Inhibition of Human Drug Metabolizing Cytochrome P450 by Buprenorphine

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The effects of buprenorphine, a powerful mixed agonist/antagonist analgesic, on several cytochrome P450 (CYP) isoform specific reactions in human liver microsomes were investigated to predict drug interaction of buprenorphine in vivo from in vitro data. The following eight CYP-catalytic reactions were used in this study: CYP1A1/2-mediated 7-ethoxyresorufin O-deethylation, CYP2A6-mediated coumarin 7-hydroxylation, CYP2B6-mediated 7-benzyloxyresorufin O-debenzylation, CYP2C8/9-mediated tolbutamide methylhydroxylation, CYP2C19-mediated S-mephenytoin 4-hydroxylation, CYP2D6-mediated bufuralol 1′-hydroxylation, CYP2E1-mediated chlorozoxazone 6-hydroxylation, and CYP3A4-mediated testosterone 6β-hydroxylation. Buprenorphine strongly inhibited the CYP3A4- and CYP2D6-catalyzed reactions with Ki values of 14.7 μM and 21.4 μM, respectively. The analgesic also weakly inhibited specific reactions catalyzed by CYP1A1/2 (Ki = 132 μM), CYP2B6 (Ki = 133 μM), CYP2C19 (Ki = 146 μM), CYP2C8/9 (IC50 > 300 μM), and CYP2E1 (IC50 > 300 μM), but not CYP2A6 mediated pathway. In consideration of the Ki values obtained in this study and the therapeutic concentration of buprenorphine in human plasma, buprenorphine would not be predicted to cause clinically significant interactions with other CYP-metabolized drugs.

Key words buprenorphine; cytochrome P450 inhibition; in vitro drug–drug interaction; human liver microsome

Buprenorphine is a synthetic derivative of morphine alkaloid thebaine with mixed agonist/antagonist analgesic properties.1,2 The analgesic effects are 30 times more potent than those of morphine, and it produces little physical dependence.1,2 Buprenorphine is used for the treatment of chronic pain in post-operative pain and terminal cancer patients,3) and recently it has been proposed for the management of opioid addicts.4)

Buprenorphine is extensively metabolized in humans by a combination of phase I and phase II reactions to form norbuprenorphine and norbuprenorphine (Fig. 1).5) Following subcutaneous, sublingual, and oral buprenorphine administration to human subjects, no free parent drug has been detected in urine.5) Norbuprenorphine is produced by N-dealkylation of the N-cyclopropylmethyl group, which is a principal metabolic pathway for buprenorphine.

Oxidative metabolism of drugs is mostly catalyzed by cytochrome P450 (CYP) enzymes which comprise a large family of hemoproteins.6,7 More than 15 isozymes have been identified in human liver, and several forms play important roles in the metabolism of drugs.8) It is of great importance that the CYP isoforms responsible for the metabolism of drugs be identified and further investigated as to whether a drug has inhibitory effect on the catalytic activity of CYP isoforms in order to consider possible drug interactions. Recently, Iribarne et al.9) and Kobayashi et al.10) reported that buprenorphine N-dealkylation, which is a principal metabolic pathway for buprenorphine, in human liver microsomes is mainly catalyzed by CYP3A4. However, it is unclear whether buprenorphine has an inhibitory effect on the catalytic activity of CYP isoforms. This study examines the characteristic properties of buprenorphine on the catalytic activities of the CYP isoforms, CYP1A1/2, CYP2A6, CYP2B6, CYP2C8/9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 using specific reaction probes for the enzymes.

MATERIALS AND METHODS

Chemicals Buprenorphine hydrochloride was supplied by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). Coumarin, tolbutamide, chlorozoxazone, glucose-6-phosphate disodium salt:hydrate, and β-NADP sodium salt were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Other chemicals were obtained from the following sources: 7-ethoxyresorufin, 4-hydroxytolbutamide, S-(+)-mephenytoin, (±)-4′-hydroxymephenytoin, 1′-hydroxybufuralol maleate salt, (±)-bufuralol hydrochloride salt, 6-hydroxychlorozoxazone, and 6β-hydroxytestosterone from Salford Ultrafine Chemical & Research, Ltd. (Guildhall Close, Manchester, England); 7-benzyloxyresorufin from Molecular Probes (Eugene, Oregon, U.S.A.); 7-hydroxycoumarin from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); resorufin, testosterone, and magnesium chloride hexahydrate from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); glucose-6-phosphate dehydrogenase (approx. 350 U/mg, 1 mg/ml) from Boehringer Mannheim GmbH (Mannheim, Germany). All other reagents and solvents were of high analytical grade.

