Short Communication

Assessment of Potential Interactions Between Dopamine Receptor Agonists and Various Human Cytochrome P450 Enzymes Using a Simple In Vitro Inhibition Screen

ABSTRACT:

The selectivity of inhibition for four dopamine receptor agonists (pramipexole, ropinirole, pergolide, and bromocriptine) on six human cytochrome P450 enzyme activities were evaluated using a simple in vitro inhibition screen. Drug-P450 interactions characterized as potent (i.e., greater than 50% inhibition of control enzyme activity) were then further examined to determine an IC50 for the interaction. Of the dopamine receptor agonists tested, three drugs, ropinirole, pergolide, and bromocriptine, were found to inhibit the activity of at least one human cytochrome P450 enzyme, while the remaining dopamine agonist, pramipexole, was devoid of any potent P450 interaction. None of the agonists tested inhibited the P450 marker activities of 2C9, 2C19, and 2E1. However, partial inhibition was observed between ropinirole and CYP1A2 and pergolide and CYP3A4. In contrast, potent interactions were observed between CYP2D6 and pergolide and ropinirole, as well as with CYP3A4 and bromocriptine. The results of this study indicate several drug P450 interactions; however, the likelihood of an in vivo interaction with these drugs remains to be established.

Parkinson’s disease is a debilitating neurologic disorder which affects approximately 100–150 people per 100,000 population usually in the fifth or sixth decade of life (1,2). Current drug therapy is aimed at correcting or preventing the neurochemical imbalances that occur in the basal ganglia of the brain (3). Treatment is usually accomplished with the use of a combination of drugs from a variety of therapeutic classes which include compounds that act directly upon the dopamine receptor. Dopamine agonists are useful in the treatment of Parkinson’s disease especially in patients who encounter disabling motor fluctuations (“on/off-effect”) often associated with levodopa therapy (4). Given the median age of a Parkinson patient and multi-drug therapy often associated with this particular age group (5), the ability to predict potential drug-drug interactions and in turn avoid inappropriate concurrent therapy is important in the successful management of this disease.

Several in vitro experimental approaches exist for identifying potential drug-drug interactions associated with a new chemical entity (NCE) (6). One method is to screen the ability of the NCE to serve as an inhibitor of various P450 metabolic activities. The in vitro screening approach has two distinct advantages: first, knowledge of the metabolic characteristics for the new drug is not rate limiting; and second, the initial inhibition screen identifies both metabolic and noncatalytic interactions.

The intent of the current study was to compare four anti-Parkinson agents with respect to their relative potential to inhibit the activities of various cytochrome P450 enzymes using a simple in vitro inhibition screen. To this end, pergolide, ropinirole, pramipexole, and bromocriptine (fig. 1) were evaluated as possible catalytic inhibitors of the activities (as determined by a decrease in formation of a specific metabolite for a select P450 marker substrate) for CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. Based upon a defined inhibition criteria, interactions that were judged to be significant were further evaluated to more fully characterize the magnitude of the drug-drug interaction.

Materials and Methods

Reagents. Pramipexole, ropinirole, and [14C]delavirdine were obtained from within Pharmacia & Upjohn (Kalamazoo, MI). Other [14C]substrates for the various cytochrome P450 isoform marker activities: [14C](S)-mephenytoin, [14C]diclofenac, and [14C]chlorzoxazone were purchased from Amersham Corp. (Arlington Heights, IL); [14C]testosterone was obtained from DuPont NEN (Boston, MA); [14C]p-nitrophenol, bromocriptine, pergolide, and NADPH were purchased from Sigma Chemical Co. (Milwaukee, WI); cDNA expressed CYP isoforms, CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 were purchased from Gentest (Woburn, MA).

