Raltegravir Is a Substrate for SLC22A6: a Putative Mechanism for the Interaction between Raltegravir and Tenofovir

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The identification of transporters of the HIV integrase inhibitor raltegravir could be a factor in an understanding of the pharmacokinetic-pharmacodynamic relationship and reported drug interactions of raltegravir. Here we determined whether raltegravir was a substrate for ABCB1 or the influx transporters SLC01A2, SLC01B1, SLC01B3, SLC22A1, SLC22A6, SLC10A1, SLC15A1, and SLC15A2. Raltegravir transport by ABCB1 was studied with CEM, CEMVBL100, and Caco-2 cells. Transport by uptake transporters was assessed by using a Xenopus laevis oocyte expression system, peripheral blood mononuclear cells, and primary renal cells. The kinetics of raltegravir transport and competition between raltegravir and tenofovir were also investigated using SLC22A6-expressing oocytes. Raltegravir was confirmed to be an ABCB1 substrate in CEM, CEMVBL100, and Caco-2 cells. Raltegravir was also transported by SLC22A6 and SLC15A1 in oocyte expression systems but not by other transporters studied. The $K_m$ and $V_{max}$ for SLC22A6 transport were 150 μM and 36 pmol/oocyte/h, respectively. Tenofovir and raltegravir competed for SLC22A6 transport in a concentration-dependent manner. Raltegravir inhibited 1 μM tenofovir with a 50% inhibitory concentration (IC50) of 14.0 μM, and tenofovir inhibited 1 μM raltegravir with an IC50 of 27.3 μM. Raltegravir concentrations were not altered by transporter inhibitors in peripheral blood mononuclear cells or primary renal cells. Raltegravir is a substrate for SLC22A6 and SLC15A1 in the oocyte expression system. However, transport was limited compared to endogenous controls, and these transporters are unlikely to have a great impact on raltegravir pharmacokinetics.

HIV infection and AIDS continue to be a major cause of worldwide mortality in the 21st century. A UNAIDS/WHO report in 2009 estimated that 33.4 million people worldwide were infected with HIV in 2008, with AIDS-related deaths numbering 2 million. Recent attempts to develop a vaccine for HIV have been largely unsuccessful (18). This, combined with increasing drug resistance, has emphasized the need to develop new drugs with unique mechanisms of action.

Raltegravir represents a new class of antiretroviral treatment (8), targeting the HIV-1 integrase enzyme by binding to the active site and preventing viral DNA insertion into the host genome (11). Recent trials have shown raltegravir to have a sustained antiretroviral effect and good tolerability in treatment-experienced HIV-1 patients (33). The primary route of raltegravir metabolism is glucuronidation via UGT1A1, and raltegravir is not a substrate or an inhibitor of the major cytochrome P450 enzymes (19, 24). However, the involvement of human drug transporters in raltegravir absorption, disposition, metabolism, and excretion (ADME) has not been fully investigated. Raltegravir has been described as being an ABCB1 substrate (25), but there are no data yet in the public domain. Raltegravir has shown higher concentrations (1.7-fold) in semen (4) and lower concentrations (0.04- to 0.39-fold) in cerebrospinal fluid (7, 41) than in plasma, which may be facilitated by drug transporters present at membrane barriers.

There are important reasons why raltegravir should be screened for potential transport by known drug transporters. First, by regulating intracellular permeation, drug transporters could be an important factor in an understanding of the lack of a clear pharmacokinetic-pharmacodynamic (PK-PD) relationship of raltegravir, i.e., the similar virological response observed for patients given a wide range of raltegravir doses (29). Second, a knowledge of the mechanisms that control raltegravir disposition may help rationalize or even anticipate drug-drug interactions in the clinic. Since raltegravir represents the first member of a new drug class, possessing a unique chemical structure containing a diketo acid derivative (34), class-specific trends in drug transport may be evident, such as those reported previously for protease inhibitors with ABCB1 (26, 37) or nucleoside reverse transcriptase inhibitors with organic anion and cation transporters (35, 36). Finally, knowledge of which transporters are involved in raltegravir ADME will identify candidate genes for future pharmacogenetic studies.

