Effects of Human Immunodeficiency Virus Protease Inhibitors on the Intestinal Absorption of Tenofovir Disoproxil Fumarate In Vitro

Leah Tong, Truc K. Phan, Kelly L. Robinson, Darius Babusis, Robert Strab, Siddhartha Bhoopathy, Ismael J. Hidalgo, Gerald R. Rhodes, and Adrian S. Ray

Gilead Sciences, Inc., Foster City, California 94404, and Absorption Systems, L. P., Exton, Pennsylvania 19341

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Human immunodeficiency virus protease inhibitors (PIs) modestly affect the plasma pharmacokinetics of tenofovir (TFV; −15% to +37% change in exposure) following coadministration with the oral prodrug TDF disoproxil fumarate (TDF) by a previously undefined mechanism. TDF permeation was found to be reduced by the combined action of ester cleavage and efflux transport in vitro. Saturable TDF efflux observed in Caco-2 cells suggests that at pharmacologically relevant intestinal concentrations, transport has only a limited effect on TDF absorption, thus minimizing the magnitude of potential intestinal drug interactions. Most tested PIs increased apical-to-basolateral TDF permeation and decreased secretory transport in MDCKII cells overexpressing P-glycoprotein (Pgp; MDCKII-MDR1 cells) and Caco-2 cells. PIs were found to cause a multifactorial effect on the barriers to TDF absorption. All PIs showed similar levels of inhibition of esterase-dependent degradation of TDF in an intestinal subcellular fraction, except for amprenavir, which was found to be a weaker inhibitor. All PIs caused a dose-dependent increase in the accumulation of a model Pgp substrate in MDCKII-MDR1 cells. Pgp inhibition constants ranged from 10.3 μM (lopinavir) to >100 μM (amprenavir, indinavir, and darunavir). Analogous to hepatic cytochrome P450-mediated drug interactions, we propose that the relative differences in perturbations in TFV plasma levels when TDF is coadministered with PIs are based in part on the net effect of inhibition and induction of intestinal Pgp by PIs. Combined with prior studies, these findings indicate that intestinal absorption is the mechanism for changes in TFV plasma levels when TDF is coadministered with PIs.

Tenofovir disoproxil fumarate (TDF; Viread, Gilead Sciences, Inc.), a prodrug of the nucleotide reverse transcriptase inhibitor tenofovir (TFV), is used to effectively deliver TFV across the gut wall. Following absorption, TDF rapidly degrades to TFV and TDF is not observed in the systemic circulation. When administered by itself, TFV has poor oral bioavailability (7). TDF is a common once-a-day backbone for use with human immunodeficiency virus (HIV) protease inhibitors (PIs). Clinical trials have shown TDF with emtricitabine in combination with either lopinavir (LPV) or atazanavir (ATV), each boosted with ritonavir (RTV, or “r” when referred to as a boosting agent), to be efficacious and well tolerated (22, 30).

Polypharmacy in HIV patients creates the potential for drug interactions (8, 35). No interaction between PIs and TDF would be anticipated due to the lack of cytochrome P450 involvement in the elimination pathway of TDF or TFV (25). However, modest changes in TFV plasma pharmacokinetic parameters have been reported for TDF coadministered with PIs. As summarized in Table 1, PIs can be categorized into three different groups based on their effects on TFV plasma pharmacokinetics. The first group encompasses PIs that cause modest increases in TFV plasma exposure (area under the concentration-time curve from 0 h to time t [AUC0-t]) when coadministered with TDF and includes saquinavir (SQV)/r, darunavir (DRV)/r, LPV/r, the experimental PI brecanavir (BRV)/r, and ATV/r. The second group of PIs do not change the TFV AUC0-t, although slight increases in the TFV maximal or minimal plasma concentration (Cmax and Cmin, respectively) have been reported, and include indinavir (IDV) and nelfinavir (NFV). The third group includes PIs that cause slight decreases in TFV levels. Tipranavir (TPV)/r causes a dose-dependent decrease in the TFV Cmax. Fosamprenavir (FPV; the phosphate prodrug of amprenavir [APV]) dosed alone causes a decrease in the TFV AUC0-t, Cmax, and Cmin, while FPV dosed as FPV/r causes a lesser decrease in TFV plasma Cmax with no change in the AUC0-t or Cmin.

