n = 16 livers) was preincubated with an aliquot (20 μl) of undiluted (for anti-CYP2C9 mAb) or diluted (1:32, for anti-CYP3A4 mAb) mouse ascites to examine the effect of antibody treatment on the correlation of hydroxy celecoxib formation with the corresponding CYP form-selective activity. The final concentrations of celecoxib and human liver microsomes were 15 μM and 0.1 mg/ml, respectively.

**Kinetic Analysis.** Estimates of apparent Kₘ and Vₘₐₓ values were obtained by fitting the untransformed data to a one- or two-enzyme model (GraFit 3; Leatherbarrow, 1992). After initial kinetic parameter estimates were obtained, the data were also analyzed by linear transformation (Eadie-Hofstee plot) to confirm a single Kₑ model.

**HPLC-UV Analysis.** Celecoxib and its metabolites were separated on a reversed phase C18 column (4.6 × 150 mm, 5 μm, Betasil, Keystone, Bellefonte, PA) using a Shimadzu LC-10AS HPLC system. The mobile phase consisted of 0.05% aqueous phosphoric acid (solvent A) and acetonitrile/H₂O (90:10) in 0.05% phosphoric acid (solvent B) and was delivered at a constant flow rate of 1.0 ml/min. The initial mobile phase consisted of 30% of solvent B, which increased linearly to 80% over 13 min, and the elution of celecoxib and hydroxy celecoxib was monitored by UV detection (254 nm). Hydroxy celecoxib, the internal standard (diclofenac), and celecoxib eluted at 10.2, 13.5, and 14.8 min, respectively, under these conditions. Due to the unavailability of hydroxy celecoxib standard, its quantification was achieved by using the calibration curve for carboxy celecoxib (assuming a similar extinction coefficient). Calibration curves were prepared by adding known quantities of carboxy celecoxib and the internal standard to control incubation mixtures. The lower limit of quantification in this study was 25 pmol/ml. The assay was linear over a carboxy celecoxib concentration range of 25 pmol/ml to 10 nmol/ml.

**Liquid Chromatography (LC)-Mass Spectrometry (MS) Analysis.** HPLC separation was carried out on an HP-1050 gradient system (Hewlett-Packard, Palo Alto, CA) using a Betasil C18 column (2 × 150 mm, 5 μm). The mobile phase consisted of 0.2% aqueous acetic acid with pH adjusted to 7.6 with NH₄OH (solvent A) and acetonitrile (solvent B) and was delivered at a constant flow rate of 0.2 ml/min. The initial mobile phase consisted of 10% of solvent B, which was held for 3 min and then linearly increased to 80% over 35 min.

The column eluant was coupled directly to a Finnigan MAT LCQ ion trap mass spectrometer. Mass spectral analyses were performed using electrospray ionization in the negative mode. The electrospray ionizing voltage was set at 4.5 kV, and the heated capillary temperature was maintained at 230°C for all analyses. Collision energy value was set at 25% for the MS/MS experiments. Under these conditions, hydroxy celecoxib yielded an [M-H]⁻ ion at m/z 396 on MS analysis. MS/MS of this parent ion produced intense fragment ions at m/z 332 (M-H-SO₂-HCOH-HF)⁻, m/z 302 (M-H-SO₂-HCOH)⁻, and m/z 282 (M-H-SO₂-HCOH-HF)⁻.

**Results**

**Metabolism of Celecoxib.** A typical UV chromatogram of the extract after incubation of celecoxib with NADPH-fortified human liver microsomes is shown in Fig. 2. After incubation, one major metabolite (retention time, 10.2 min) was detected and identified (LC-MS/MS) as hydroxy celecoxib (Fig. 1). No carboxy celecoxib was detected, although minor amounts of the corresponding aldehyde were detected by LC-MS (data not shown).

Overall, the formation of methyl hydroxy celecoxib conformed to saturable kinetics, and a representative Michaelis-Menten plot (pool of n = 10 different livers) is presented in Fig. 3. A slight but significant decrease in hydroxy celecoxib formation was observed at higher celecoxib concentrations. Similar results were obtained with microsomal preparations from human liver microsomes (HHM-0259). Inset, Eadie-Hofstee plot. The mAb against human CYP2C9 showed inhibition of hydroxy celecoxib in human liver microsomes exhibited monophasic enzyme kinetics over the substrate concentration range of 1 to 20 μM (Fig. 3, inset).

