In Vitro Investigations into the Roles of Drug Transporters and Metabolizing Enzymes in the Disposition and Drug Interactions of Dolutegravir, a HIV Integrase Inhibitor

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ABSTRACT

Dolutegravir (DTG; S/GSK1349572) is a potent HIV-1 integrase inhibitor with a distinct resistance profile and a once-daily dose regimen that does not require pharmacokinetic boosting. This work investigated the in vitro drug transport and metabolism of DTG and assessed the potential for clinical drug-drug interactions. DTG is a substrate for the efflux transporters P-glycoprotein (Pgp) and human breast cancer resistance protein (BCRP). Its high intrinsic membrane permeability limits the impact these transporters have on DTG’s intestinal absorption. UDP-glucuronosyltransferase (UGT) 1A1 is the main enzyme responsible for the metabolism of DTG in vivo, with cytochrome P450 (P450) 3A4 being a notable pathway and UGT1A3 and UGT1A9 being only minor pathways. DTG demonstrated little or no inhibition (IC\textsubscript{50} values < 30 \mu M) in vitro of the transporters Pgp, BCRP, multidrug resistance protein 2, organic anion transporting polypeptide 1B1/3, organic cation transporter (OCT) 1, or the drug metabolizing enzymes CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, UGT1A1, or 2B7. Further, DTG did not induce CYP1A2, 2B6, or 3A4 mRNA in vitro using human hepatocytes. DTG does inhibit the renal OCT2 (IC\textsubscript{50} < 1.9 \mu M) transporter, which provides a mechanistic basis for the mild increases in serum creatinine observed in clinical studies. These in vitro studies demonstrate a low propensity for DTG to be a perpetrator of clinical drug interactions and provide a basis for predicting when other drugs could result in a drug interaction with DTG.

HIV RNA in serum, patients living longer, and fewer patients dying from the disease (Palella et al., 1998, 2006). This outcome changes the focus of HIV therapy from one mainly focused on managing the viral disease to one of also considering other comorbidities, including the treatment of an aging HIV population with conditions such as cardiovascular disease and diabetes. Thus, as newer HIV agents come to the market, the drug-drug interaction (DDI) assessment needs to include a broader number of co-medications than previously considered for the treatment of HIV disease.

Management of DDIs in HIV subjects is challenging, because the major mechanisms of DDIs in antiretroviral combination therapy involve cytochrome P450 (P450) enzymes, ATP-binding cassette efflux transporters, and solute carrier uptake transporters (Zembruski et al., 2011a). Most PIs (e.g., lopinavir, fosamprenavir, darunavir) are substrates or inhibitors of CYP3A4, and many of these agents require boosting with ritonavir, a very potent CYP3A4 inhibitor (Hull and Montaner, 2011). Inhibition of metabolic enzymes is not limited to only PIs or the CYP enzyme family, but other antiviral drugs such as atazanavir are potent inhibitors of both CYP3A4 and UDP-glucuronosyltransferase.
Dolutegravir (DTG) is a tricyclic carbamoyl pyridone integrase inhibitor that blocks the strand transfer step during the integration of the HIV-1 DNA into the genome of the host cell. The unique features of DTG include a superior resistance profile and once-daily, low-milligram dosing that does not require pharmacokinetic boosting (Min et al., 2010). Like other antiretroviral agents, it is expected that DTG will be coadministered in combination with a wide variety of other drugs, including agents other than antivirals for non-HIV–related comorbidities. Because of the potential overlapping transporter and metabolic pathways of DTG and drugs coadministered to treat HIV infection or other diseases, it is critical to evaluate the potential for clinical DDIs, especially in the HIV polypharmacy setting. Therefore, the objective of this study was to investigate the mechanisms for the potential role of transporters and metabolizing enzymes in the disposition of DTG and to provide a basis for predicting when other drugs could result in a drug interaction with DTG and when DTG might cause interactions with other coadministered drugs.

