P-Glycoprotein and Cytochrome P-450 3A Inhibition: Dissociation of Inhibitory Potencies

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ABSTRACT

Many P-glycoprotein (P-gp) inhibitors studied in vitro and in vivo are also known or suspected to be substrates and/or inhibitors of cytochrome P-450 3A (CYP3A). Such overlap raises the question of whether CYP3A inhibition is an intrinsic characteristic of P-gp inhibitors, a matter of concern in the development and rational use of such agents. Thus, the purpose of the present study was to determine whether the ability to inhibit P-gp and CYP3A is, in fact, linked and whether specific P-gp inhibitors with limited ability to inhibit CYP3A can be identified. Therefore, the potency of a series of 14 P-gp inhibitors was assessed by measuring their inhibition of the transepithelial flux across Caco-2 cells of digoxin, a prototypical P-gp substrate. CYP3A inhibition was determined from the impairment of nifedipine oxidation by human liver microsomes. Determination of the apparent K<sub>i</sub> values for CYP3A inhibition and the IC<sub>50</sub> for P-gp and CYP3A inhibition allowed comparison of the relative inhibitory potency of the compounds on the two proteins’ function. The IC<sub>50</sub> for P-gp inhibition ranged from 0.04 to 3.8 μM. All compounds inhibited CYP3A with apparent K<sub>i</sub> values of between 0.3 and 76 μM and IC<sub>50</sub> between 1.5 and 50 μM. However, no correlation was found between the extent of P-gp inhibition and CYP3A inhibition, and the ratio of the IC<sub>50</sub> for CYP3A inhibition to the IC<sub>50</sub> for P-gp inhibition varied from 1.1 to 125. These results demonstrate that, although many P-gp inhibitors are potent inhibitors of CYP3A, a varying degree of selectivity is present. The development and use of P-gp inhibitors with minimal or absent CYP3A inhibitory effects should decrease the impact of drug interactions on the therapeutic use of such compounds.

INTRODUCTION

P-gp<sup>3</sup> is an ATP-dependent efflux drug transporter that is constitutively expressed in normal tissues including the gastrointestinal epithelium, the canalicular membrane of the liver, the kidney (1, 2), and capillary endothelial cells in the central nervous system (3, 4). Because of such tissue localization and its broad substrate specificity, P-gp appears to play a key role in absorption, distribution, and elimination of many drugs including anticancer agents (5–7). In addition, overexpression in malignant cells has been associated with the development of the multidrug resistance phenomenon for many anticancer drugs (8, 9). In vitro, the efficacy of such chemotherapeutic agents can be restored by pharmacological inhibition of P-gp (10–12), and many chemicals have been shown to possess such an effect. Clinically, however, definitive benefit of P-gp inhibition in anticancer drug treatment still remains to be established and is complicated by the fact that many chemomodulators also result in interactions with drug-metabolizing enzymes (13–19). For example, plasma concentrations of doxorubicin almost doubled during PSC833 administration (18), and similar increases in plasma concentration of etoposide are also produced by this P-gp inhibitor (19).

In addition to their effects as P-gp inhibitors, many chemomodulating agents also inhibit cytochrome P-450 3A (CYP3A) activity (20, 21), which is the most abundant cytochrome P-450 enzyme present in human liver and intestine and is known to be involved in the metabolism of a large number of drugs including anticancer agents (22). Some anticancer drugs such as taxol are metabolized by CYP3A to products that lack antitumor activity (23); in contrast, CYP3A-mediated metabolism of tamoxifen, methoxymorpholinodoxorubicin, cyclophosphamide, and ifosfamide (CYP3A2B) produce metabolites, some of which have antitumor activity greater than the parent compound (24–26). For drugs requiring activation, the administration of P-gp inhibitors, which are also CYP3A inhibitors, may result in a reduced therapeutic effect, despite the P-gp inhibition-mediated, enhanced intracellular accumulation of the parent drug. Conversely, for drugs, the elimination of which is dependent on CYP3A, inhibition of CYP3A by P-gp inhibitors may cause excessive drug accumulation and increased toxicity, resulting in the need to reduce the dose of chemotherapeutic agent (13–19). Accordingly, an understanding of the likelihood of CYP3A interactions with P-gp inhibitors is essential for their rational use.