The goal of the research described here was to determine if PIs can alter the intestinal absorption of TFV, causing the slight perturbations in the level of TFV observed in the systemic circulation. These studies were undertaken in light of the following observations suggesting that PIs may affect TFV absorption: (i) TFV absorption can be increased by a high-fat meal (3, 25); (ii) TFV intestinal absorptive flux was previously reported to be limited in vitro by esterase activity and efflux transport, based on increased Caco-2 cell permeability observed in the presence of fruit extracts and the transport inhibitor cyclosporine A (CsA) (43); (iii) esterase activity has also been noted to affect the absorption of TFV prodrugs in vivo, based on the correlation between increased esterase stability and higher oral bioavailability in the dog for different ester prodrugs of TFV (42); and (iv) PIs are known to inhibit the intestinal efflux transporter P-glycoprotein (Pgp) (27). Detailed in vitro studies were designed to identify if Pgp is in-
TABLE 1. Reported effects of coadministration of PIs with TDF (300 mg once daily) on the plasma pharmacokinetics of TFV in clinical drug interaction studies

<table>
<thead>
<tr>
<th>PI(s)</th>
<th>Dosing regimen (mg/24 h)</th>
<th>AUC</th>
<th>% Change</th>
<th>C\textsubscript{max}</th>
<th>C\textsubscript{min}</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATV/r</td>
<td>300/100 OD</td>
<td>↑ 37</td>
<td>↑ 34</td>
<td>↑ 29</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>BRV/r</td>
<td>300/100 BID</td>
<td>↑ 32</td>
<td>↑ 24</td>
<td>NR\textsuperscript{a}</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>LPV/r</td>
<td>400/100 BID</td>
<td>↑ 32</td>
<td>↑ 15</td>
<td>↑ 51</td>
<td>23, 26</td>
<td></td>
</tr>
<tr>
<td>DRV/r</td>
<td>300/100 BID</td>
<td>↑ 22</td>
<td>↑ 24</td>
<td>↑ 37</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>SQV/r</td>
<td>1,000/100 BID</td>
<td>↑ 14</td>
<td>↑ 15</td>
<td>↑ 23</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>IDV</td>
<td>800 TID</td>
<td>↔ 14</td>
<td>↑ 14</td>
<td>NR\textsuperscript{b}</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>NFV</td>
<td>1,250 BID</td>
<td>↔ 23</td>
<td>↑ 9</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPV/r</td>
<td>500/100 BID</td>
<td>↔ 38</td>
<td>38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPV/r</td>
<td>700/100 BID</td>
<td>↓ 18</td>
<td>↔ 29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPV</td>
<td>1,400 BID</td>
<td>↓ 15</td>
<td>↓ 25</td>
<td>↓ 12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} ↔, ↑, and ↓ denote a statistically significant lack of change, an increase, and a decrease in TFV plasma pharmacokinetics, respectively.

\textsuperscript{b} OD, BID, and TID denote once-, twice-, and thrice-daily dosing, respectively.

\textsuperscript{c} NR, not reported.

