Inhibition of Methadone and Buprenorphine N-Dealkylations by Three HIV-1 Protease Inhibitors

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(Received August 15, 1997; accepted November 24, 1997)

This paper is available online at http://www.dmd.org

ABSTRACT:

Ritonavir, indinavir, and saquinavir, all human immunodeficiency virus-1 protease inhibitors with a potent antiviral effect during triple therapy, are extensively metabolized by liver cytochrome P450 3A4. As this P450 isoform is involved in the metabolism of about 50% of drugs, coadministration of protease inhibitors with other drugs may lead to serious effects due to enzyme inhibition. Among these drugs, methadone and buprenorphine, both metabolized by P450 3A4, are potential candidates to drug interactions. In this study, metabolic interactions between these protease inhibitors and methadone or buprenorphine were studied in vitro in a panel of 13 human liver microsomes. Ritonavir was the most potent competitive inhibitor with $K_i$ about 50 and 20 nM for methadone and buprenorphine metabolisms, respectively. Indinavir and saquinavir also inhibited methadone N-demethylation ($K_i$ about 3 and 15 μM, respectively) and buprenorphine N-dealkylation ($K_i$ about 0.8 and 7 μM, respectively). The rank order of inhibition potency against metabolism of methadone and buprenorphine was ritonavir > indinavir > saquinavir. There is obvious potential for clinically significant drug interactions, particularly with ritonavir. In brief, caution should be advised if human immunodeficiency virus-1 protease inhibitors are coadministered with methadone and buprenorphine.

Recent studies demonstrated that a new class of protease inhibitors, associated with two nucleoside analogs during triple therapy, were very efficient against HIV virus (Barry et al., 1997). These drugs, namely ritonavir, indinavir, and saquinavir, are potent and specific inhibitors of HIV-1 protease, one of the major enzymes encoded by the retrovirus. These protease inhibitors are extensively metabolized by the liver P450 3A4 enzyme (Chiba et al., 1996, 1997; Fitzsimmons and Collins, 1997; Kumar et al., 1996) that represent about 30% of total P450 in human liver (Shimada et al., 1994). Thus, these drugs have a high first-pass effect (Barry et al., 1997), resulting in the excretion of less than 5% of the unchanged form. As patients with HIV disease are likely to be taking a multiple prolonged drug regimen, this may lead to drug interactions as a result of competition for the same substrate binding site of enzyme. As P450 3A4 is involved in the metabolism of quite half of the drugs currently on the market (Güengerich, 1996; Li et al., 1995), numerous drug interactions can be expected. Among these drugs, methadone and buprenorphine, now widely used in the treatment of opioid addiction (Dole and Nyswander, 1965; Mello and Mendelson, 1980), are likely to be coadministered with protease inhibitors to acquired acquired immunodeficiency syndrome (AIDS) patients. Furthermore, as methadone and buprenorphine are specific substrates of P450 3A4 (Iribarne et al., 1996, 1997a), these two drugs are potential candidates to interact with protease inhibitors.

The aim of this study was to compare the inhibitory potential of the HIV protease inhibitors ritonavir, indinavir, and saquinavir against methadone and buprenorphine N-dealkylations catalyzed by P450 3A4 in a panel of human liver microsomes. As the prediction of in vivo metabolic drug interaction from in vitro data has made significant advances in the last decade (Bertz and Granneman, 1997), in vitro data allow us to estimate the likelihood of metabolic interactions between more than three protease inhibitors and two opioid substitutes.

Materials and Methods

Chemicals. Ritonavir (ABT-538) was a gift from Abbott Laboratories, indinavir (MK-639) was from Merck Research Laboratories, and saquinavir (RO-31–8959) was from Roche Research Center. Methadone was purchased from Sigma-Chemie (Saint-Quentin-Fallavier, France), and buprenorphine was a gift from Schering-Plough (Herouville-Saint-Clair, France). All solvents were from the highest quality available from Merck (Darmstadt, Germany). Stock solutions of protease inhibitors were 1 mM ritonavir in methanol, 1 mM saquinavir, and 1 mM indinavir both in H2O/acetonitrile (1/1, v/v).