Materials and Methods

GlucoSmithKline Active Pharmaceutical Ingredient Chemistry and Analysis (Stevenage, UK) supplied [14C]DTG (59 mCi/mmol), GF120918 (elacridar), and [14C]cimetidine (57 mCi/mmol). [3H]Digoxin (5 Ci/mmol) and [3H]amprolavin (19 Ci/mmol) were purchased from GE Healthcare/Amersham Biosciences (Piscataway, NJ). [3H]Estradiol 17β-d-glucuronide (EG; 50 Ci/ mmol) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). Cryopreserved human hepatocytes (pool of 3 donors; 2 male and 1 female) were obtained from CellzDirect (Durham, NC). Pooled human liver microsomes (human liver microsomes [HLMs]; prepared from 15 donors) were obtained from Xenotech, LLC (Lenexa, KS), and the micromolar protein concentration and P450 content were provided by the manufacturer. Supersomes, containing P-glycoprotein (Pgp), human breast cancer resistance protein (BCRP), and multidrug resistance associated protein-2 transporter (MRP2) and the organic anion transporting polypeptide (OATP) uptake transporters (Dixit et al., 2007). Coadministration of Pgp or BCRP inhibitors, such as ritonavir or saquinavir, may increase the oral absorption of an HIV co-medication that is a substrate for these transporters or allow entry into more restricted tissues such as the brain or placenta. Alternatively, absorption can be reduced via induction of these transporters. Clinically significant Pgp interactions have been reported with the PIs tipranavir/ritonavir and the Pgp substrate lopamiderap, resulting in a 50% reduction in the area under the curve (AUC) of lopamiderap (Mukwaya et al., 2005).

DTG was tested in triplicate in vitro using the plasma substrate [3H]HJEG and membrane vesicles prepared from baculovirus-infected S9 cells expressing human MRP2. (Invitrogen). Vesicles (50 μg protein) were preincubated for 5 minutes at 37°C in reaction buffer with DTG or M2 (0.1–100 μM) or positive control benzomorpane (0.1–100 μM) and reactions initiated by the addition of 10 mM MgATP solution containing 50 μM [3H]HJEG. Additional incubations were performed in the absence of inhibitor and in the presence of 10 mM MgAMP solution for passive transport. After 3 minutes, reactions were terminated by the addition of chilled stopping buffer (40 mM MOPS-Tris, 70 mM KCl), samples were transferred to 96-well glass fiber filter plates and washed, and radioactivity was measured using LSC.

OATP Assays. For OATP1B1 studies, Chinese hamster ovary cell line heterologously expressing the human OATP1B1 transporter obtained from the University of Zurich was used. Human embryonic kidney (HEK) MSRII cells (American Type Culture Collection, Manassas, VA) transduced with BacMam baculovirus containing the human OATP1B3 transporter were used for the OATP1B3 inhibition assay. Cell culture and inhibition studies were completed as described previously (Polli et al., 2008). DTG was tested in triplicate (0.3–100 μM), and rifamycin SV (10 μM) was used as a positive control inhibitor.

Organic Cation Transporter Inhibition Assays. Organic cation transporter (OCT) 1 inhibition screening studies were conducted at Absorption Systems LP (Exton, PA) using HEK293 cells expressing OCT1. [14C]Metformin (10 μM) was incubated with DTG (10 μM) or positive control inhibitor (10 μM repaglinide or 100 μM quinidine) for 10 minutes at 37°C in a humidified incubator. Incubations were performed in triplicate. Cells were lysed with 75% ethanol and radioactivity measured by LSC in a Tri-Carb 2900TR liquid scintillation analyser (PerkinElmer Life and Analytical Sciences). OCT2 inhibition studies were conducted at Optivia (Menlo Park, CA). MDCKII cells grown on permeable supports were transferred with a vector containing OCT2. The transport of [14C]metformin (10 μM) was determined by LSC. DTG was tested in triplicate (0.1–30 μM). Cimetidine was used as a positive control inhibitor (100 and 300 μM).

Identification of the Enzymes Involved in the Metabolism of DTG. [14C] DTG (50 μM) was incubated with pooled cryopreserved human hepatocytes (1.5 million cells/ml) in Weymouth’s medium containing 5% fetal bovine serum for 4 and 20 hours at 37°C in a CO2 incubator. Reactions were terminated with an equal volume of acetonitrile, samples centrifuged, and supernatants analyzed by radio-HPLC. Incubations with media but no cells were used as negative controls. Positive control incubations were performed with
7-ethoxycoumarin to confirm phase I and II metabolism of the hepatocytes. The effect of a selective CYP3A4 inhibitor, azelaolin, on the metabolism of DTG was evaluated by preincubating azelaolin (5 μM) with HLMs (2 mg/ml), 0.1 M phosphate buffer, pH 7.4, and cofactor for 10 minutes before adding [14C]DTG to start the reaction. Reactions were terminated after 2 hours by adding an equal volume of acetonitrile. Control incubations were performed in the absence of azelaolin, cofactor, or HLMs. P450 reaction phenotyping experiments were conducted by preincubating Supersomes expressing individual human P450 enzymes, CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 (150 pmol/ml), for 5 minutes at 37°C with [14C]DTG (5 μM) in 0.1 M phosphate buffer, pH 7.4. Reactions were initiated with the addition of cofactor (an NADPH-regenerating system consisting of 1 mM NADPH, 5.5 mM glucose 6-phosphate, and 1.2 units/ml glucose-6-phosphate dehydrogenase) and terminated after 2 hours by the addition of an equal volume of acetonitrile. Incubations were performed with no cofactor and with vector control membranes.

