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Regular Article

Celecoxib is a substrate of CYP2D6: Impact on celecoxib metabolism in individuals with CYP2C9*3 variants

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ABSTRACT

Celecoxib was characterized as a substrate of human cytochrome P450 (CYP) 2D6 in vitro. In recombinant CYP2D6, celecoxib hydroxylation showed atypical substrate inhibition kinetics with apparent K_m, K_i, and V_{max} of 67.2 μM, 12.6 μM, and 1.33 μM/min, respectively. In human liver microsomes (HLMs), a concentration-dependent inhibition of celecoxib hydroxylation by quinidine was observed after CYP2C9 and CYP3A4 were inhibited. In individual HLMs with variable CYP2D6 activities, a significant correlation was observed between celecoxib hydroxylation and CYP2D6-selective dextromethorphan *O*-demethylation when CYP2C9 and CYP3A4 activities were suppressed ($r = 0.97$, $P < 0.0001$). Molecular modeling showed two predominant docking modes of celecoxib with CYP2D6, resulting in either a substrate or an inhibitor. A second allosteric binding antechamber, which stabilized the inhibition mode, was revealed. Modeling results were consistent with the observed substrate inhibition kinetics. Using HLMs from individual donors, the relative contribution of CYP2D6 to celecoxib metabolism was found to be highly variable and dependent on CYP2C9 genotypes, ranging from no contribution in extensive metabolizers with CYP2C9*1*1 genotype to approximately 30% in slow metabolizers with allelic variants CYP2C9*1*3 and CYP2C9*3*3. These results demonstrate that celecoxib may become a potential victim of CYP2D6-associated drug-drug interactions, particularly in individuals with reduced CYP2C9 activity.

1. Introduction

Celecoxib, a nonsteroidal anti-inflammatory drug, is widely used for relieving the symptoms of inflammation. By selectively inhibiting cyclooxygenase-II, celecoxib avoids common adverse effects of other drugs in its class, such as gastrointestinal bleeding, ulceration, and perforation [1].

All major metabolites of celecoxib originate from methyl hydroxylation, mediated primarily by human cytochrome P450 (CYP) 2C9 (Fig. 1) [2]. The predominant role of CYP2C9 and the relatively minor role of CYP3A4 in the metabolic pathway of celecoxib have been previously demonstrated in vitro by Tang et al. [3]. Considerable activity was also detected in the recombinant CYP2D6 system; however, the lack of CYP2D6-selective inhibition by quinidine and the absence of a correlation with dextromethorphan *O*-demethylation in human liver microsomes (HLMs) led researchers to conclude that celecoxib was an unlikely substrate of CYP2D6 [3]. In contrast, the inhibition of CYP2D6 by celecoxib has been well recognized [4]. As a result, the current drug label for celecoxib identifies it only as an inhibitor, but not as a substrate, of CYP2D6 with regard to potential drug-drug interactions [5].

In the current study, we thoroughly characterized the CYP2D6-mediated metabolism of celecoxib to its hydroxyl metabolite using three primary experimental approaches: recombinant enzymes, enzyme-selective inhibitors, and correlation with enzyme-selective probe activity in HLMs. In addition, we substantiated our novel findings using molecular modeling.

The molecular structure of CYP2D6 is well established. According to extensive structural biology studies [6–8], the key requirements for a typical ligand of CYP2D6 are the interaction between Asp301 and Glu216 in the catalytic site of the enzyme, and the presence of a basic nitrogen atom at a distance of 5–7 Å from the site of metabolism or inhibition [9,10]. This latter structural feature is absent in celecoxib (Fig. 1); none of its three nitrogen atoms are favorable for having a positive charge at physiological pH. Nevertheless, a rather unique interaction

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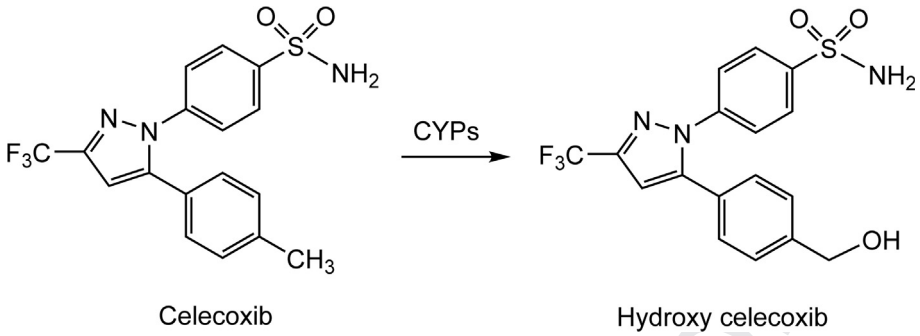

Fig. 1. The major metabolic pathway of celecoxib: methyl hydroxylation mediated by cytochrome P450.

between celecoxib and CYP2D6 was demonstrated in this study by docking the drug to an ensemble of published X-ray crystal structures of CYP2D6.