P450 Inhibition Screen. The ability of the four dopamine receptor agonists (pramipexole, ropinirole, bromocriptine, and pergolide) to inhibit P450 en-
### Table 1

Effects of the anti-parkinson drugs on the activities for the following P450 enzymes; CYP1A2, 2C9, 2C19, 2D6, 2E1, and 3A4

<table>
<thead>
<tr>
<th>Pergolide</th>
<th>Ropinirole</th>
<th>Pramipexole</th>
<th>Bromocriptine</th>
</tr>
</thead>
<tbody>
<tr>
<td>P450 enzyme</td>
<td>Marker Activity</td>
<td>Marker Activity</td>
<td>Marker Activity</td>
</tr>
<tr>
<td>chordzoxazone 6-hydroxylation</td>
<td>102.6 ± 2.7</td>
<td>102.2 ± 1.8</td>
<td>103.8 ± 1.4</td>
</tr>
<tr>
<td>diclofenac 4'-hydroxylation</td>
<td>100.2 ± 4.9</td>
<td>94.9 ± 1.8</td>
<td>106.3 ± 14.1</td>
</tr>
<tr>
<td>mephentyoin 4-hydroxylation</td>
<td>102.6 ± 4.3</td>
<td>93.9 ± 1.8</td>
<td>102.8 ± 10.1</td>
</tr>
<tr>
<td>delavirdine N-demethylation</td>
<td>101.8 ± 5.3</td>
<td>101.4 ± 5.5</td>
<td>99.3 ± 4.7</td>
</tr>
<tr>
<td>testosterone 6β-hydroxylation</td>
<td>60.9 ± 1.7</td>
<td>60.9 ± 2.8</td>
<td>60.9 ± 2.8</td>
</tr>
</tbody>
</table>

Values represent the mean (± SD) of triplicate determinations measuring the percent P450 activity remaining compared with control (minus inhibitor) in cDNA CYP microsomal incubations in the presence of inhibitor (10 or 100 µM).

### Results and Discussion

One means of rapidly identifying potential drug-drug interactions is through the use of in vitro screening techniques. These experiments are carried under the acknowledgment that in vitro methods are useful in predicting a lack of inhibition or potent inhibition of drug metabolizing enzymes but often lack the subtlety to predict the magnitude of a particular interaction in vivo. For the present study [14C]-P450 marker substrates were incubated with cDNA expressed P450 microsomal preparations in the presence of four different dopamine receptor agonists: pergolide, ropinirole, pramipexole, and bromocriptine. The use of radiolabeled compounds in conjunction with HPLC radiochemical detection minimized the possibility of chromatographic and/or spectrometric interferences that might compromise product quantitation. The criteria for the choice of P450 marker substrate required that the compound possess a high affinity towards the specific P450 enzyme, that it was extensively metabolized to form a single product, and that both product and marker substrate were separated using a generic HPLC system. In these experiments, incubations with cDNA CYP microsomes instead of human liver microsomes were performed to minimize the total amount of protein in the incubation mixture. Incubations were carried out at substrate concentrations equivalent to the known apparent affinity constant (K_i) for P450 marker substrate to the metabolizing enzyme. Assessment of the extent of inhibition of enzymes was investigated against six different cDNA expressed human cytochrome P450 enzyme systems (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4). Incubations were conducted in quadruplicate and each incubation contained cDNA CYP microsomal protein (0.1–0.15 mg), NADPH (1 mM), [14C]-CYP marker substrate ([S] = K_i), and one of the dopamine agonists (0, 10, or 100 µM) in a final volume of 0.2 ml of 100 mM, pH 7.4 potassium phosphate buffer (table 1). Samples were preincubated for 4 min at 37°C, and the reactions were initiated by the addition of NADPH. Incubation reactions were allowed to proceed for 30–60 min and then terminated by addition of 100 µl acetonitrile. Sample tubes were vortex mixed for 10 sec, then centrifuged at 14000 x g for 15 min at 27°C, and the subsequent incubation supernatant (300 µl) transferred to HPLC autoinjector vials and capped. Formation of CYP marker metabolite was quantitated by HPLC using radiochemical detection as previously described (7) or outlined below.