Liver Microsomes Preparation Pooled human liver microsomes from 10 donors were prepared at the Biomedical
Research Institute, Human and Animal Bridge Discussion Group (HAB, Chiba, Japan). Human liver samples were legally procured from National Disease Research Interchange (NDRI, Pennsylvania, U.S.A.) through the international partnership between NDRI and HAB. The study was conducted in accordance with the Declaration of Helsinki. Human livers were homogenized with 0.25 M sucrose containing 3 mM Tris and 0.1 mM EDTA (pH 7.4), and microsomes were isolated by differential centrifugation using an ordinary method. Washed microsomes were resuspended in 100 mM Tris–HCl buffer (pH 7.4) containing 1 mM EDTA and 20% glycerol at protein concentrations of 20 mg/ml in the livers.

Determination of Human CYP Activities

7-Ethoxyresorufin O-deethylase activity by CYP1A1/2, coumarin 7-hydroxylase activity by CYP2A6, 7-benzoxylresorufin O-debenzylation activity by CYP2B6, tolbutamide methylhydroxylase activity by CYP2C8/9, S-mephenytoin 4-hydroxylase activity by CYP2C19, bufuralol 1′-hydroxylase activity by CYP2D6, chlorzoxazone 6-hydroxylation activity by CYP2E1, and testosterone 6β-hydroxylation activity by CYP3A4 were determined as described.[11]

Standard incubation mixtures of 0.5 ml contained microsomal protein (0.1—0.5 mg), 0.1 M potassium phosphate buffer (pH 7.4), 0.1 mM EDTA, NADPH generating system (2.5 mM β-NADPH, 25 mM glucose-6-phosphate, 2 units of glucose-6-phosphate dehydrogenase, and 10 mM magnesium chloride), and substrates with or without buprenorphine. Buprenorphine was dissolved in dimethyl sulfoxide and organic solvent concentration was 1 (v/v) % in the reaction system. Product formation was determined using HPLC and a fluorescence spectrophotometer. Assay methods were validated in this study. Calibration curves for resorufin, 7-hydroxyresorufin, 1′-methylhydroxyresorufin, 7-ethoxyresorufin, 1′-hydroxybufuralol, 6-hydrochlorzoxazone, and 6β-hydroxytestosterone were established with respective calibration ranges of 0.2—200 μM (γ = 0.9996), 0.025—5 μM (γ = 1.0000), 0.05—10 μM (γ = 0.9992), 0.025—5 μM (γ = 0.9996), 0.025—5 μM (γ = 0.9995), 0.25—100 μM (γ = 0.9994), and 0.03—30 μM (γ = 0.9999).

The substrate concentrations used to estimate the kinetic parameters for each assay were 0.01—10 μM 7-ethoxyresorufin, 0.1—100 μM coumarin, 0.03—10 μM 7-benzoxylresorufin, 25—1000 μM tolbutamide, 5—500 μM S-mephenytoin, 5—200 μM bufuralol, 10—400 μM chlorzoxazone, and 10—250 μM testosterone. For the determination of the residual activity in the presence of buprenorphine (30—300 μM), the concentrations of substrates were 0.5 μM 7-ethoxyresorufin, 2 μM coumarin, 1 μM 7-benzoxylresorufin, 100 μM tolbutamide, 100 μM S-mephenytoin, 20 μM bufuralol, 100 μM chlorzoxazone, and 100 μM testosterone. Selective CYP inhibitors were used in this study to validate that the assays were working properly. 7,8-Benzoflavone,[12] furafylline,[13] orphenadrine,[14] quercetin,[15] sulfaphenazole,[16] tranylcypromine,[17] quinidine,[18] diethylthiocarbamate,[19] and ketoconazole,[20] which are inhibitors of CYP1A1, 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4 activities, inhibited the respective enzyme activities (data not shown). Diethylthiocarbamate is also known to be a specific inhibitor of CYP2A6[22] and the present study confirmed the potent inhibitory capability of this compound on CYP2A6 mediated metabolism (data not shown).