Although the indisputably predominant role of CYP2C9 in the metabolism of celecoxib should keep potential contribution of CYP2D6 low in a general population, both enzymes are well known for their polymorphism; reported enzyme activities vary widely, from nonfunctional to extremely high in many ethnic populations [11–14]. Therefore, it is conceivable that the metabolic contribution of CYP2D6 could increase to a level significant enough to impact the metabolism of celecoxib in certain populations of CYP2C9 slow metabolizers with either extensive or ultra-high CYP2D6 activity. To investigate this novel concept, we assessed the CYP2D6 relative contribution for metabolism (f_m) of celecoxib in individual HLMs obtained from CYP2C9-genotyped poor and intermediate metabolizers and compared the results to wild-type extensive metabolizers.

2. Materials and methods

2.1. Chemicals

Celecoxib and celecoxib-d7 were purchased from Santa Cruz Biotechnologies (Dallas, TX). Hydroxy celecoxib, dextromethorphan, and dextrorphan were purchased from Toronto Research Chemicals (Toronto, Canada). Quinidine and sulfaphenazole were purchased from Sigma-Aldrich (St. Louis, MO). Recombinant CYP isoforms (Supersomes™) and potassium phosphate buffer (PPB) were purchased from Corning Life Sciences (Tewksbury, MA). Mixed-gender pooled HLMs and single-donor genotyped HLMs were purchased from Xenotech LLC (Lenexa, KS) and BioreclamationIVT (Baltimore, MD). Reduced nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Oriental Yeast Company (Andover, MA). All other chemicals, reagents, and solvents were of either analytical grade or high-performance liquid chromatography (HPLC) grade.

2.2. Analytical methods

Analyses of celecoxib and hydroxy celecoxib were performed using liquid chromatography with tandem mass spectrometry (LC-MS/MS). The AB Sciex 4000 QTrap triple quadrupole mass spectrometer (Framingham, MA) was interfaced with Shimadzu HPLC systems, including LC-10AD binary pumps and the SIL-HTC autosampler (Shimadzu Corporation, Kyoto, Japan). The HPLC column used was Zorbax XDB-C18 5μ 2.1 × 50 mm (Agilent, Santa Clara, CA). HPLC resolution was achieved with a gradient consisting of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) in the following steps: $t = 0$ min, percent solvent B (%B) = 10; $t = 0.5$ min, %B = 99; $t = 2.5$ min, %B = 99; and $t = 2.6$ min, %B = 10, with a total run time of 5 min. The flow rate was 500 μl/min, and the injection volume was 5 μl. The mass spectrometer was operated in positive ion scan mode with a Turbo Spray ionization source. The ionization spray voltage was set to 5000 V, and the source temperature was maintained at 650 °C. The reaction was assessed in the multiple reaction monitoring (MRM) mode with the transition of mass-to-charge ratio (m/z) of 382 → 362 for celecoxib, m/z 398 → 378 for hydroxy celecoxib, and 261 → 117 for (*R*)-propranolol. The analytes were quantitated using a standard curve containing known amounts of the metabolite. Data processing was conducted using the AB Sciex Analyst™ 1.6 software (Framingham, MA).

The quinidine inhibition study was analyzed using a similar method, except the gradient was longer to avoid any potential ion suppression to the analyte. The gradient consisted of the following steps: $t = 0$ min, %B = 10; $t = 3$ min, %B = 99; $t = 5$ min, %B = 99; and $t = 5.1$ min, %B = 10, with a total run time of 7.5 min. Stable isotope-labeled celecoxib-d7 was used as the internal standard (IS) with an MRM transition of m/z 389 → 368. For the CYP2D6 activity assay, the formation of dextrorphan from dextromethorphan as a CYP2D6-selective probe reaction was monitored using the same LC-MS/MS instrument, except using a Sunfire C18 5μ 2.1 × 150 mm column (Waters, Milford, MA). The gradient consisted of the following steps: $t = 0$ min, %B = 1; $t = 3$ min, %B = 80; $t = 4$ min, %B = 99; and $t = 4.1$ min, %B = 1, with a total run time of 7.5 min. MRM transitions of m/z 272 → 215 for dextromethorphan and m/z 258 → 157 for dextrorphan were used for quantitation.

2.3. Determination of kinetic parameters of celecoxib in recombinant CYP2D6

The initial stock solution of celecoxib was prepared in methanol. Incubations were carried out in deep-well 96-well plates containing recombinant CYP2D6 (10 pmol/ml), celecoxib (0.098–200 μM) and PPB (100 mM, pH 7.4) at a final volume of 200 μl. After pre-incubating in a water bath at 37 °C with gentle shaking for 5 min, the reactions were initiated by adding an NADPH solution (final concentration 2 mg/ml) and were continued for 0, 2, 5, and 10 min. At the end of the incubations, the reaction samples were quenched by adding 200 μl of acetonitrile/methanol (1:1 by volume) containing (*R*)-propranolol (10 μg/l) as IS. After mixing and centrifugation at 1500 × *g* for 10 min at 4 °C, the supernatant was analyzed by LC-MS/MS for the formation of hydroxy celecoxib. The incubations were conducted in duplicate and repeated twice to calculate the mean and standard deviation.

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