**MATERIALS AND METHODS**

**Reagents.** TDF, its monoester intermediate metabolite (monoPoc TFV), and TFV were obtained from Gilead Sciences, Inc. (Foster City, CA). SQV was purchased from Moravek Biochemicals, Inc. (Brea, CA). NFV (mesylate salt), DRV (sulfate salt), RTV, APV, and LPV were purchased from Toronto Research Chemicals, Inc. (North York, Ontario, Canada). ATV (sulfate salt) was isolated from capsules. BRV and DRV were chemically synthesized. The transport inhibitor CsA, the Pgp substrate calcine-AM, and the esterase inhibitor di-isopropyl fluorophosphate. All PI inhibition studies were done at 50 \(\mu\)M in the presence or absence of PIs or the known esterase inhibitor di-isopropyl fluorophosphate. All PI inhibition studies were done at 50 \(\mu\)M, except for those with RTV, where the dose dependence of inhibition was assessed at concentrations of 10, 50, and 100 \(\mu\)M. The potent esterase inhibitor di-isopropyl fluorophosphate was incubated at 2 \(\mu\)M for 30 min prior to the addition of acetonitrile containing 0.4% formic acid in Caco-2 cell assays by reversed-phase chromatography on a BEH C\(_18\) column (1.7 \(\mu\)m by 50 mm by 2.1 mm), with a gradient from 1% to 80% acetonitrile over 3.5 min and a flow rate of 0.9 ml/min, on an Acquity ultra-performance LC system coupled to a Micromass Quatro Premier XE MS/MS system running in positive ionization and multiple reaction monitoring modes (the column, LC system, and mass spectrometer were all purchased from Waters). Water and 0.1% acetic acid in water were used as the puffer solvent. The protein precipitate was removed by centrifugation, and the supernatant was analyzed by LC/MS/MS as described below.

**Quantitation of TDF and its metabolites by LC/MS/MS.** Levels of TDF and its metabolites were quantified using LC/MS/MS methods by comparing the relative peak areas of samples to standard curves for known concentrations prepared with appropriate sample matrices. Levels of TDF and monoPoc TFV were determined in Caco-2 cell permeability assays and intestinal S9 stability assays by reversed-phase chromatography on a BEH C\(_18\) column (1.7 \(\mu\)m by 50 mm by 2.1 mm), with a gradient from 1% to 80% acetonitrile over 3.5 min and a flow rate of 0.9 ml/min, on an Acquity ultra-performance LC system coupled to a Micromass Quatro Premier XE MS/MS system running in positive ionization and multiple reaction monitoring modes (the column, LC system, and mass spectrometer were all purchased from Waters). Water and 0.1% acetic acid in water were used as the puffer solvent. The protein precipitate was removed by centrifugation, and the supernatant was analyzed by LC/MS/MS as described below.

**Data analysis.** The A-to-B and B-to-A apparent permeability coefficients (\(P_{app}\)) for Caco-2 and MDCKII cells were calculated using the equation \(P_{app} = (dC/dt) \times (V_{in}/A \times C_{o})\), where \(dC/dt\) is the change in concentration over time, as measured at 60 and 120 min, in \(\mu\)M/s; \(V_{in}\) is the volume of the receiver well, in \(\text{cm}^3\); \(A\) is the area of the cell monolayer, in \(\text{cm}^2\); and \(C_{o}\) is the initial concentration in the donor well, in \(\mu\)M. The percent recovery was calculated by comparing the total amount of material in the donor and receiver chambers at the end of the experiment relative to the amount added to the donor chamber at the beginning of the experiment. The efflux ratio (ER) was defined as the B-to-A \(P_{app}\) divided by the A-to-B \(P_{app}\). The binding constant for efflux saturation was calculated by comparing the ER measured at the lowest concentration of TFV tested (1 \(\mu\)M) to that observed at higher concentrations, using the following equation: efflux saturation = \((ER_{1} - 1)/(ER_{1} - 1)(ER_{1} - 1)\times 100\%\), where \(ER_{1}\) was the donor chamber at 0 and 2 h. Cells were dosed on the apical (A) or basolateral (B) side to determine A-to-B and B-to-A permeability, respectively, and were incubated at 37°C with 5% CO\(_2\) in a humidified incubator. Each determination was performed in duplicate, and the permeation of control compounds (atenolol, propranolol, and digoxin) was determined to meet acceptance criteria for each assay plate.

Biochemical permeability studies were done using confluent monolayers of the human colon carcinoma cell line Caco-2 (16) seeded in 12-well plates essentially as previously reported (37). The effects of PIs on the permeation of TDF were studied following a 30-min preincubation of cell monolayers with 20 \(\mu\)M PIs in transport buffer to allow for saturation of transporter binding sites. Following preincubation, fresh assay buffer containing inhibitor and TDF was added, and the assay was started. Each determination was performed in duplicate, and the permeation of control compounds (atenolol, propranolol, and digoxin) was determined to meet acceptance criteria for each batch of assay plates. Levels of TDF and its metabolites in Caco-2 and MDCKII cell permeability studies were analyzed by liquid chromatography coupled to triple-quadrupole mass spectrometry (LC/MS/MS) as described below.