There have been a number of studies undertaken to evaluate the pharmacokinetic interactions between raltegravir and co-administered drugs. Most studies have shown raltegravir metabolism and disposition to be largely unaffected. For example, etravirine (1), maraviroc (3), darunavir (2), and rifabutin (6) had no or a relatively modest effect on raltegravir plasma concentrations. In addition, despite ritonavir being an inducer of both ABCB1 (9) and UGT1A1 (12), the drug caused only a minimal reduction in the raltegravir plasma concentration...
Similarly, tipranavir combined with ritonavir had little impact on raltegravir pharmacokinetics (13). However, there are interactions between raltegravir and coadministered drugs that have a more marked effect on raltegravir disposition.

Atazanavir is an inhibitor of UGT1A1 (42), and the coadministration of atazanavir (400 mg once a day [QD]) with raltegravir (100 mg single dose) resulted in increased raltegravir plasma area under the concentration-time curve (AUC), $C_{\text{max}}$, and $C_{\text{min}}$ values of 72%, 53%, and 95%, respectively (20). This interaction was also confirmed in a separate study (43). Efavirenz and rifampin are inducers of UGT1A1 expression (14, 40) and have been shown to decrease raltegravir plasma exposure (22, 39). Other interactions were reported previously for omeprazole (21) and fosamprenavir (28).

One intriguing interaction is that of tenofovir, causing a moderate increase in the raltegravir AUC and $C_{\text{max}}$ by 49% and 64%, respectively (38). Although the increase in the raltegravir plasma concentration is unlikely to have any important clinical significance (i.e., no increase in toxicity), the mechanism of the interaction is currently unexplained. Interestingly, tenofovir is an anionic compound when charged and is therefore a substrate of the organic anion uptake transporters SLC22A6 and SLC22A8 (36).

The aim of this study was to confirm the transport of raltegravir by ABCB1 (phosphoglycoprotein) and to investigate potential transport by major human drug influx transporters (25), with the exception of CNTs (concentrative nucleoside transporters) and ENTs (equilibrative nucleoside transporters), since these are specific to nucleotides. The influx transporters characterized are SLC15A2 (PEPT2). Since tenofovir is a substrate for SLC15A2 and SLC22A6 and SLC22A8 (36).

Bidirectional transport of raltegravir using Caco-2 cell monolayers. Caco-2 monolayer experiments were performed as previously described (17), with slight modifications. When confluent, Caco-2 cells (passage 30) were seeded onto polycarbonate membrane transwells at a density of $5 \times 10^6$ cells/cm². The medium was replaced every 2 days, and plates were used in the experiments after 21 days. Monolayer integrity was checked by using a Millicell-ERS instrument (Millipore) to determine the transepithelial electrical resistance (TEER) across the monolayer. A TEER of >600 was deemed acceptable. On the day of the experiment, the TEER was assessed, and the medium in each plate was replaced with warm transport buffer (HBSS containing 25 mM HEPES and 0.1% [wt/vol] FCS) for each direction and 100 µl supernatant aliquots were taken and added to scintillation vials in order to calculate extracellular drug concentrations. The remaining supernatant was discarded, and the cells were washed with ice-cold HBSS and centrifuged (2,000 rpm at 1° C for 1 min), and 100 µl supernatant aliquots were taken and added to scintillation vials in order to calculate extracellular drug concentrations. The remaining supernatant was discarded, and the cells were washed with ice-cold HBSS and centrifuged (2,000 rpm at 1° C for 1 min). This HBSS wash was repeated a total of 3 times, after which the HBSS was discarded and 100 µl tap water was added to lyse the cells. The incubations were counted for 5 min, and 100 µl samples were added to scintillation vials. Four milliliters of scintillation fluid was added to all the scintillation vials, which were then loaded into a liquid scintillation analyzer (Tri-Carb). Using supernatant and intracellular radioactivity readings, cellular accumulation ratios (CARs) (ratio of drug in the cell pellet compared with drug in the supernatant, assuming a 1 ml volume per cell) were calculated for raltegravir and saquinavir in each cell line as described previously (22).
were tested alongside [3H]raltegravir to ensure successful transporter expression. The temperature in a 60-rpm shaker for 1 h). Radiolabeled positive-control drugs into Barth’s solution containing calcium (88 mM NaCl, 1 mM KCl, 15 mM HEPES, 0.1 mM MgSO₄ · 7H₂O, 100 U penicillin, 100 µg streptomycin [pH 7.4]) and stored in a cold room at 8°C. Healthy cells were selected and injected with transporter cRNA (50 ng per oocyte; 1 ng/nl) or sterile water (50 nl) and maintained in Barth’s solution containing calcium to allow expression (5 days) for SLC22A6-injected oocytes and 3 days for all other conditions; 18°C). Barth’s solution was replaced daily, and damaged oocytes were removed.