Human Liver Microsomal Preparations. Human liver samples were from multi-organ donors who died after traffic accidents. The medical history for each donor on drug taking before death was not known. Sampling was made in accordance to French law. Microsomal fractions were prepared by differential centrifugations as previously described (Berthou et al., 1991). Two banks of human liver microsomes were used. Samples termed BrX were previously characterized by specific monoxygenase activities specific to P450 3A, including tamoxifen N-demethylation, testosterone 6β-hydroxylation, estradiol 2-hydroxylation, toremifene N-demethylation, methadone N-demethylation, nifedipine oxidation, and buprenorphine N-dealkylation (Berthou et al., 1994; Iribarne, 1996, 1997a; Jacquot et al., 1991). Other activities specific to P450 1A, 2D6, 2E1, 2C9, and 2C19 have been also characterized for this bank of microsomes (Berthou et al., 1991; Iribarne et al., 1996). Samples termed BrX were characterized only by nifedipine oxidation, 4-nitrophenol hydroxylation, and ethoxyresoruﬁn O-deethylisation activities.

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1 Abbreviations used are: HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome.
**Table 1**

Nature of inhibition mechanism of three protease inhibitors on methadone and buprenorphine N-dealkylations

<table>
<thead>
<tr>
<th>Protease Inhibitor</th>
<th>[I] Plasma (µM)</th>
<th>C_{max} (µM)</th>
<th>t_{1/2} (h)</th>
<th>Substrate</th>
<th>Type of Inhibition</th>
<th>K_i (µM)</th>
<th>α</th>
<th>Predicted % of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ritonavir</td>
<td>15</td>
<td>15.5</td>
<td>3.2</td>
<td>Buprenorphine</td>
<td>Competitive</td>
<td>0.02</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Ritonavir</td>
<td>(600 mg × 2)</td>
<td></td>
<td></td>
<td>Methadone</td>
<td>Competitive</td>
<td>0.05</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Indinavir</td>
<td>4.7</td>
<td>11</td>
<td>1.8</td>
<td>Buprenorphine</td>
<td>Mixed-type</td>
<td>0.8</td>
<td>3.3</td>
<td>85%</td>
</tr>
<tr>
<td>Indinavir</td>
<td>(800 mg × 3)</td>
<td></td>
<td></td>
<td>Methadone</td>
<td>Noncompetitive</td>
<td>3</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Saquinavir</td>
<td>0.011</td>
<td>0.086</td>
<td>13</td>
<td>Buprenorphine</td>
<td>Competitive</td>
<td>7</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Saquinavir</td>
<td>(600 mg)</td>
<td></td>
<td></td>
<td>Methadone</td>
<td>Mixed-type</td>
<td>15</td>
<td>10</td>
<td>No</td>
</tr>
</tbody>
</table>

Two microsomal preparations, Br67 and Br062, were used. The 1-ml incubation mixture consisted of variable concentrations of methadone or buprenorphine in 0.1 M phosphate buffer, pH 7.4, with 0.5 mg of microsomal proteins and variable amounts of protease inhibitor. After 5 min of preincubation at 37°C, reaction was started by 1 mM NADPH addition. The enzymatic reaction was stopped after 20 min at 37°C by addition of 100 µl of 2 M NaOH for methadone and by addition of 1 ml of 0.5 M phosphate buffer, pH 9.0, for buprenorphine metabolism.

Materials and Buprenorphine N-Dealkylations. Metabolisms of methadone and buprenorphine were performed as previously described in this laboratory (Iribarne et al., 1996, 1997a). Briefly, the 1-ml reaction mixture consisted of variable concentrations of methadone (100–2000 µM) or buprenorphine (20–500 µM) in 0.1 M potassium phosphate buffer, pH 7.4. It was incubated 20 min at 37°C with 0.5 mg of microsomal protein, 1 mM NADPH, and variable amounts of protease inhibitor. After organic extraction, metabolite analysis was performed by reversed phase high performance liquid chromatography-UV detection at 210 nm, as previously described (Iribarne et al., 1996, 1997a).