**Pgp inhibition assay.** The inhibition of Pgp-mediated transport was studied using the Pgp substrate calcine-AM and MDCKII-MDR1 cells. Studies were done with a 90-well fluorescence assay essentially as described previously (37). Twofold serial dilutions of PIs, with a starting concentration of 50 \(\mu\)M (seven individual concentrations were tested), were added simultaneously with calcine-AM to parental and MDCKII-MDR1 cells. All studies were done in duplicate.

**Inhibition of TDF degradation in a human intestinal subcellular fraction.** Human intestinal S9 (a subcellular fraction) was purchased from Xenotech LLC (Lenexa, KS). Reactions were done with a 1-mg/ml protein concentration. TDF was incubated at 2 \(\mu\)M in the presence or absence of PIs or the known esterase inhibitor di-isopropyl fluorophosphate. All PI inhibition studies were done at 50 \(\mu\)M, except for those with RTV, where the dose dependence of inhibition was assessed at concentrations of 10, 50, and 100 \(\mu\)M. The potent esterase inhibitor di-isopropyl fluorophosphate was incubated at 2 \(\mu\)M. Reactions were done in duplicate at 37°C. After 30 and 60 min, TDF degradation was stopped by the addition of 67% acetonitrile in water. The protein precipitate was removed by centrifugation, and the supernatant was analyzed by LC/MS/MS as described below.

**Bidirectional permeability studies were done using confluent monolayers of the human colon carcinoma cell line Caco-2 (16) seeded in 12-well plates essentially as previously reported (37). The effects of PIs on the permeation of TDF were studied following a 30-min preincubation of cell monolayers with 20 \(\mu\)M PIs in transport buffer to allow for saturation of transporter binding sites. Following preincubation, fresh assay buffer containing inhibitor and TDF was added, and the assay was started. Each determination was performed in duplicate, and the permeation of control compounds (atenolol, propranolol, and digoxin) was determined to meet acceptance criteria for each assay plate. Levels of TDF and its metabolites in Caco-2 and MDCKII cell permeability studies were analyzed by liquid chromatography coupled to triple-quadrupole mass spectrometry (LC/MS/MS) as described below.

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measured at 1 μM and ER, was determined at the respective test concentration. The amount of PI required to inhibit Pgp-dependent calcein-AM transport was determined by fitting the percent increase in intracellular fluorescence in the presence of different PI concentrations to a hyperbolic curve. The percent inhibition of TDF degradation was then obtained by comparing the observed rate of TDF degradation with the addition of dimethyl sulfoxide (control) to that obtained in the presence of inhibitor. Where appropriate, the statistical significance of differences observed was assessed using two-tailed Student’s t tests.

RESULTS

Dose-dependent effect of efflux transport on TDF permeation. TDF (formula weight, 635) is given as a single 300-mg tablet once a day, and assuming rapid dissolution into approximately 250 ml of intestinal content, the pharmacologically relevant intestinal concentration should be around 2 mM. The dose-dependent bidirectional permeation of TDF was determined at concentrations approaching those anticipated in the gastrointestinal tract, using the Caco-2 cell intestinal permeability model. The A-to-B $P_{app}$ of TDF increased linearly >10-fold between concentrations of 1 and 1,000 μM (Fig. 1A). Over this TDF concentration range, the ER for TDF was found to be saturable, yielding an apparent binding constant for efflux saturation of 144 μM (concentration required to cause 50% saturation) (Fig. 1B), reflecting a decrease in the ER, from 25 at 1 μM TDF to 2.6 at 1,000 μM TDF.