The purpose of the present study was, therefore, to determine the quantitative relationship between P-gp and CYP3A inhibition of a series of compounds with established P-gp inhibitory properties.

MATERIALS AND METHODS

Materials. With the exception of PSC833, which was a gift from Novartis (Basel, Switzerland), the remaining 13 compounds studied (Fig. 1) were provided by Pfizer (Groton, CT). All compounds were dissolved in DMSO. [3H]Digoxin (specific activity, 15 Ci/mmol; radiochemical purity, >97%) was obtained from DuPont-New England Nuclear (Boston, MA). Nifedipine and quinidine were purchased from Sigma Chemical Co. (St. Louis, MO). All tissue culture media and reagents were purchased from Life Technologies, Inc. (Gaithersburg, MD).

Transport in Cultured Caco-2 Cells. Caco-2 cells were grown with DMEM (high glucose) supplemented with 10% FCS, 2 mM l-glutamine, 100 units penicillin/ml, 100 μg of streptomycin/ml, and 1% nonessential amino acids (Life Technologies, Inc.), and incubated at 37°C under 5% CO<sub>2</sub>. Caco-2 cells were plated at a density of 1 × 10<sup>5</sup> cells/12 mm well on 0.4 μm size polycarbonate membrane filters (Transwell; Costar Corp., Cambridge, MA). Cells were supplemented with fresh media every 2 days and used in the transport studies on the fourth day after plating. Transepithelial resistance was measured in each well using a Millicell ERS ohm meter (Millipore, Bedford, MA); wells registering a resistance of 200 ohms or greater, after correcting for the resistance obtained in control blank wells, were used. Transport experiments were carried out using the same protocol described previously (27). Briefly, 1–2 h before the start of the transport experiments, the medium in each compartment was replaced with a serum-free medium (Optitroin; Life Technologies, Inc.). Then, digoxin transport in the basal-to-apical direction as well as the apical-to-basal direction was measured in two corresponding wells by replacing the media with 700 μl of serum-free medium (Optitroin) containing [3H]Digoxin (5 μM) in either the apical compartment of the first well or in the basolateral compartment of the corresponding second well. The digoxin concentration corresponded to about one-third of the reported K<sub>M</sub> for P-gp-mediated
transport (28). [3H]Digoxin appearing in the opposite compartment after 0.5, 1, 2, and 2.5 h was quantified in 25-μl aliquots taken from each compartment by liquid scintillation counting, following the addition of 5 ml of scintillation fluid (ScintiVerse BD; Fisher Scientific, Fair Lawn, NJ), and expressed as percentage of added radioactivity. Inhibition of digoxin translocation was determined after the addition of the test compound in equal concentrations to both the apical and basal compartments. The DMSO concentration in both incubations with and without inhibitor was 10%; preliminary experiments demonstrated that 10% DMSO in Caco-2 cells did not affect the translocation process (data not shown). The extent of inhibition was estimated using the assumption that complete inhibition of P-gp-mediated digoxin transport would result in the loss of net basal-to-apical versus apical-to-basal translocation differences. Accordingly, the degree of inhibition at each time point was determined from the relationship:

\[
\text{\% inhibition} = 1 - \frac{(i_{\text{basal-to-apical}} - a_{\text{apical-to-basal}})}{a_{\text{basal-to-apical}} - a_{\text{apical-to-basal}}} \times 100
\]

where \(i\) and \(a\) represent the percentage of digoxin transport in the presence and absence of an inhibitor, respectively. Because digoxin transport during the studied time points was linear, the percentage of inhibition calculated at each time point was averaged. The extent of inhibition at various inhibitor concentrations (0.001, 0.01, 0.1, 1, 5, 10, 20, and 100 μM) was used to determine the IC\(_{50}\) according to the Hill equation (Prism program; Graphpad, San Diego, CA). Experiments were performed in triplicate, with each experiment carried out on a different day.