For UGT reaction phenotyping experiments, HLMs (1 mg/ml) were incubated with alanethicin (50 μg/mg microsomal protein) in 0.1 M Tris-HCl, pH 7.4 buffer, and 2 mM MgCl₂ for 15 minutes on ice. [14C]DTG (16 μg/mg microsomal protein) was added to HLMs for 5 minutes at 37°C and reactions initiated with cofactor (5 mM UDP glucuronic acid (UDPGA)). Incubations were performed, in duplicate, for 0–4 hours. The effect of atazanavir, a UGT1A1 inhibitor (Zhang et al., 2005) on [14C]DTG glucuronidation was evaluated in HLMs preincubated with [14C]DTG (10 μM) and atazanavir (0.1–30 μM) for 5 minutes. Reactions were initiated by cofactor and incubated for 2 hours. [14C] DTG incubations with recombinant human UGT enzymes (UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, or 2B15; at 1 mg/ml protein) were conducted by activating the enzymes with alanethicin as described above. Reactions were started with UDPGA and incubations performed for 2 and 4 hours. Control incubations were performed 1) in the absence of cofactor and 2) with UGT insect cell control Supersomes (lacking UGT activity) (BD Gentest). The in vitro enzyme kinetic parameters were determined for glucuronide formation of [14C]DTG (2.0, 5.0, 10, 25, 50, and 100 μM) in HLMs and human UGT1A1 enzymes. Incubations were performed for 2 hours and processed as described previously.

HPLC Radiochemical Profiling and Metabolite Identification. Radiochemical profiles of human hepatocytes, HLMs, and recombinant P450 and UGT incubations were generated using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) coupled with a model 625 radiochemical detector (PerkinElmer Life and Analytical Sciences) in a 0.1 M Tris-HCl, pH 7.4 buffer containing 5 mM MgCl₂, probe substrate, 25 μg/ml alanethicin, and 0.25 mg/ml UGT enzymes. Reactions were initiated with 2 mM UDPGA. Sample analysis was performed using fluorescence detection with a SpectraMax M2e microplate reader (Molecular Devices, Sunnyvale, CA). The decrease in scopoletin or HFC fluorescence due to the production of the glucuronide metabolite was monitored at excitation and emission wavelengths of 410 and 590 nm, respectively, with measurements taken every 2 min over the course of 30 min. Rates of scopoletin or HFC glucuronidation were calculated by monitoring a decrease in substrate fluorescence in the absence and presence of inhibitor and expressed as the rate (slope). Rates of metabolite production at each concentration of inhibitor were expressed as a percentage of the mean uninhibited control rate for each assay. Nonlinear regression analyses of the data were performed using GraFit (v.5.0.8; Eriothicus Software Limited, London, UK) to calculate IC₅₀ values.

UGT Inhibition by DTG. The ability of DTG to inhibit UGT1A1 and 2B7 was evaluated by measuring the glucuronidation of scopoletin (20 μM; UGT1A1) and 7-hydroxy-4-(trifluoromethyl) coumarin (HFC; 15 μM; UGT2B7) in UGT Supersomes (BD Gentest) using fluorescence detection (Collier et al., 2000). DTG (0.1–100 μM), positive control inhibitor atazanavir (UGT1A1), or gemfibrozil (UGT2B7) was incubated in duplicate in a 96-well OptiPlate (PerkinElmer Life and Analytical Sciences) in a 0.1 M Tris-HCl, pH 7.4 buffer containing 5 mM MgCl₂, probe substrate, 25 μg/ml alanethicin, and 0.25 mg/ml UGT enzymes. Reactions were initiated with 2 mM UDPGA. Sample analysis was performed using fluorescence detection with a SpectraMax M2e microplate reader (Molecular Devices, Sunnyvale, CA). The decrease in scopoletin or HFC fluorescence due to the production of the glucuronide metabolite was monitored at excitation and emission wavelengths of 410 and 590 nm, respectively, with measurements taken every 2 min over the course of 30 min. Rates of scopoletin or HFC glucuronidation were calculated by monitoring a decrease in substrate fluorescence in the absence and presence of inhibitor and expressed as the rate (slope). Rates of metabolite production at each concentration of inhibitor were expressed as a percentage of the mean uninhibited control rate for each assay. The IC₅₀ values were calculated using GraFit software.