Effects of PIs on bidirectional permeation of TDF through monolayers of MDCKII-MDR1 and Caco-2 cells. Lower than pharmacologically relevant concentrations of TDF were used in cell-based permeability studies in order to increase efflux, facilitating the accurate assessment of the relative effects of PIs on TDF permeation. Having established the Pgp-dependent efflux of TDF, the effects of PIs on the efflux of TDF in MDCKII-MDR1 cells were assessed. PIs decreased the efflux of TDF in Pgp-overexpressing cells (Fig. 2). The PIs BRV, NFV, LPV, and RTV were the most potent inhibitors of TDF efflux, while DRV, IDV, and APV showed smaller effects. We could not assess the effects of TPV due to insufficient material,
but it has been reported to be a weak Pgp inhibitor (Aptivus package insert; Boehringer Ingelheim).

In order to study the effects of select PIs on the permeation of TDF in a more physiologically relevant model of gastrointestinal absorption, bidirectional permeability studies were done with Caco-2 cells (16). The effects of LPV, ATV, RTV, SQV, IDV, and APV on the permeation of TDF were compared to those of the potent transport inhibitor, CsA (10 μM), and the esterase inhibitor di-isopropyl fluorophosphate (DFP; 2 μM). All PIs except APV and IDV caused significant increases in the TDF A-to-B $P_{app}$, all PIs except IDV caused a significant inhibition of TDF efflux. Results for PIs are organized left to right in order of increasing efflux inhibition, and the ER for each treatment condition is indicated above the respective bars. Values represent the means ± standard deviations for three independent experiments done in duplicate. The statistical significance of changes in the A-to-B $P_{app}$ values and ERs compared to the values for Caco-2 cells in the absence of coincubation was determined by using Student’s unpaired two-tailed t tests, assuming equal variance (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). While the change in TDF A-to-B $P_{app}$ caused by CsA did not reach statistical significance based on unpaired analyses of all data, it did cause a reproducible increase in side-by-side assays, as illustrated by Student’s paired two-tailed t test (#, $P = 0.03$).

![Graph](http://aac.asm.org/)

**FIG. 3.** Effects of select PIs on bidirectional permeation of 50 μM TDF through monolayers of Caco-2 cells. The effects of 20 μM APV, IDV, SQV, ATV, LPV, and RTV were compared to those of a potent transport inhibitor, CsA (10 μM), and the esterase inhibitor di-isopropyl fluorophosphate (DFP; 2 μM). All PIs except APV and IDV caused significant increases in the TDF A-to-B $P_{app}$. All PIs except IDV caused a significant inhibition of TDF efflux. Results for PIs are organized left to right in order of increasing efflux inhibition, and the ER for each treatment condition is indicated above the respective bars. Values represent the means ± standard deviations for three independent experiments done in duplicate. The statistical significance of changes in the A-to-B $P_{app}$ values and ERs compared to the values for Caco-2 cells in the absence of coincubation was determined by using Student’s unpaired two-tailed t tests, assuming equal variance (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). While the change in TDF A-to-B $P_{app}$ caused by CsA did not reach statistical significance based on unpaired analyses of all data, it did cause a reproducible increase in side-by-side assays, as illustrated by Student’s paired two-tailed t test (#, $P = 0.03$).

Inhibition of intestinal esterase hydrolysis of TDF by PIs. The potential for inhibition of TDF hydrolysis by PIs was assessed in a human intestinal subcellular fraction (S9). The major product of TDF degradation in S9 was the intermediate monooester metabolite of TFV (monoPoc TFV), with lower levels of TFV (data not shown). RTV caused a dose-dependent decrease in TDF degradation (Fig. 4A). Di-isopropyl fluorophosphate and PIs were found to inhibit TDF degradation (Fig. 4B) and, concomitantly, to decrease the appearance of its metabolites. PIs were tested at a concentration near their solubility limits to model pharmacologically relevant concentrations potentially formed following dosing in the gastrointestinal tract (50 μM). While most PIs showed a similar inhibition of TDF degradation, IDV and DRV reproducibly showed less marked inhibition (<40% inhibition). The results for APV did not reach statistical significance.