### HPLC Analysis of Select P450 Marker Metabolite Formation

Quantitation of select P450 enzyme activities (CYP2C9: diclofenac oxidation to 4-hydroxydiclofenac; CYP2C19: (S)-mephenytoin hydroxylation to (S)-4'-hydroxymephenytoin; and CYP2E1: oxidation of para-nitrophenol to 4-nitrocatechol) were achieved using identical HPLC systems. Briefly the HPLC system was equipped with a PE410 pump, a PE ISS-100 autosampler (Perkin Elmer, Norwalk, CT) and a Radiomatic Flo-One Model A-500 radioactivity flow detector (Packard Instrument Co., Meriden, CT). Analytical separations of CYP2C9, 2C19, and 2E1 marker substrates and metabolites were performed on a Zorbax SB-C8 column (250 × 4.6 mm; Mac-Mod Analytical, Chadds Ford, PA). The mobile phase consisted of solvent A (90:10 water:methanol containing 0.5% acetic acid) and solvent B (10:90 water:methanol containing 0.5% acetic acid). Initial mobile phase conditions (10% B) at a rate of 1.0 mL/min with a linear gradient to 90% B in 40.0 min, the final conditions were held for 5.0 min.

#### Data Analysis

Regression analysis of dopamine receptor agonist inhibition of CYP biomarker metabolite formation across varying substrate and inhibitor concentrations was accomplished using the graphing/statistical program Prism (version 1.3, GraphPad Software, San Diego, CA).

### References


### Notes

Values represent the mean (± SD) of triplicate determinations measuring the percent P450 activity remaining compared with control (minus inhibitor) in cDNA CYP microsomal incubations in the presence of inhibitor (10 or 100 µM).
a specific P450 enzyme activity fell into two categories, weak or no interactions and potent interactions. The latter was defined as a decrease in P450 enzyme activity greater than 34% at an inhibitor concentration of 10 μM. The basis of the inhibition criteria relies upon the relationship between the Michaelis Menten equation (E1) and the equation for a competitive inhibitor (E2) (8, 9). Using (E1), if the specific P450 marker substrate concentration is equal to its apparent \( K_M \) for that enzyme, then the rate of metabolite formation, by definition, will be equal to half the maximal velocity (\( V_{max} \)) for the reaction. However, in the presence of a new drug, using (E2) with a substrate concentration equivalent to that used in (E1), the concentration of the NCE required to inhibit 34% of the select enzyme activity must be equal to the \( K_I \) for the NCE towards that particular enzyme. Restated, with the inhibitor concentrations used in the current study, we are able to context whether a drug will be a potent (\( K_I < 10 \, \mu M \)) or weak inhibitor (\( K_I > 100 \, \mu M \)) of a specific P450 enzyme.

The results listed in table 1 illustrate a large degree of variability in P450 isoform inhibition among the dopamine receptor agonists: pergolide, ropinirole, pramipexole, and bromocriptine. For example, inhibition results presented in table 1 reveal that ropinirole (10 μM) was able to decrease the metabolic activity of CYP1A2 by about 36%, while the remaining compounds tested lacked any inhibitory effect. The observed interaction between ropinirole and CYP1A2 was anticipated, however, since CYP1A2 has been implicated in the metabolism of ropinirole (10).

None of the dopamine receptor agonists tested inhibited CYP2C9, CYP2C19, and CYP2E1 activity (table 1). Interestingly, each compound did inhibit CYP2D6 activity although the extent of inhibition was quite different (table 1). However, based upon the chemical structures of each compound (fig. 1) with respect to the active site restriction associated with CYP2D6, which requires a basic nitrogen 5–7 A from the site of metabolism (11), it is not surprising that each compound interacted with this enzyme. Although each compound did inhibit CYP2D6, these in vitro observations do not imply that these compounds are metabolized by CYP2D6. It is possible for a compound to be metabolized by one particular P450 enzyme and interact in a noncatalytic fashion with another as has been shown for the interaction between CYP2D6 and quinidine (12). Characterization of the magnitude of inhibition by pergolide and ropinirole reveal that both drugs potently inhibit CYP2D6 (table 2). Calculated IC50 values for each interaction suggest that pergolide was a more potent inhibitor than ropinirole (IC50 = 0.08 and 0.54 μM, respectively). With respect to the clinical consequences for the observed CYP2D6 inhibition by pergolide and ropinirole, a meaningful comparison between the in vitro inhibition data with known in vivo plasma concentrations for each drug was not available.