For the determination of the Ki values, the ranges of 7-ethoxyresorufin and buprenorphine concentrations were 0.25—1 μM and 50—150 μM, respectively; those of 7-benzoxylresorufin and buprenorphine concentrations were 0.75—3 μM and 30—300 μM; those of S-mephenytoin and buprenorphine concentrations were 100—400 μM and 30—300 μM; those of bufuralol and buprenorphine concentrations were 20—80 μM and 10—100 μM; and those of testosterone and buprenorphine concentrations were 50—200 μM and 10—100 μM, respectively.

Data Analysis

Apparent K_m and V_max values for the formation of metabolites were calculated by a nonlinear regression analysis using a computer program, WinNonlin Standard (Version 2.1, Scientific Consulting, Inc., Apex, NC, U.S.A.). The method of Dixon[21] was used to calculate inhibition constants (Ki).

RESULTS AND DISCUSSION

Multiple drug therapy is a common therapeutic practice, particularly for patients with various diseases. Whenever 2 or more drugs are administered concurrently, there is the possibility of drug interaction. Many drug interactions are clinically caused by inhibition of drug metabolizing enzymes, cytochrome P450s (CYPs), leading to decreased metabolic clearance and increased exposure to the inhibited drug. The inhibition of CYP enzymes should thus be examined to assess the potential for drug interactions.

The current study examined the in vitro ability of buprenorphine to inhibit the metabolism of substrates for CYP1A1/2, CYP2A6, CYP2B6, CYP2C8/9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 (Table 1 and Fig. 2). Buprenorphine had strong inhibitory effects on CYP3A4-mediated testosterone 6β-hydroxylation and CYP2D6-mediated bufuralol 1′-hydroxylation with Ki values of 14.7 μM and 21.4 μM, respectively. The type of inhibition was considered to be competitive from Dixon plots. In addition, buprenorphine had weak inhibitory effects on CYP1A1/2-mediated 7-ethoxyresorufin O-deethylation (Ki = 132 μM), CYP2B6-mediated 7-benzoxylresorufin O-debenzylation (Ki = 133 μM), CYP2C19-mediated S-mephenytoin 4-hydroxylation (Ki = 146 μM), CYP2C8/9-mediated tolbutamide methylhydroxylation (IC_50 > 300 μM), and CYP2E1-mediated chlorzoxazone 6-hydroxylation (IC_50 > 300 μM), while it did not inhibit CYP2A6-mediated coumarin 7-hydroxylation at concentrations up to 300 μM. Buprenorphine is a substrate of CYP3A4[9,10] and thus may have the strongest inhibitory effect on CYP3A4 catalyzed reactions.

The lowest Ki value (CYP3A4; Ki = 14.7 μM) was used to estimate conservatively the potential for inhibitory drug interactions with buprenorphine during clinical use of the drug. Peak plasma concentration of buprenorphine after an intravenous dose of 1.2 mg, a sublingual dose of 4 mg, or a buccal dose of 4 mg in healthy volunteers was 24.40 to 55.83 ng/ml (0.0522 to 0.119 μM), 1.93 to 7.20 ng/ml (0.00413 to 0.0154 μM), and 0.25 to 3.90 ng/ml (0.000535 to 0.00834 μM), respectively.[22] Oral dose of 40 mg or subcutaneous dose of 2 mg to a patient produced peak levels of 0.57 to 4.69 ng/ml (0.00122 to 0.0100 μM) and 0.2 to 8.74 ng/ml (0.000428 to 0.0187 μM), respectively.[23] Plasma concentration of buprenor-
Table 1. Effect of Buprenorphine on the Cytochrome P450 Catalyzed Oxidase Activities in Human Liver Microsomes