**Measurement of CYP3A Activity.** CYP3A activity was determined in human liver microsomes prepared from human liver sample HL.110 (Nashville Regional Organ Procurement Agency, Nashville, TN) as described (29) with the total cytochrome P-450 content measured as described by Omura and Sato (30). CYP3A activity was assessed in duplicate by the formation rate of the nifedipine metabolite (dehydronifedipine). Briefly, the incubation medium consisted of microsomes containing 100 pmol cytochrome P-450, 1.5 mM NADPH, nifedipine at various concentrations (15, 20, 30, 60, and 200 μM), and 10 μl of various inhibitor concentrations diluted in DMSO, resulting in final inhibitor concentrations between 0.5 and 40 μM in a total volume of 500 μl of 0.1 M phosphate buffer (pH 7.4). Control incubations without addition of inhibitor were also performed in the presence of 2% DMSO. At this concentration of DMSO, nifedipine oxidation was reduced by 25% (data not shown). To avoid variability in CYP3A activity due to differing DMSO concentrations, 2% DMSO was used in all incubation procedures. Incubations were performed at 37°C and stopped after 10 min by the addition of 1 ml of CH\(_2\)Cl\(_2\). The nifedipine metabolite was measured by HPLC-UV as described elsewhere (31).
with an interassay variability of <5%. The Michaelis-Menten kinetics (apparent $V_{\text{max}}$, apparent $K_m$) were calculated using a nonlinear regression computer program ("kcat"; BioMetallics, Princeton, NJ). The type of inhibition and the inhibition constant (apparent $K_i$) were derived from appropriate reploting of the Lineweaver-Burk plot (32). IC$_{50}$s were calculated by the same procedure as for P-gp inhibition, using a nifedipine concentration of 20 µM.

Statistics. Significance of inhibition for CYP3A and P-gp were determined using a Students’ $t$ test or Mann-Whitney $U$ test, with $P < 0.05$ being taken as the minimum level of significance accepted. Data are expressed as means ± SD.

RESULTS

P-gp Inhibition. The effect of P-gp inhibition on the basal-to-apical and apical-to-basal digoxin translocation across Caco-2 cells is shown in Fig. 2, which also illustrates the extremes of potency found for P-gp inhibition with CP114416 and CP99542. The IC$_{50}$s for inhibition of digoxin transport for the various compounds are presented in Fig. 3A.

CYP3A Inhibition. The apparent $K_m$ and apparent $V_{\text{max}}$ values for nifedipine oxidation in the presence of 2% DMSO without any inhibitor were 30 µM and 7 nmol/nmol cytochrome P-450/min, respectively. All of the compounds except the cyclosporine derivatives acted as mixed-function inhibitors, whereas the cyclosporine derivatives were competitive inhibitors. The apparent $K_i$ values ranged from 0.32 to 76 µM (Table 1), and the IC$_{50}$ values ranged from 1.5 to 50 µM (Fig. 3A).

Except for CP117227, which produced hyperbolic inhibition, all compounds exhibited linear inhibition. CP117227 inhibited CYP3A by 35% at 40 µM. Of its weak CYP3A inhibitory effects and limited solubility in DMSO, the IC$_{50}$ for verapamil could not be determined, but at concentrations up to 40 µM, the maximal inhibition by verapamil of CYP3A-mediated nifedipine oxidation was 30%. On the basis of the inhibition produced by the range of verapamil concentrations, the concentration required to produce 25% inhibition was calculated.

Comparison of P-gp with CYP3A Inhibition. The relative inhibition ratio of IC$_{50}$ (CYP3A):IC$_{50}$ (P-gp) varied widely (1.06–125; Fig. 3B). However, a statistically significant correlation of the CYP3A and P-gp inhibition IC$_{50}$s was not observed ($r^2 = 0.17$, $P = 0.16$; $n = 12$).