Drug accumulation in transporter RNA-injected X. laevis oocytes. Drug accumulation studies using X. laevis oocytes were performed as described previously, with slight modifications (15). Unless otherwise stated, radiolabeled drug was incubated in Hanks balanced salt solution (pH 7.4) with 2345 oocytes per condition in a 48-well Nunc flat-bottom plate (500 µl; 0.33 µM/cell; 1 µM room temperature in a 60-rpm shaker for 1 h). Radiolabeled positive-control drugs were tested alongside [3H]raltegravir to ensure successful transporter expression. Positive-control drugs used were [3H]estrone-3-sulfate for SLC01A2, SLC01B1, and SLC01B3; [3H]aminohippuric acid for SLC22A6; [3H]uric acid for SLC10A1; [3H]glycyrrhetic acid (100 µM) for SLC37A1, SLC37A2, and SLC7A11; and [3H]lactate ammonium for SLC22A1. All incubations were terminated by transferring the oocytes and cell membrane transwells at a density of 5 × 10⁴ cells/cm² for 37°C in 5% CO₂). Once incubation was complete, an extracellular sample was taken, and incubations were terminated by washing each well with cold HBSS (4°C; 3 ml) three times to remove excess drug. Cells were lysed with 0.5 ml tap water, and contents were analyzed by liquid scintillation as described above for the CEM experiments.

Determination of transporter mRNA expression in primary renal proximal tubule cells and whole kidney. mRNA from primary renal proximal tubule cells were isolated by using TRI reagent according to the manufacturer’s protocol. mRNA was then reverse transcribed by using the TaqMan reverse transcription kit according to the manufacturer’s protocol. Real-time PCR using TaqMan array plates was performed with SLC22A6, SLC22A1, SLC22A3, ABCC1, ABCC2, ABCC3, ABCC4, and ABCC10. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene.

RESULTS

Toxicity of raltegravir was assessed with Caco-2 cells line. Raltegravir was not cytotoxic in CEM or Caco-2 cell lines at the tested concentrations. Cell viability (percent mean viability compared to the drug-free control ± SD; n = 6) was unaffected by concentrations of up to 100 µM in the Caco-2 (104.9% ± 12.7%) and CEM (113.9% ± 13.0%) cell lines. A concentration of 1 µM raltegravir was used in subsequent experiments.
experiments with these cells to minimize the risk of transporter saturation, as previously recommended (17).

ABC1 expression levels in CEM and CEMVBL100 cell lines. The expression of ABC1 in CEM and CEMVBL100 cell lines was determined by using flow cytometry. CEMVBL100 cells had a significantly higher level of ABC1 cell surface expression (relative fluorescence = 246.7 ± 9.3; P < 0.001) than in CEM cells (relative fluorescence = 1.5 ± 0.5).

Effect of the ABC1 inhibitor taricin on the accumulation of raltegravir and saquinavir using CEM and CEMVBL100 cell lines. The cellular accumulation of [3H]raltegravir was determined with CEM and ABC1-overexpressing CEMVBL100 cells, and the effect of the ABC1 inhibitor taricin on this accumulation was investigated (Fig. 1A). The level of [3H]raltegravir accumulation was lower in CEMVBL100 cells (CAR = 1.4 ± 0.2) than in CEM cells (CAR = 2.1 ± 0.2; P = 0.02). This difference was reversed when CEMVBL100 cells were treated with taricin (CAR = 2.0 ± 0.4). The control ABC1 substrate [3H]saquinavir had a lower level of accumulation in CEMVBL100 cells (CAR = 19.0 ± 5.6) than in CEM cells (CAR = 37.5 ± 2.1; P = 0.02) (Fig. 1B). This difference was also reversed when CEMVBL100 cells were treated with taricin (CAR = 37.8 ± 8.5).