**Inhibition of Pgp by PIs.** The relative inhibition of Pgp by PIs was studied by monitoring the effects of coincubation on the Pgp-dependent accumulation of the fluorescent compound calcein (metabolite of the Pgp substrate calcein-AM) in MDCKII-MDR1 cells. All PIs showed a dose-dependent inhibition of Pgp. The effects of PIs ranged from the most potent, LPV, to the least potent, DRV, with observed inhi-
TABLE 2. Inhibition of Pgp-dependent efflux of calcein-AM following incubation of MDCKII-MDR1 cells with the Pgp inhibitor CsA or with PIs

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CsA</td>
<td>9.34 ± 4.33</td>
</tr>
<tr>
<td>LPV</td>
<td>10.3 ± 4.4</td>
</tr>
<tr>
<td>NFV</td>
<td>19.9 ± 21.1</td>
</tr>
<tr>
<td>BRV</td>
<td>24.1 ± 9.2</td>
</tr>
<tr>
<td>RTV</td>
<td>39.6 ± 19.4</td>
</tr>
<tr>
<td>ATV</td>
<td>67.8 ± 27.7</td>
</tr>
<tr>
<td>SQV</td>
<td>100 ± 68</td>
</tr>
<tr>
<td>APV</td>
<td>&gt;100</td>
</tr>
<tr>
<td>IDV</td>
<td>&gt;100</td>
</tr>
<tr>
<td>DRV</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

*Values represent means ± standard deviations for 3 to 10 independent assays, each including seven inhibitor concentrations, done in duplicate. HIV PIs were tested at concentrations of up to 50 μM in the absence of serum proteins. All PIs showed significant dose-dependent inhibition of Pgp during the assay, although many did not attain ≥25% inhibition at 50 μM, and their IC_{50} values are reported as >100 μM.

**DISCUSSION**

The observation of efflux in Caco-2 cells illustrates that TDF is a substrate for one or more intestinal efflux transport pumps. The differential transport of TDF observed in parental and Pgp-overexpressing MDCKII cells suggests that this highly expressed intestinal efflux pump is at least partly responsible for this phenomenon. There is a synergistic interaction between intestinal Pgp transport and cytochrome P450 3A4 degradation in limiting the oral bioavailability of substrates for both proteins (34, 46). The interaction is facilitated by Pgp increasing the intracellular residence time of compounds in intestinal cells and allowing for further degradation by cytochrome P450 3A4. Our results support the hypothesis that a similar interplay between intestinal Pgp and esterase activity may play a role in limiting the intestinal absorption of TDF. However, the measurement of an apparent binding constant for efflux transport of 144 μM, combined with the high solubility of TDF, suggests that efflux is largely saturated during TDF absorption (Fig. 1B). Of primary importance in the current discussion of drug interactions, this suggests the potential for only minimal changes in TDF absorption and resulting TFV circulating levels upon coadministration with agents affecting either esterase cleavage or Pgp transport.

PIs were established to be inhibitors of the molecular barriers to TDF absorption, based on results in bidirectional permeability, intestinal extract stability, and fluorescent Pgp inhibition assays. Their multifactorial effect on efflux transport and esterase-dependent permeation of TDF through Caco-2 cell monolayers was likely the reason that SQV, ATV, LPV, and RTV caused similar or greater effects on the TDF A-to-B P_{app} than did the near complete blockage of efflux by CsA. Evidence for the role of Pgp in the efflux component of this interaction can be drawn from the effects of PIs on Pgp-dependent efflux in MDCKII-MDR1 cells. However, these results do not eliminate the potential for a contribution by other intestinal efflux pumps known to be expressed in Caco-2 cells (16). RTV, ATV, and LPV were observed in both MDCKII-MDR1 and Caco-2 cells to increase the A-to-B P_{app} of TDF and to decrease TDF efflux, likely explaining the presence of these PIs in combinations causing increases in TFV exposure in vivo. Alternatively, the lower inhibition caused by APV and IDV in transepithelial permeability, esterase activity, and Pgp transport assays may explain why FPV and IDV do not cause increased TFV levels in patients.