**Immunoinhibition of Hydroxy Celecoxib Formation.** The mAb against human CYP2C9 significantly inhibited the formation of hydroxy celecoxib in human liver microsomes, yielding up to >85% inhibition in a concentration-dependent manner (Fig. 4A). The mAbs against human CYP3A4 suppressed hydroxy celecoxib formation by ~10% at low concentrations, and its inhibitory effect diminished as the concentration of ascites increased. When the volume of ascites...
reached >5 μl, a small but significant rise in the rate of hydroxy celecoxib formation was observed (Fig. 4B). To avoid stimulation of celecoxib hydroxylase activity, therefore, the ascites fluid containing anti-CYP3A4 was diluted (1:32) in subsequent experiments. The presence of both antibodies in the incubation system almost completely inhibited the formation of hydroxy celecoxib (90–95% inhibition) as shown in Fig. 4C. The treatment of a panel of human liver microsomal samples (n = 16 subjects) with antibodies against CYP2C9 and CYP3A4 inhibited the formation of hydroxy celecoxib by 72 to 92% and 0 to 27%, respectively (Fig. 5).

**Correlation Studies.** The formation rates of hydroxy celecoxib were determined in 16 different human liver microsomal preparations. There was considerable interindividual variability in the values obtained with a mean ± S.D. activity (nanomoles per minute per milligram of protein) of 0.48 ± 0.17. As shown in Fig. 6, A and B, the rate of hydroxy celecoxib formation correlated with tolbutamide methyl-hydroxylation (CYP2C9) and testosterone 6β-hydroxylation (CYP3A4/5) activities (r = 0.92, P < .01 and r = 0.55, P < .05, respectively). Interestingly, the suppression of one of these CYPs by its corresponding antibody improved the correlation with the second CYP form. Namely, inhibition of celecoxib hydroxylation by antibody against CYP3A4 slightly improved the correlation with tolbutamide methyl-hydroxylation activity (r = 0.93, P < .005, Fig. 6C). However, inhibition of celecoxib hydroxylation by antibody against CYP2C9 significantly enhanced the correlation with testosterone 6β-hydroxylation activity (r = 0.96, P < .005, Fig. 6D). The correlation of hydroxy celecoxib formation with activities selective for other CYP isoforms [e.g., 7-ethoxyresorufin O-dealkylation, CYP1A2; coumarin 7-hydroxylation, CYP2A6; 7-ethoxy-4-trifluoromethylcoumarin O-deethylation, CYP2B6; taxol-6-hydroxylation, CYP2C8; (S)-mephenytoin 4′-hydroxylation, CYP2C19; dextromethorphan-O-demethylation, CYP2D6; and chlorzoxazone 6-hydroxylation, CYP2E1] was not statistically significant (Table 2).

**Chemical Inhibition.** Based on the immunoinhibition and correlation data, three selective chemical inhibitors (SLF, KTZ, and QND) were chosen to substantiate the contribution of CYP2C9 and CYP3A4 in the metabolism of celecoxib. As displayed in Fig. 7, SLF (selective for CYP2C9) and KTZ (selective for CYP3A4) significantly inhibited the formation of hydroxy celecoxib in a concentration-dependent manner, whereas QND (selective for CYP2D6) did not show any significant effect (inhibition, <5%).

**Celecoxib Metabolism by cDNA-Expressed Human Cytochromes P450.** The CYP reaction phenotype of celecoxib was further evaluated with rCYP proteins. Consistent

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**Table 1**

Apparent kinetic parameters for the formation of hydroxy celecoxib in the presence of human liver microsomes

<table>
<thead>
<tr>
<th>Microsome Preparation</th>
<th>K&lt;sub&gt;m&lt;/sub&gt; (μM)</th>
<th>V&lt;sub&gt;max&lt;/sub&gt; (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMM0259*</td>
<td>5.1</td>
<td>0.43</td>
</tr>
<tr>
<td>HG30</td>
<td>2.8</td>
<td>1.37</td>
</tr>
<tr>
<td>HG56</td>
<td>3.4</td>
<td>0.55</td>
</tr>
<tr>
<td>HG66</td>
<td>3.9</td>
<td>0.45</td>
</tr>
<tr>
<td>Mean (S.D.)</td>
<td>3.8 (0.9)</td>
<td>0.70 (0.45)</td>
</tr>
</tbody>
</table>

* Pool of livers (n = 10 different subjects).
with the results obtained with native human liver microsomes, a decrease in celecoxib methyl hydroxylase activity was also observed when the substrate concentrations exceeded 15 μM (Fig. 8A). Fitting the data acquired at substrate concentrations of ≤15 μM yielded an apparent $K_m$ value of 4.1 μM, which was comparable to that observed with native human liver microsomes. By comparison, the rCYP3A4-catalyzed metabolism of celecoxib was best described by a simple Michaelis-Menten equation and was characterized by a higher $K_m$ value (18 versus 4.1 μM) and a lower $V_{max}$ value (1.4 versus 17 pmol/min/pmol rCYP) compared with rCYP2C9 (Fig. 8B).