Effect of the ABC1 inhibitor taricin on the bidirectional transport of raltegravir and digoxin using a Caco-2 monolayer. The $P_{\text{app}}$ values obtained for [3H]raltegravir and [3H]digoxin with and without taricin are given in Fig. 1C. All $P_{\text{app}}$ and efflux ratio calculations were made by using the samples taken after 120 min of incubation as sink conditions were maintained. [3H]raltegravir showed significantly higher transport in the B→A direction ($P_{\text{app}} = 13.4 \times 10^{-6} \pm 2.1 \times 10^{-6}$) compared to the A→B direction ($P_{\text{app}} = 7.3 \times 10^{-6} \pm 2.2 \times 10^{-6}$; P = 0.02). The efflux ratio (B→A/A→B) of [3H]raltegravir at 120 min was 1.9. The presence of taricin reduced the efflux ratio of [3H]raltegravir to 1.3 (P = 0.30). The control compound [3H]digoxin showed significantly higher levels of transport in the B→A direction ($P_{\text{app}} = 12.9 \times 10^{-6} \pm 0.6 \times 10^{-6}$) compared to the A→B direction ($P_{\text{app}} = 2.1 \times 10^{-6} \pm 0.3 \times 10^{-6}$; P < 0.001). The efflux ratio of [3H]digoxin at 120 min was 6.3. The presence of taricin reduced the efflux ratio of [3H]digoxin to 0.9 (P = 0.58).

Accumulation of raltegravir in oocytes by uptake transporters. [3H]raltegravir transport by the investigated uptake transporters is given in Table 1. The level of [3H]raltegravir accumulation was significantly higher in SLC22A6-injected oocytes (0.44 ± 0.12 pmol/oocyte; n = 18) than in H2O-injected oocytes (0.20 ± 0.05 pmol/oocyte; n = 19; P < 0.001). [3H]raltegravir accumulation was also significantly higher in SLC15A1-injected oocytes (0.26 ± 0.12 pmol/oocyte; n = 19) than in H2O-injected oocytes (0.17 ± 0.03 pmol/oocyte; n = 19; P = 0.003). The positive-control compounds [3H]estrone-3-sulfate, [3H]aminohippuric acid, [3H]taurocholic acid, [3H]glycyl sarcosine, and [3H]tetraethyl ammonium all showed significant increases in levels of accumulation in transporter RNA-injected oocytes compared to H2O-injected oocytes.

### Table 1. Accumulation of 1 μM raltegravir and various positive-control compounds in oocytes

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Drug</th>
<th>Mean drug concn (pmol/oocyte) ± SD</th>
<th>RNA/H2O ratio (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC01A2</td>
<td>RAL</td>
<td>0.39 ± 0.14</td>
<td>1.11 (0.09)</td>
</tr>
<tr>
<td></td>
<td>ESS</td>
<td>1.48 ± 0.63</td>
<td>5.48 (&lt;0.001)</td>
</tr>
<tr>
<td>SLC01B1</td>
<td>RAL</td>
<td>0.34 ± 0.05</td>
<td>1.08 (0.09)</td>
</tr>
<tr>
<td></td>
<td>ESS</td>
<td>2.68 ± 1.55</td>
<td>7.97 (&lt;0.01)</td>
</tr>
<tr>
<td>SLC01B3</td>
<td>RAL</td>
<td>0.63 ± 0.19</td>
<td>0.97 (0.09)</td>
</tr>
<tr>
<td></td>
<td>ESS</td>
<td>0.56 ± 0.23</td>
<td>2.60 (&lt;0.01)</td>
</tr>
<tr>
<td>SLC22A6</td>
<td>RAL</td>
<td>0.44 ± 0.12</td>
<td>2.22 (&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>AHA</td>
<td>0.44 ± 1.24</td>
<td>56.9 (&lt;0.01)</td>
</tr>
<tr>
<td>SLC10A1</td>
<td>RAL</td>
<td>0.20 ± 0.03</td>
<td>1.03 (0.57)</td>
</tr>
<tr>
<td></td>
<td>TCA</td>
<td>0.20 ± 0.12</td>
<td>3.51 (&lt;0.01)</td>
</tr>
<tr>
<td>SLC15A1</td>
<td>RAL</td>
<td>0.26 ± 0.12</td>
<td>1.52 (&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>GLY</td>
<td>0.45 ± 0.13</td>
<td>4.22 (&lt;0.01)</td>
</tr>
<tr>
<td>SLC15A2</td>
<td>RAL</td>
<td>0.19 ± 0.04</td>
<td>1.08 (0.16)</td>
</tr>
<tr>
<td></td>
<td>GLY</td>
<td>0.29 ± 0.19</td>
<td>2.09 (&lt;0.01)</td>
</tr>
<tr>
<td>SLC22A1</td>
<td>RAL</td>
<td>0.21 ± 0.03</td>
<td>1.21 (0.06)</td>
</tr>
<tr>
<td></td>
<td>TEA</td>
<td>0.34 ± 0.06</td>
<td>1.99 (&lt;0.01)</td>
</tr>
</tbody>
</table>