Induction of P450 mRNA by DTG in Human Hepatocytes. Primary human hepatocytes from a single donor were commercially obtained plated in a sandwich configuration on a collagen substrate with Matrigel overlay (CellDirect); three separate donors were tested. Hepatocytes were treated for 2 days with DTG (1, 5, 10, 20, 30, and 40 μM), vehicle control (0.1% dimethyl sulfoxide), or prototypical inducers, β-naphthoflavone (CYP1A2) (35 μM), phenobarbital (CYP2B6) (1 mM), and rifampicin (CYP3A4) (20 μM). After culture and test compound treatment, cells were lysed and total RNA was extracted. mRNA expression for each P450 was determined using quantitative real-time polymerase chain reaction technology (TaqMan) (Bowen et al., 2000). Relative differences in mRNA expression were assessed based on different cycling threshold values, which were determined by the ABI 7900 Real Time PCR System Sequence Detection Software (SDS v1.3).

Calculations. For monolayer efflux studies, the flux of DTG and probe substrates was calculated using the following equation:

\[ J = \frac{V(dC/dt)}{A} \]

where \( J \) is the flux (nmol/cm²/h), \( V \) is the receptor volume (ml), \( C \) is the receiver drug concentration (nmol/ml), \( t \) is the incubation time in hours, and \( A \) is the membrane surface area (cm²).

The passive permeability of DTG, probe substrates, and Lucifer yellow in the presence of GF120918 was determined using the following equation as described previously (Rautio et al., 2006):

\[ P_{eff} = - \left( \frac{V_{d}V_{k}}{V_{d} + V_{k}} \right) \ln \left( 1 - \left( \frac{V_{d} + V_{k}C_{d}(t)}{V_{d}C_{p}(0) + V_{k}C_{d}(t)} \right) \right) \times 10^{7} \text{nm/s} \]

where \( P_{eff} \) is the permeability coefficient at pH 7.4, \( V_{d} \) and \( V_{k} \) are donor and receiver well volumes, respectively (ml), \( A \) is the membrane surface area (cm²), \( t \) is the incubation time (seconds), \( C_{d}(t) \) is the measured concentration in the

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receiver well at time $t$ (nmol/ml), and $C_d(t)$ is the measured concentration in the donor well at time $t$ (nmol/ml).

For transporter and P450 inhibition studies, the IC$_{50}$ values (the concentration of inhibitor required for 50% inhibition of the monolayer transport, cellular uptake, or metabolite production rates) were calculated with GraFit (version 5.08; Erithacus Software Limited) using:

$$y = \frac{\text{Range}}{1 + \left(\frac{x}{IC_{50}}\right)^\gamma} + \text{background}$$

where $y$ = the rate of transport, uptake, or metabolite generation of an appropriate probe (expressed as a percentage of the uninhibited control), Range = the rate in the absence of test compound, $x$ = the slope factor, $x$ = the inhibitor concentration ($\mu$M), and background = the uninhibited rate (expressed as a percentage of the total rate).

For UGT1A1 enzyme kinetic determinations, nonlinear regression analysis of the ether glucuronide formation was performed using GraFit. Michaelis-Menten parameters $V_{max}$, $K_m$, and $CL_i$ were calculated using the following equations:

$$v = \frac{V_{max} \cdot [S]}{K_m + [S]} \quad CL_i = \frac{V_{max}}{K_m}$$

where $V_{max}$ is maximal rate of metabolite produced, $K_m$ is equal to the substrate concentration at which the reaction rate is half its maximal value, $[S]$ is the substrate concentration, and $CL_i$ is the intrinsic clearance.