DISCUSSION

P-gp inhibition has a potential for therapeutic benefit in the treatment of drug-resistant cancer. In addition, we have shown recently that the disruption of P-gp at the blood/brain barrier results in the enhanced penetration of HIV protease inhibitors and possibly other drugs into the central nervous system (27). Optimal P-gp inhibitor therapy will require drugs that are sufficiently potent to inhibit P-gp at therapeutically achievable plasma concentrations while producing minimal inhibition of CYP3A at these concentrations. To identify such drugs, it is necessary to compare their relative potency as P-gp and CYP3A inhibitors to define their relative selectivity. Drugs that are relatively selective for P-gp will be less likely to produce metabolically determined drug interactions (15). In the past, little attention has been given to the potential for inhibitors of P-gp to also inhibit CYP3A activity (16), despite the potential for such inhibition to alter the therapeutic efficacy of anticancer drugs in at least two ways: (a) through reduced cytotoxicity of anticancer agents, which are activated by CYP3A; and (b) through increased systemic toxicity of anticancer drugs, the elimination of which is mediated by CYP3A (19, 22). In addition, because of the dual expression of P-gp and CYP3A in the gastrointestinal epithelial cells, P-gp inhibition combined with CYP3A inhibition may produce a substantial increase in oral bioavailability of drugs over and above that produced by P-gp inhibition.
alone. Thus, an understanding of the relative effects of putative P-gp inhibitors on CYP3A activity is essential for rational development and therapy with these compounds.

This study demonstrates that there is a large range in the potency of these compounds as both P-gp and CYP3A inhibitors. All of the compounds exhibited some inhibitory effect on CYP3A. However, an important finding was that no significant correlation exists between the ability of the compounds to inhibit P-gp and their ability to inhibit CYP3A. Although some of the most potent P-gp inhibitors were also the most potent CYP3A inhibitors (e.g., CP114416), conversely, some relatively potent CYP3A inhibitors were relatively poor P-gp inhibitors (e.g., CP99542; Fig. 3A). Thus, the molecular recognition sites of P-gp and CYP3A differ in ways that result in differential effects of compounds at the two sites.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mechanism of CYP 3A inhibition</th>
<th>apparent Kᵢ (μM)</th>
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<tbody>
<tr>
<td>CP114146</td>
<td>Linear, mixed function</td>
<td>0.32</td>
</tr>
<tr>
<td>CP100356</td>
<td>Linear, mixed function</td>
<td>13.0</td>
</tr>
<tr>
<td>CP114769</td>
<td>Linear, mixed function</td>
<td>2.0</td>
</tr>
<tr>
<td>CP101556</td>
<td>Linear, mixed function</td>
<td>5.9</td>
</tr>
<tr>
<td>FK506</td>
<td>Linear, competitive</td>
<td>1.1</td>
</tr>
<tr>
<td>CP99542</td>
<td>Linear, competitive</td>
<td>1.6</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>Linear, competitive</td>
<td>4.9</td>
</tr>
<tr>
<td>PSC833</td>
<td>Linear, mixed function</td>
<td>16</td>
</tr>
<tr>
<td>Verapamil</td>
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<td>76</td>
</tr>
<tr>
<td>CP12379</td>
<td>Linear, mixed function</td>
<td>13</td>
</tr>
<tr>
<td>CP69042</td>
<td>Linear, mixed function</td>
<td>23</td>
</tr>
<tr>
<td>CP117227</td>
<td>Hyperbolic, mixed function</td>
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</tr>
<tr>
<td>CP147478</td>
<td>Linear, mixed function</td>
<td>0.75</td>
</tr>
<tr>
<td>Quinidine</td>
<td>Linear, mixed function</td>
<td>51</td>
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Fig. 3. A, IC₅₀ for P-gp and CYP3A inhibition [for CP117227 and verapamil, inhibitor concentrations that produced 25% CYP3A inhibition (IC₃₀), respectively]. B, the selectivity ratio for CYP3A to P-gp inhibition [IC₅₀ (CYP3A)/IC₅₀ (P-gp)] The higher the ratio, the less risk for CYP3A-mediated drug interactions with a similar P-gp inhibitory effect.
REFERENCES


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