*Results are expressed as the mean drug concentrations per oocyte (pmol/oocyte) (n = 2 biological replicates; n = 4 experimental replicates per biological replicate) ± standard deviations. Also shown are the ratios of drug accumulation between transporter RNA-injected and H2O-injected oocytes. RAL, raltegravir; ESS, estrone-3-sulfate; AHA, aminohippuric acid; TCA, taurocholic acid; GLY, glycyl sarcosine; TEA, tetraethyl ammonium.
Determination of the time-dependent accumulation of raltegravir, tenofovir, and aminohippuric acid in SLC22A6-injected oocytes. All drugs tested had a greater accumulation rate in SLC22A6-injected oocytes than in H2O-injected oocytes (Fig. 2A, B, and C). [3H]raltegravir concentrations continued to increase in SLC22A6-injected oocytes throughout the 4-h incubation period, whereas saturation was reached in H2O-injected oocytes after 2 h. Both [3H]tenofovir and [3H]aminohippuric acid showed virtually no accumulation in H2O-injected oocytes.

Determination of raltegravir and tenofovir \( K_m \) and \( V_{max} \) values using SLC22A6-injected oocytes. An incubation time of 1 h was chosen for subsequent kinetic studies. [3H]raltegravir and [3H]tenofovir kinetics were determined for SLC22A6 in the oocyte expression system (Fig. 2D and E). The raltegravir \( K_m \) and \( V_{max} \) were calculated to be 150 \( \mu M \) and 36 pmol/oocyte/h, respectively. The raltegravir CL\(_{int} \) (\( V_{max}/K_m \)) was calculated to be 0.2 \( \mu l/oocyte/h \). The tenofovir \( K_m \) and \( V_{max} \) were calculated to be 25 \( \mu M \) and 129 pmol/oocyte/h, respectively. The tenofovir CL\(_{int} \) was calculated to be 5.2 \( \mu l/oocyte/h \).

Competition between raltegravir and tenofovir for SLC22A6 transport. When incubated at 1 \( \mu M \) for 1 h, [3H]raltegravir showed a significantly higher level of accumulation in SLC22A6-injected oocytes than in H2O-injected oocytes (1.73 ± 0.46 pmol/oocyte versus 0.54 ± 0.06 pmol/oocyte; \( n = 5; P < 0.014 \)) (Fig. 3A). The coinoculation of [3H]raltegravir with 1, 3, 10, 30, 100, and 300 \( \mu M \) tenofovir resulted in levels of [3H]raltegravir accumulation in SLC22A6-injected oocytes of 1.78 ± 0.41 pmol/oocyte, 1.63 ± 0.62 pmol/oocyte, 1.48 ± 0.58 pmol/oocyte, 1.28 ± 0.27 pmol/oocyte, 0.96 ± 0.28 pmol/oocyte, and 0.83 ± 0.31 pmol/oocyte, respectively (Fig. 3C). There was a statistically significant decrease (\( P < 0.05 \)) in the level of [3H]raltegravir accumulation when concentrations of 100 \( \mu M \) tenofovir and higher were added to the incubation mixture.