Kinetic Analysis and K_i Determinations. For each K_i determination, three kinetics were carried out. The first one did not contain protease inhibitor, but the same amount of solvent was needed to add inhibitor. The second and the third experiments contained inhibitor at two different final concentrations: saquinavir 5 and 10 µM, indinavir 1 and 5 µM, and ritonavir 50 and 100 nM. K_i and V_{max} were determined with Enzpack 3 Biosoftware (Cambridge, UK).

Once K_i values were measured, incubations with a panel of 13 human liver microsomes were carried out with concentrations of protease inhibitors equal to the K_i and, with 500 µM methadone or 100 µM buprenorphine, respective K_i values of these substrates (Iribarne et al., 1996, 1997a). Results are expressed as a percentage of the corresponding control values.

Prediction of in Vivo Drug Interactions. The prediction of metabolic drug interactions was based upon the assumption of competition for the same substrate binding site of enzyme. The efficacy of the inhibition will depend on the K_i value of the inhibitor in relation to the K_m value of the drug, assuming that plasma concentrations of inhibitor and drug reflect liver concentrations (Bertz and Granneman, 1997; Iribarne et al., 1997b; Ring et al., 1995). The relation between these parameters is function of the mechanism of inhibition competitive, noncompetitive, or mixed-type (David, 1995; Ring et al., 1995). Therefore, percentage of inhibition can be semi-quantitatively predicted.

Results and Discussion

Metabolisms of ritonavir, indinavir, and saquinavir are characterized by very low K_m values, about 20 µM with human liver microsomes but only about 0.5 µM with pure recombinant P450 3A4 (Kumar et al., 1996), 1–2 µM (Chiba et al., 1997) and less than 0.5 µM (Fitzsimmons and Collins, 1997), respectively, whereas methadone and buprenorphine are characterized by high K_m values, 500 and 70 µM (Iribarne et al., 1996, 1997a), respectively. Therefore, it can be predicted that methadone or buprenorphine are not able to inhibit metabolism of protease inhibitors, even at high concentrations. K_i Determinations. Ritonavir. Ritonavir was shown not to be a mechanism-based inhibitor of P450 3A4 as demonstrated by the following experiment. A preincubation of microsomal proteins in presence of 1 mM NADPH and 100 nM ritonavir in 0.1 M potassium phosphate buffer, pH 7.4, was carried out for 20 min at 37°C. This preincubation medium was then diluted 20-fold in a mixture containing 500 µM methadone, 1 mM NADPH in 0.1 M potassium phosphate buffer, pH 7.4. No significant inhibition of methadone N-dealkylation was observed following such a preincubation step.

Preliminary studies showed that ritonavir inhibited methadone N-dealkylation and buprenorphine N-dealkylation in a concentration-dependent manner. Kinetics of methadone and buprenorphine were studied in absence or presence of 50 or 100 nM ritonavir. This inhibition was characterized by a concentration-dependent increase in K_i value, whereas V_{max} was unchanged. This was consistent with a competitive inhibition mechanism. Ritonavir was a very potent competitive inhibitor of methadone N-dealkylation, with an apparent K_i of 50 nM, and buprenorphine N-dealkylation, with an apparent K_i of 20 nM (table 1).

The measured inhibition constants were comparable with IC_{50} values previously reported for nifedipine oxidation (IC_{50} = 70 nM), 17α-ethynylestradiol 2-hydroxylation (IC_{50} = 2 µM), terfenadine hydroxylation (IC_{50} = 140 nM) (Kumar et al., 1996), and testosterone 6β-hydroxylation (K_i = 19 nM) (Eagling et al., 1997). These data confirm that ritonavir is a very potent inhibitor of all P450 3A4-catalyzed reactions.