**In Vitro-In Vivo Extrapolation of DTG as a Victim of UGT1A1 and CYP3A4 Inhibition.** A mechanistic static equation based on previously described models (Hinton et al., 2008) was used to predict the magnitude of DTG exposure in vivo when coadministered with the clinically relevant UGT1A1 and CYP3A4 inhibitors atazanavir and ritonavir:

$$\frac{AUC_i}{AUC} = \frac{\frac{f_{m,\text{CTPAA}}}{K_{\text{LATZ,CTPAA}}} + \frac{f_{m,\text{RTV}}}{K_{\text{RTV,CTPAA}}} + \frac{f_{m,\text{ATZ}}}{K_{\text{LATZ,UGT1A1}}} + \frac{f_{m,\text{RTV}}}{K_{\text{RTV,UGT1A1}}}}{1 + \frac{\left[\text{LATZ}\right]}{K_{\text{LATZ,CTPAA}}} + \frac{\left[\text{RTV}\right]}{K_{\text{RTV,CTPAA}}} + \frac{\left[\text{ATZ}\right]}{K_{\text{LATZ,UGT1A1}}} + \frac{\left[\text{RTV}\right]}{K_{\text{RTV,UGT1A1}}}} + (1 - f_{m,\text{CTPAA}} - f_{m,\text{UGT1A1}})$$

Unbound hepatic inlet concentrations of atazanavir, $[I]_{\text{LATZ}}$ (0.74 $\mu$M), and ritonavir, $[I]_{\text{RTV}}$ (0.016 $\mu$M), were estimated according to methods previously described in the literature (Ito et al., 1998). The atazanavir inhibition constants ($K_i$) for UGT1A1 and CYP3A4 are $K_{\text{LATZ,UGT1A1}}$ (0.2 $\mu$M) and $K_{\text{LATZ,CTPAA}}$ (2.35 $\mu$M), respectively (Busti et al., 2004). Ritonavir inhibition constants for CYP3A4 and UGT1A1 are $K_{\text{RTV,CTPAA}}$ (0.03 $\mu$M) and $K_{\text{RTV,UGT1A1}}$ (9.5 $\mu$M) (Zhang et al., 2005; Granfors et al., 2006). The $K_{\text{RTV,UGT1A1}}$ was estimated by dividing the reported IC$_{50}$ value in half, assuming competitive inhibition. $F_{ug}$, the fraction of substrate escaping gut-wall metabolism, was assumed to be 1 based on DTG’s in vitro CLs and high passive permeability. $f_m$ is the fraction of the substrate dose metabolized by a specific enzyme (e.g., CYP3A4 or UGT1A1).

**Results**

**ATP-Binding Cassette Transporter Substrate Assays.** To determine whether $^{14}$C]DTG is a substrate for human Pgp and BCRP, the in vitro bidirectional transport across MDCKII monolayers expressing these transporters was studied. In addition, the passive permeability was determined by inclusion of GF120918, a Pgp and BCRP inhibitor. The efflux ratio for $^{14}$C]DTG across the MDCKII-MDR1 monolayers was 3.5 and decreased to 0.74 in the presence of GF120918, consistent with DTG being a substrate for Pgp (Table 1). For BCRP, the efflux ratio of $^{14}$C]DTG across the MDCKII-BCRP monolayers was 3.1 and decreased to 0.80 in the presence of GF120918 (Table 1), consistent with DTG being a substrate for BCRP. The in vitro passive membrane permeability of $^{14}$C]DTG across the MDCKII monolayers was 333 nm/s (P5.5 B→A + GF120918) using Dulbecco’s modified Eagle’s medium as the transport buffer and was 265 (P5.5[abs]) and 252 (P7.4 [abs]) nm/s using fasted state-simulated intestinal fluid buffer, at pH 5.5/7.4 (donor/receiver) and 7.4/7.4, respectively. These results demonstrate that DTG has high membrane permeability independent of pH.

**ABC and Solute Carrier Transporter Inhibition Assays.** The inhibition of Pgp and BCRP by DTG was assessed by determining the B→A transport of $^{3}$H]digoxin and $^{14}$C]cimetidine across MDCKII-MDR1 or MDCKII-BCRP monolayers, respectively. DTG at a concentration of 100 $\mu$M decreased the Pgp-mediated transport of $^{3}$H]digoxin by 29% and inhibited BCRP-mediated transport of $^{14}$C]cimetidine by 43% at 30 $\mu$M and 50% at 100 $\mu$M. However, due to solubility limitations of DTG, an IC$_{50}$ value could not be determined (Table 2). MRP2 inhibition by DTG and its ether glucuronide, M2, was assessed by measuring the uptake of $^{3}$H]EJG in MRP2 membrane vesicles. At all concentrations tested (0.1–100 $\mu$M), no inhibition of $^{3}$H]EJG uptake in MRP2 vesicles was observed (IC$_{50}$ > 100 $\mu$M; Table 2).