Similar results were seen when [3H]tenofovir accumulation was investigated in the presence of various concentrations of raltegravir. When incubated at 1 \( \mu M \) for 1 h, [3H]tenofovir showed a significantly higher level of accumulation in SLC22A6-injected oocytes than in H2O-injected oocytes (2.11 ± 0.37 pmol/oocyte versus 0.03 ± 0.01 pmol/oocyte; \( n = 5; P < 0.009 \)) (Fig. 3B). The coinoculation of [3H]tenofovir with 1, 3, 10, 30, 100, and 300 \( \mu M \) raltegravir resulted in levels of [3H]tenofovir accumulation in SLC22A6-injected oocytes of...
2.11 ± 0.14 pmol/oocyte, 2.07 ± 0.38 pmol/oocyte, 1.47 ± 0.43 pmol/oocyte, 0.78 ± 0.50 pmol/oocyte, 0.49 ± 0.06 pmol/oocyte, and 0.28 ± 0.08 pmol/oocyte, respectively (Fig. 3D). There was a statistically significant decrease \( (P < 0.05) \) in levels of \([3H]\)tenofovir accumulation when concentrations of 10 μM raltegravir and higher were added to the incubation mixtures.

**Effect of transporter inhibitors on the accumulation of raltegravir in peripheral blood mononuclear cells.** The cellular accumulation of \([3H]\)raltegravir in peripheral blood mononuclear cells was determined (Fig. 4A). Cellular accumulation was not significantly altered by coincubation with tariquidar (CAR = 3.78 ± 1.56; \( P = 0.77 \)), probenecid (CAR = 3.26 ± 0.98; \( P = 0.77 \)), or glycyl sarcosine (CAR = 3.23 ± 0.83; \( P = 0.56 \)).

**Interactions in primary renal proximal tubule epithelial cells.** The levels of cellular accumulation of \([3H]\)raltegravir (CAR = 2.01 ± 0.20) (Fig. 4B), \([3H]\)tenofovir (CAR = 3.45 ± 0.76) (Fig. 4C), and \([3H]\)aminohippuric acid (CAR = 0.50 ± 0.02) (Fig. 4D) in renal proximal tubule epithelial cells were determined. \([3H]\)raltegravir cellular accumulation was not significantly altered by treatment with 1 mM probenecid (CAR = 2.19 ± 0.45; \( P = 0.56 \)) or 100 μM tenofovir (CAR = 2.07 ± 0.08; \( P = 0.66 \)). \([3H]\)tenofovir showed a high level of cellular accumulation, which was not significantly altered by treatment with 1 mM probenecid (CAR = 2.59 ± 0.56; \( P = 0.19 \)) or 100 μM raltegravir (CAR = 3.15 ± 0.74; \( P = 0.65 \)). For \([3H]\)aminohippuric acid there was a trend toward a lower level of cellular accumulation when incubated with 1 mM probenecid (CAR = 0.44 ± 0.03; \( P = 0.08 \)).

**Transporter expression in primary renal proximal tubule cells versus whole kidneys.** All transporters tested showed lower or undetectable levels of expression in primary renal proximal tubule cells compared to whole kidney (Fig. 4E). Importantly, SLC22A6 was undetectable in primary renal cells.