The rates of hydroxy celecoxib formation in the presence of several rCYPs are listed in Table 3. On normalization of the data with respect to the abundance of each enzyme in native human liver microsomes (Rodrigues, 1999), it was found that the majority (86%) of the hydroxylase activity was attributable to CYP2C9, although CYP3A (6%) did contribute. The normalized data were in agreement with the results of the immunoinhibition experiments.

**Discussion**

In the present study, it has been shown that the first step in the hepatic biotransformation of celecoxib (≤25 μM) involves a methyl hydroxylation reaction mediated principally by CYP2C9. The involvement of CYP2C9 is well supported by several lines of evidence: 1) a good correlation between the rate of hydroxy celecoxib formation and tolbutamide methyl-hydroxylase activity in a panel of human liver microsomes, 2) extensive inhibition of celecoxib metabolism by mAbs against CYP2C9 and SLF (a selective inhibitor of CYP2C9), and 3) a high turnover in the presence of human rCYP2C9. However, based on the results of this study, it is concluded that a member or members of the human liver microsomal CYP3A subfamily play a minor role (~10%). For example, the correlation of celecoxib hydroxylase with testosterone 6β-hydroxylase was improved in the presence of anti-CYP3A4 mAb versus tolbutamide methyl-hydroxylase activity (panel of human liver microsomes, r = 0.96 versus 0.55). In addition, KTZ at 0.1 μM (a specific inhibitor of CYP3A) and anti-CYP3A4 mAbs significantly inhibited the formation of hydroxy celecoxib by 10%. The greater inhibitory effect seen at a higher concentration of KTZ (≥1 μM) may be attributable to the nonselective inhibition of CYP2C9 (Boobis, 1995).

The involvement of other CYP forms in the metabolism of celecoxib is unlikely. rCYP2D6 showed considerable activity (2.9 pmol/min/pmol rCYP2D6), but there was no correlation between celecoxib metabolism and dextromethorphan O-demethylase activity and no inhibition in the presence of QND...
(a selective inhibitor of CYP2D6) in native human liver microsomes. Similarly, although appreciable activity was observed with rCYP2C19 (3.2 pmol/min/pmol rCYP2C19), the poor correlation between celecoxib hydroxylase and \((S)\)-mephenytoin 4\(^{\text{\footnotesize{9}}}\)-hydroxylase in human liver microsomes did not support the involvement of the enzyme. However, it is possible that CYP2C19 may play a more prominent role in some individuals with higher CYP2C19 levels (Lasker et al., 1998).

Moreover, metabolism by CYP2C8 in human liver microsomes was ruled out because of the weak correlation with taxol hydroxylase activity (Table 2). It should be noted that SLF would not be expected to inhibit (<10\%) CYP2C8 and CYP2C19 at the concentrations used in the present study (Mancy et al., 1996).

The decrease in hydroxy celecoxib formation rate at higher substrate concentrations may be suggestive of substrate inhibition and the fact that simple Michaelis-Menten kinetics do not adequately model the kinetic data. In fact, the experimental data were best modeled by incorporation of a term for substrate inhibition. Unfortunately, the poor aqueous solubility of celecoxib prevented the use of high substrate concentrations, and it was not possible to better define the inhibition term. By comparison, other investigators have been able to use a wider range of substrate concentrations to

TABLE 2
Correlation of various CYP activities with the formation of hydroxy celecoxib in human liver microsomes