<table>
<thead>
<tr>
<th>CYP</th>
<th>Reaction</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (pmol/min/mg)</th>
<th>% Control activity 30 µM</th>
<th>% Control activity 100 µM</th>
<th>% Control activity 300 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1/2</td>
<td>7-Ethoxyresorufin O-deethylase</td>
<td>0.519</td>
<td>15.0</td>
<td>95.3</td>
<td>74.2</td>
<td>12.3</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>Coumarin 7-hydroxylation</td>
<td>1.73</td>
<td>163</td>
<td>82.6</td>
<td>69.1</td>
<td>86.1</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>7-Benzoxoxyresorufin O-debenzylation</td>
<td>1.59</td>
<td>4.83</td>
<td>89.4</td>
<td>57.3</td>
<td>32.0</td>
</tr>
<tr>
<td>CYP2C8/9</td>
<td>Tolbutamide methylhydroxylation</td>
<td>442</td>
<td>84.9</td>
<td>87.7</td>
<td>62.4</td>
<td>54.8</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>S-Mephenytoin 4-hydroxylation</td>
<td>86.0</td>
<td>8.54</td>
<td>86.4</td>
<td>69.7</td>
<td>39.0</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Bufuralol 1'-hydroxylation</td>
<td>21.7</td>
<td>61.0</td>
<td>42.1</td>
<td>22.3</td>
<td>0</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>Chlorozaxone 6-hydroxylation</td>
<td>115</td>
<td>2600</td>
<td>83.4</td>
<td>74.7</td>
<td>58.4</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Testosterone 6β-hydroxylation</td>
<td>136</td>
<td>5540</td>
<td>51.1</td>
<td>21.1</td>
<td>9.4</td>
</tr>
</tbody>
</table>

The control activities were 7.25, 74.8, 1.44, 8.52, 2.39, 10.9, 109, and 1490 pmol/min/mg for CYP1A1/2, CYP2A6, CYP2B6, CYP2C8/9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 catalyzed reactions, respectively. Enzyme incubations and metabolite analysis were carried out in duplicate.

Fig. 2. Dixon Plots for the Inhibition of CYP1A1/2-Mediated 7-Ethoxyresorufin O-Deethylase (A), CYP2B6-Mediated 7-Benzoxoxyresorufin O-Debenzylation (B), CYP2C19-Mediated S-Mephenytoin 4-Hydroxylation (C), CYP2D6-Mediated Bufuralol 1'-Hydroxylation (D), and CYP3A4-Mediated Testosterone 6β-Hydroxylation (E) Activities by Buprenorphine in Human Liver Microsomes.

Each experiment was performed in duplicate using pooled microsomes and each value is shown.
protein binding of buprenorphine was determined in the in vitro studies, suggesting buprenorphine to possibly have little or no inhibitory effect on the metabolism of drugs oxidized by CYP1A1/2, CYP2B6, CYP2C8/9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in vivo. Actually, there are no clinical reports that buprenorphine causes drug–drug interactions with other CYP-metabolized drugs.

To extrapolate in vitro inhibition data to in vivo situations, free concentrations of drug and inhibitor at the site of metabolism are important.24,25) However this factor is difficult to evaluate in vitro. Often in vivo and/or in vitro unbound plasma concentration of a drug and/or inhibitor is used to approximate concentrations of both at an enzyme site. Plasma protein binding of buprenorphine was determined in vitro to be >95%26) and the drug primarily bound to α- and β-globulin fractions.3) The free (unbound) concentration of buprenorphine is 120 times less than the apparent value and high plasma protein binding or high plasma protein binding of buprenorphine indicated intracellular binding or the accumulation of buprenorphine in hepatocytes on the inhibition may not significantly affect the assessment of metabolic drug interactions made in this study.

In conclusion, the results of in vitro experiments performed with human liver microsomes indicated that, although buprenorphine inhibited CYP1A1/2, CYP2B6, CYP2C8/9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4-mediated catalytic activity, this compound, at expected therapeutics concentrations, would not be predicted to cause clinically significant interactions with other CYP-metabolized drugs.

REFERENCES