There is, however, a partial disconnect between the increase in TDF permeation observed for most PIs in vitro and the spectrum of effects that PIs and their combinations have on plasma TFV levels clinically. This inconsistency is most apparent in the decreases in TFV levels observed when TDF is coadministered with TPV/r, FPV/r, and most significantly, FPV. A clue to the mechanism for the decreased TFV levels observed with these PIs is evident in the drug interaction between the strong intestinal Pgp inducer rifampin (14, 15) and TDF. When TDF was coadministered with rifampin, a slight decrease in TFV plasma levels (16% C_{max} and 12% AUC_{0-24}) was observed (9). The similarity in the effects of rifampin and these PIs suggests a common mechanism for the decreased TFV levels. Consistent with this hypothesis, TPV is known as a strong inducer of metabolizing enzymes (Aptivus product insert; Boehringer Ingelheim). While they are less marked inducers than TPV or rifampin, both NFV and APV induce intestinal Pgp after repeat dosing in the rat (18). Studies with a colonic cell line have shown the rank order of Pgp induction of tested PIs to be RTV > NFV > APV > SOV > IDV (33), and NFV also induces Pgp expression in CD4^{+} and CD8^{+} lymphocytes in vitro (11). Indeed, the intestinal Pgp induction potential of NFV, APV, rifampin, and TPV may not be coincidental with their minimal (NFV) or slightly detrimental (FPV, rifampin, and TPV) effects on TFV circulating levels.

The ability of PIs to both inhibit and induce Pgp illustrates that their net effect on intestinal Pgp activity will be a balance between their inhibition and induction potentials. Digoxin levels have been shown to be sensitive to intestinal Pgp activity (14, 17), and the strong Pgp inducers and weak Pgp inhibitors TPV/r and rifampin have been shown to reduce digoxin’s AUC_{0-24} (14, 45). Alternatively, the digoxin AUC_{0-24} is increased by the potent Pgp inhibitors RTV and LPV/r (21, 31). Similarly, net inhibition of intestinal Pgp has been observed for LPV/r, based on its effects on the oral bioavailability of the Pgp substrate fexofenadine in healthy volunteers (44). While it was observed to be an inducer of Pgp, the net effect of ATV on Pgp activity in vitro has been shown to be inhibition (32). A model for the effects of PIs on TDF absorption, including intestinal esterase inhibition and the net effect of intestinal Pgp inhibition and induction, is presented in Fig. 5.

It has been proposed that the clinically observed changes in TFV circulating levels are due to PI inhibition of the multidrug resistance-related protein 2 (MRP2)-dependent apical renal...
transport of TFV in the kidney during the process of active tubular secretion. Furthermore, blockage of MRP2-mediated renal elimination of TFV has been proposed to cause the accumulation of TFV in renal proximal tubule cells, resulting in an alteration in the renal safety profile of TFV (20, 28, 39, 47). However, two independent reports have shown that the renal transport of TFV is likely mediated by MRP4, not MRP2 (19, 36, 37). Further studies have shown that PIs have minimal potential to inhibit the active renal tubular secretion of TFV mediated by influx from the blood via the human organic anion transporters 1 and 3 and by efflux from proximal tubule cells into the urine by MRP4 (6). Renal accumulation of TFV due to PIs is also not consistent with other observations, including (i) the low incidence of TDF-related renal adverse events in the context of combination therapy, with or without PIs (the TDF renal safety profile was recently reviewed by Sax et al. [41] and by Gitman et al. [13]); and (ii) the lack of overlapping recognition of TFV and PIs by renal efflux transporters (6), unlike the common interaction of PIs and TFV with the intestinal efflux transporter Pgp illustrated here.

We have shown that all PIs have the potential to modulate the intestinal absorption of TDF. The differential changes in circulating TFV levels observed clinically with different PIs are likely related to the relative abilities of PIs to (i) inhibit TDF hydrolysis in intestinal tissue, (ii) inhibit Pgp-mediated efflux of TDF, and (iii) induce Pgp expression. Our work also suggests that the magnitude of perturbations in TDF absorption caused by concomitant agents should be limited by partial saturation of intestinal transport.

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REFERENCES