**DISCUSSION**

The results from CEM/CEM VBL accumulation and Caco-2 bidirectional transport experiments confirm that raltegravir is transported by ABCB1. The extent of raltegravir transport by ABCB1 was small compared to the transport of the positive controls saquinavir and digoxin. Indeed, FDA guidelines recommend that a drug should achieve an efflux ratio of at least 2 in Caco-2 cell monolayers and show greater than a 50% reduction in the efflux ratio when an ABCB1 inhibitor is used in order for ABCB1 transport to be considered relevant in vivo (16). In our Caco-2 experiment raltegravir achieved an efflux ratio of only 1.9 and a reduction of 32% when tariquidar was used to inhibit ABCB1. The low rate of raltegravir transport by ABCB1 may explain the absence of major drug interactions with known potent ABCB1 inhibitors. This is consistent with a
Raltegravir showed significantly increased levels of accumulation in SLC15A1- and SLC22A6-injected oocytes compared to H$_2$O-injected controls, but accumulation was not higher in oocytes expressing SLCO1A2, SLCO1B1, SLCO1B3, SLC15A2, and SLC10A1. In SLC22A6-injected oocytes, both raltegravir and tenofovir inhibited the accumulation of the other in a concentration-dependent manner (Fig. 3C and D). No competition was observed for H$_2$O-injected oocytes, which supports the hypothesis that raltegravir and tenofovir are competing for SLC22A6 transport and are not having nonspecific effects on oocyte membrane permeability. IC$_{50}$ values of 27.3 μM and 14.0 μM were determined for tenofovir and raltegravir, respectively. The IC$_{50}$ obtained for raltegravir was much lower than the observed $K_m$ for SLC22A6 (IC$_{50}$ of 14.0 μM versus a $K_m$ of 147 μM). These results suggest that raltegravir is a more efficient SLC22A6 inhibitor than it is a substrate.

Previous studies indicated that SLC22A6 and SLC15A1 are absent from PBMCs (5), and so these transporters are unlikely to explain the unusual PK-PD relationship for raltegravir. Indeed, our studies of PBMCs with known inhibitors of ABCB1, SLC22A6, and SLC15A1 revealed no significant interaction with raltegravir.

Wenning et al. (38) previously studied the interaction of raltegravir (400 mg twice daily) and tenofovir (300 mg once daily). The study showed increased raltegravir AUC (49%) and $C_{\text{max}}$ (64%) values but no effect on the raltegravir $C_{\text{min}}$ and a decrease in the tenofovir AUC (10%), $C_{\text{max}}$ (23%), and $C_{\text{min}}$ (13%) (38). SLC22A6 is expressed predominantly in the proximal tubule of the kidney on the basolateral (blood-facing) surface, thereby facilitating the removal of drugs out of the blood and into the urine (31). Therefore, a possible mechanism of interaction is the inhibition of SLC22A6-mediated raltegravir transport at the kidney-proximal tubule by tenofovir, resulting in increased raltegravir plasma concentrations. In order to investigate interactions at the level of the kidney, we conducted a number of experiments with primary renal proximal cells. No interaction between tenofovir and raltegravir was observed for these cells, but neither was an interaction with the positive-control substrate and inhibitor. Subsequent analyses revealed the expression of SLC22A6 to be undetectable, unlike kidney tissue. Furthermore, all transporters that were assessed were at lower levels of expression than in kidney tissue, and the absence of a robust primary cell model for these studies is a limitation.

Since only a small percentage (around 30%) of raltegravir excreted via the kidney is in the parent form, with the remaining 70% being the glucuronide metabolite (24), it is important to determine if the raltegravir glucuronide is also transported by SLC22A6 and inhibited by tenofovir. It would also be interesting to investigate the transport and inhibitory potential of tenofovir diphosphate for SLC22A6 and whether this affects raltegravir transport to the same degree.

The Xenopus laevis oocyte expression system has several advantages when investigating drug transport. The large size and high level of protein production of oocytes provide robust and reliable data. Also, the level of expression of endogenous primary and secondary active xenobiotic transporters in oocytes is low (32). However, there are also disadvantages. The temperature must be maintained at 18°C during protein expression and at around room temperature during any accumulation experiments to avoid degradation, and this may impact transporter kinetics. Also, as in other models, the expression of the investigated transporters is superphysiological. Therefore, although they allow an investigation of low-affinity or high-permeability substrates, this means that caution should be taken when extrapolating the data to in vivo observations.

In summary, our studies have shown raltegravir to have minimal interactions with known drug transporters. Raltegravir is transported by ABCB1 in vitro, although the rate of transport is low and the potential for interactions is expected to be small. Raltegravir is a substrate for SLC22A6 and SLC15A1 in X. Laevis oocyte expression systems and competes with tenofovir for SLC22A6 transport. Polymorphisms in SLC22A6...
have previously been described and now warrant study in the context of raltegravir.

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