Taking into account the competitive inhibition mechanism, it can be predicted that coadministration of ritonavir should be able to completely inhibit methadone N-dealkylation and buprenorphine N-dealkylation. This prediction was made using values of K_m and K_i determined in vitro, together with the actual substrate and inhibitor concentration encountered in vivo. These concentrations were found to be 15 µM ritonavir (Lea and Faulds, 1996), methadone (1 µM) (Iribarne et al., 1996; Verebely et al., 1975), and buprenorphine (10 nM) (Subutex. Monographie; Schering-Plough, France). The in vitro model probably underestimated the degree of inhibition expected in vivo. Indeed, the partition of protease inhibitors, known to be lipophilic, between plasma and liver is expected to increase liver enzyme concentration vs. plasma (Von Moltke et al., 1994). This assertion is validated by the knowledge of pharmacokinetic properties of protease inhibitors, especially their large first-pass metabolism in the liver (Barry et al., 1997; Chiba et al., 1997; Deeks et al., 1997; Fitzsimmons and Collins, 1997).

Indinavir. Buprenorphine incubations with variable amounts of indinavir showed a concentration-dependent inhibition. K_m increased, whereas V_{max} decreased in presence of 1 or 5 µM indinavir. Indinavir was a potent mixed-type inhibitor of buprenorphine N-dealkylation with an apparent K_i of 0.8 µM (fig. 1A).

Kinetics of methadone N-dealkylation was studied in presence of 2 or 5 µM indinavir. K_m values remained constant, and V_{max} decreased, suggesting that indinavir was a noncompetitive inhibitor with an apparent K_i of 3 µM (fig. 1B).

Indinavir is a potent mixed-type inhibitor of buprenorphine N-dealkylation (K_i = 800 nM, α = 3.3 (α is K_{m}/K_i). K_{m} measures the effectiveness of the inhibitor’s binding to the enzyme substrate complex and a noncompetitive inhibitor of methadone N-dealkylation (K_i = 3 µM). These inhibition constant values are comparable with...
Inhibition of buprenorphine from 20 to 500 μM, some Br062 were incubated for 20 min with buprenorphine concentrations ranging from 20 to 500 μM, and competitive inhibition of methadone N-demethylation, applying as concentration inhibitor 4.7 μM, i.e. plasma concentration during treatment (Balani et al., 1996). On the contrary, buprenorphine N-dealkylation would be expected to be inhibited by about 85%, according to the equation of mixed-type inhibition. However, as discussed above, this predictive model underestimates the inhibitor concentration in liver, suggesting that the precision of prediction needs to be verified by in vivo pharmacokinetic studies.

Saquinavir. Preliminary studies showed that saquinavir inhibited methadone N-demethylation and buprenorphine N-dealkylation in a concentration-dependent manner. Kinetics of methadone were carried out in absence or presence of 5 or 10 μM saquinavir. This inhibition was characterized by a concentration-dependent increase in $K_m$ values and decrease in $V_{\text{max}}$ values. This is consistent with a mixed-type inhibition with a $K_i$ of 15 μM (table 1).

Saquinavir is known to be characterized by a high $V_{\text{max}}/K_m$ ratio in human liver microsomes, namely 4 ml/min x mg of microsomal protein (Fitzsimmons and Collins, 1997) vs. 18 μl/min x mg for indinavir (Chiba et al., 1997). Therefore, bioavailability seems to be limited with saquinavir, due to a high first-pass metabolism (Fitzsimmons and Collins, 1997; Noble and Faulds, 1996). Accordingly, saquinavir could be extensively metabolized under the assay conditions described above. Preliminary experiments showed that 10 μM saquinavir used as inhibitor of 500 μM methadone with 0.5 mg of microsomal proteins for a 20-min incubation period was not entirely metabolized. However, as this partial (60%) metabolism may impact the $K_i$ results, complementary experiments were performed to decrease the saquinavir metabolism by using 0.25 mg of microsomal proteins for a 10-min incubation period. These new incubation conditions allowed us to measure an $IC_{50}$ of 20 μM, in full agreement with $K_i$ of 15 μM. The nonextensive metabolism of saquinavir in presence of methadone could be explained by close values of dissociation constant $K_i$, 4 and 2 μM for methadone and saquinavir, respectively (data not shown).