<table>
<thead>
<tr>
<th>Activity</th>
<th>CYP</th>
<th>Correlation Coefficient ( (r^2) )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Ethoxyresorufin O-dealkylation</td>
<td>1A2</td>
<td>0.26</td>
<td>&gt;.3</td>
</tr>
<tr>
<td>Coumarin 7-hydroxylation</td>
<td>2A6</td>
<td>0.46</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>7-EFC ((S))-Mephenytoin</td>
<td>2B6</td>
<td>0.29</td>
<td>&gt;.2</td>
</tr>
<tr>
<td>4(^{\text{\footnotesize{9}}})-hydroxylation</td>
<td>2C19</td>
<td>0.35</td>
<td>&gt;.2</td>
</tr>
<tr>
<td>Taxol-6-hydroxylation</td>
<td>2C8</td>
<td>0.42</td>
<td>&lt;.1</td>
</tr>
<tr>
<td>Tolbutamide methyl-hydroxylation</td>
<td>2C9</td>
<td>0.92 (0.93)(^d)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Dextromethorphan O-demethylation</td>
<td>2D6</td>
<td>0.11</td>
<td>&gt;.6</td>
</tr>
<tr>
<td>Chlorzoxazone 6-hydroxylation</td>
<td>2E1</td>
<td>0.35</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Testosterone 6(^{\text{\footnotesize{b}}})-hydroxylation</td>
<td>3A4/5</td>
<td>0.55 (0.96)(^d)</td>
<td>&lt;.02</td>
</tr>
</tbody>
</table>

\(^a\) Panel consisted of microsomal preparations from 16 different human livers.  
\(^b\) 7-EFC, 7-ethoxy-4-trifluoromethylcoumarin.  
\(^c\) Hydroxy celecoxib formation was determined in the presence of anti-CYP3A4 ascites.  
\(^d\) Hydroxy celecoxib formation was determined in the presence of anti-CYP2C9 ascites.

Fig. 7. Effects of different concentrations of chemical inhibitors on human liver microsome-catalyzed celecoxib methyl hydroxylase activity (celecoxib was evaluated at a final concentration of 5 \( \mu \)M). Reaction rates are expressed as a percentage of the control velocity without inhibitor (mean ± S.D., \( n = 3 \) microsomal preparations).

TABLE 3
Formation of hydroxy celecoxib by recombinant CYP proteins expressed in insect cells

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Hydroxy Celecoxib Formation Rate</th>
<th>Mean Specific CYP Content</th>
<th>Normalized Hydroxy Celecoxib Formation Rate</th>
<th>Percentage of Total Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{pmol/min/pmol rCYP} )</td>
<td>( \text{nmol/mg protein} )</td>
<td>( \text{nmol/min/mg protein} )</td>
<td>( % )</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>12.30</td>
<td>0.096</td>
<td>1.18</td>
<td>86.1</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>0.70</td>
<td>0.108</td>
<td>0.08</td>
<td>5.8</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>0.42</td>
<td>0.045</td>
<td>0.02</td>
<td>1.4</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>3.19</td>
<td>0.019</td>
<td>0.06</td>
<td>4.4</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>2.90</td>
<td>0.010</td>
<td>0.03</td>
<td>2.1</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>0.02</td>
<td>0.049</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>15.17</td>
<td>0.343</td>
<td>1.37</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) 5 pmol rCYP/ml and 25 pmol rCYP/ml for CYP2C9 and other isoforms, respectively. Incubation at 37°C for 30 min (celecoxib concentration of 25 \( \mu \)M).  
\(^b\) Mean specific content of CYP in native human liver microsomes (Rodrigues, 1999).  
\(^c\) Rate of hydroxy celecoxib formation normalized with respect to the specific content of each CYP in native human liver microsomes.

Fig. 8. Rates of formation of hydroxy celecoxib by the recombinant CYP2C9 (A) and CYP3A4 (B). Insets, Eadie-Hofstee plots.
successfully evaluate substrate inhibition (Goeger and Anderson, 1992; Gorski et al., 1994; Tucker et al., 1994; Schmider et al., 1996; von Moltke et al., 1996; Shiraga et al., 1999). Interestingly, substrate inhibition was limited to celecoxib hydroxylase activity catalyzed by CYP2C9 and was not observed with CYP3A4. Moreover, the phenomenon was observed with the recombinant enzyme and native liver microsomes. To our knowledge, celecoxib is the first CYP2C9 substrate that exhibits substrate inhibition; no similar findings have been reported for commonly used CYP2C9 probes, such as tolbutamide, diclofenac, and phen tolbutamide, phenytoin, (Figure 4). Community pharmacogenetic research has shown that a large number of drugs, including tolbutamide, phenytoin, (S)-warfarin, losartan, sulfamethoxazole, and various NSAIDs (Goldstein and de Morais, 1994; Lasker et al., 1998; Miners and Birkett, 1998; Gill et al., 1999; McCrea et al., 1999). In summary, the results of the present study indicate that celecoxib methyl hydroxylat ion, is largely catalyzed by CYP2C9, although CYP3A4 plays a minor role.

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