Finally, it appears that both bromocriptine and pergolide were able to inhibit the 6-hydroxylation of testosterone, a marker substrate for CYP3A4 (table 1). The amount of inhibition varied between bromocriptine and pergolide, with bromocriptine being a more potent inhibitor of CYP3A4 than pergolide. In this situation the inhibition of CYP3A4 by bromocriptine and pergolide may reflect a catalytic interaction, as CYP3A4 has been previously demonstrated to metabolize structurally similar ergot compounds (13, 14). In addition, co-administration of bromocriptine and erythromycin, a marker inhibitor/substrate for CYP3A4 activity in vivo (15), resulted in a 4.6-fold increase in bromocriptine maximum plasma concentrations (16). Characterization of the magnitude of the bromocriptine/CYP3A4 interaction revealed bromocriptine was a potent inhibitor of CYP3A4 with a calculated IC50 value for the interaction of 1.69 μM (table 2). In this case, however, a prediction of the clinical consequences for the observed CYP3A4 inhibition by bromocriptine, a meaningful correlation between the in vitro inhibition data could be made based upon known in vivo bromocriptine plasma concentrations. In man a single oral dose of bromocriptine (5 mg) results in a Cmax of 0.465 ng/ml (18), taking into account that the drug is 90% protein bound, this dose will result in a maximal free drug bromocriptine plasma concentration of about 0.07 nM. Therefore, using a well-stirred model system, this concentration is approximately twenty-thousand times lower than the determined in vitro IC50 value for bromocriptine (IC50 = 1.69 μM). Thus, as long as co-administration of bromocriptine does not dramatically alter the dispositional characteristics of a drug primarily mediated by CYP3A4 (i.e. the concentration of drug available at the site of metabolism is not different in the presence or absence of bromocriptine), the current data suggest that bromocriptine should not significantly alter the metabolism of a second drug whose clearance is mediated by CYP3A4.

To place into perspective the magnitude of inhibition for CYP2D6 and 3A4 activity observed in the current screen, IC50 values were determined for the potent CYP2D6 and 3A4 inhibitors, quinidine and ketoconazole. Under these screening conditions, the determined IC50 values obtained for quinidine and ketoconazole were 0.07 and 0.01 μM, respectively (table 2). In this light, bromocriptine (IC50 = 1.69 μM) and ropinirole (IC50 = 0.54 μM) both appear to be about one order of magnitude less potent than the “benchmark” P450 inhibitors quinidine and ketoconazole. In contrast, pergolide (IC50 = 0.08 μM) was similar to quinidine in its capacity to inhibit CYP2D6 activity.

In conclusion, a simple means of screening potential drug/P450 interactions in vitro has been described. The objective of the current study was to predict potential drug-drug interactions which may be associated with various dopamine receptor agonists in vivo. For this study several dopamine receptor agonists (pergolide, ropinirole, pramipexole, and bromocriptine) were evaluated for their propensity to interact with a panel of select cytochrome P450 enzymes. Of the four dopamine receptor agonist tested, three drugs, ropinirole, pergolide, and bromocriptine, interacted with at least one human cytochrome P450 enzyme while the remaining agonist, pramipexole, was devoid of any significant P450 interaction. Unfortunately, little is known pertaining to the in vivo pharmacokinetics of these drugs other than that they are administered in low mg doses and are highly protein bound. Consequently, while we were able to detect potential P450 interactions, we are unable predict the likelihood of an in vivo interaction with these drugs based upon the current in vitro findings.

**References**