Kinetics of buprenorphine N-dealkylation were performed in absence or presence of 10 or 20 μM saquinavir. $K_m$ increased in a concentration-dependent manner, whereas $V_{\text{max}}$ was unchanged. Saquinavir was demonstrated to be a competitive inhibitor with an apparent $K_i$ of 7 μM (fig. 1C).

Saquinavir is a mixed-type inhibitor of methadone N-demethylation ($K_i = 15 \mu M, \alpha = 10$). Saquinavir has been previously described to inhibit the human small intestinal microsomal P450 3A4-dependent terfenadine metabolism with a $K_i$ value of 0.7 μM (Fitzsimmons and Collins, 1997) and testosterone 6β-hydroxylation with a $K_i$ of 3 μM (Eagling et al., 1997). Using the equation for mixed-type inhibition and the equation for competitive inhibition for methadone and buprenorphine, respectively, and applying as inhibitor concentration 11 nM, i.e. plasmatic concentration during treatment (Noble and Faulds, 1996), saquinavir is expected to be unable to inhibit methadone or buprenorphine metabolisms.

Co-Incubations of Protease Inhibitors and Methadone or Buprenorphine with Human Liver Microsomes. Methadone N-demethylation and buprenorphine N-dealkylation were highly significant correlated in the panel of 13 human liver microsomal preparations ($r = 0.93; p<0.001$) with a 5-fold interindividual variability.

Inhibition of opiate substitute metabolism by ritonavir, indinavir, or saquinavir co-incubated at concentrations equal to $K_i$ presented a great
interindividual variability among 13 human liver microsomes (table 2). But means of inhibition percentages against control activity were near 50%. Such results confirmed that protease inhibitors were incubated at their respective K<sub>i</sub> values.

The residual activity of buprenorphine dealkylation after inhibition by ritonavir was significantly correlated with total buprenorphine dealkylation (r = 0.65; p < 0.0001), whereas the residual activity of methadone demethylation was significantly correlated with total methadone demethylation (r = 0.55; p < 0.0001).

As one of the largest growing of anti-retroviral drugs, protease inhibitors are promising agents for the management of patients infected with the human immunodeficiency virus. Interactions between protease inhibitors and medications commonly prescribed to treat HIV infections or associated to treat opiate dependency represent a potential hurdle for both the clinician and the patient. Differences in the drug interaction profiles of the three protease inhibitors and the two opiate substitutes are largely due to their varying affinities for P450 3A4. Such a property has been exploited to increase bioavailability of saquinavir. Indeed, interactions between ritonavir and saquinavir or between indinavir and saquinavir with P450 3A4 allow to decrease first-pass metabolism of saquinavir, thus increasing its bioavailability (Barry et al., 1997; Merry et al., 1997) and accordingly its plasma concentration. The present study demonstrated that coadministration of some protease inhibitors with opiate substitutes would be expected to result in significantly higher amounts of opiate substitute.

In brief, use of ritonavir should be carefully controlled in case of coadministration of methadone or buprenorphine; if not, risk of opiate substitute overdose is foreseeable. Use of indinavir would be expected to strongly inhibit buprenorphine N-dealkylation. Use of saquinavir would not alter methadone or buprenorphine metabolisms.

Taking together these results (in vitro experiments and in vivo extrapolation), it seems that caution should be advised in the clinical use of methadone and buprenorphine when protease inhibitors are coadministered. However, the interactions predicted from this in vitro study need further clinical evaluation.

Acknowledgments. We thank Merck-Research Labs, Roche Research Center, and Abbott Labs for gifts of indinavir (MK639), saquinavir (RO-31-8959), and ritonavir (ABT-538), respectively. This work was supported in part by a grant from Ministère de la Santé (Programme Hospitalier de Recherche Clinique, CHU-Brest).

References