**OATP1B1 and OATP1B3 inhibition by DTG was investigated by determining the uptake of $^{3}$H]EJG in Chinese hamster ovary cell line heterologously expressing the human OATP1B1 transporter and HEK-MSR1-OATP1B3 cells, respectively. At all concentrations tested (0.3–100 $\mu$M), no inhibition of $^{3}$H]EJG uptake in MRP2 vesicles was observed (IC$_{50}$ > 100 $\mu$M; Table 2).

<p>| TABLE 1 |
| Results of transport studies for $^{14}$C]DTG (3 $\mu$M) in MDCKII-MDR1 or MDCKII-BCRP cell monolayers$^a$ |</p>
<table>
<thead>
<tr>
<th>MDCKII Cell Line</th>
<th>GF120918$^b$</th>
<th>Rate Apical to Basolateral</th>
<th>Rate B→A</th>
<th>Apical Efflux Ratio</th>
<th>Apical to Basolateral Mass Balance</th>
<th>B→A Mass Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1</td>
<td>–</td>
<td>7.3 ± 0.52</td>
<td>28 ± 3.1</td>
<td>3.8</td>
<td>84 ± 7.1</td>
<td>84 ± 4.5</td>
</tr>
<tr>
<td>MDR1</td>
<td>+</td>
<td>4.7 ± 0.04</td>
<td>3.5 ± 0.07</td>
<td>0.74</td>
<td>84 ± 1.9</td>
<td>75 ± 1.7</td>
</tr>
<tr>
<td>BCRP</td>
<td>–</td>
<td>2.5 ± 0.24</td>
<td>7.8 ± 0.29</td>
<td>3.1</td>
<td>81 ± 3.4</td>
<td>85 ± 3.5</td>
</tr>
<tr>
<td>BCRP</td>
<td>+</td>
<td>4.2 ± 0.09</td>
<td>3.3 ± 0.06</td>
<td>0.80</td>
<td>81 ± 2.1</td>
<td>76 ± 2.4</td>
</tr>
</tbody>
</table>

$^a$ Data are the mean ± S.D. from three monolayers over 90 min using Dulbecco’s Modified Eagle’s medium as the transport buffer. All donor compartments contained Lucifer yellow (100 $\mu$M) to determine monolayer integrity (pass criterion P 7 4 ± 50 nm/s). Amprenavir served as a positive control for Pgp efflux and cimetidine for BCRP efflux. The measured radiochemical purity of $^{14}$C] DTG was 98% and no metabolic or chemical degradation was detected during the studies.

$^b$ GF120918 was used in both donor and receiver compartments at 2 $\mu$M for Pgp and BCRP.
Inhibition of human ATP-binding cassette transporters and solute carriers by DTG

<table>
<thead>
<tr>
<th>Transporter</th>
<th>IC50 (μM)</th>
<th>Substrate</th>
<th>[I1]/[IC50]</th>
<th>[I2]/IC50&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pgp</td>
<td>&gt;100</td>
<td>[3H]Digoxin</td>
<td>&lt;0.08</td>
<td>&lt;5</td>
</tr>
<tr>
<td>BCRP</td>
<td>&gt;30</td>
<td>[3H]Cimetidine</td>
<td>&lt;0.26*</td>
<td>&lt;16**</td>
</tr>
<tr>
<td>OATP1B1</td>
<td>&gt;100</td>
<td>[3H]Estradiol</td>
<td>&lt;0.08</td>
<td>n/a&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>OATP1B3</td>
<td>&gt;100</td>
<td>[3H]Estradiol</td>
<td>&lt;0.08</td>
<td>n/a&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>MRP2</td>
<td>&gt;100</td>
<td>[3H]Estradiol</td>
<td>&lt;0.08</td>
<td>&lt;5</td>
</tr>
<tr>
<td>OCT1</td>
<td>NC</td>
<td>[14C]Metformin</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>OCT2</td>
<td>1.9 (0.29)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>[14C]Metformin</td>
<td>4.2&lt;sup&gt;f&lt;/sup&gt;</td>
<td>n/a</td>
</tr>
</tbody>
</table>

<sup>a</sup> [I1] = 8 μM with DTG clinical daily dose of 50mg (Song et al., 2010); *possible DDI risk based on [I1] > 0.1 assuming [I2] = total plasma concentration as described in (Zhang et al., 2008; Giacomini et al., 2010).

<sup>b</sup> [I2] = 477 μM using 250 ml gastrointestinal volume. **Possible DDI risk based on [I2] > 10 as described in (Zhang et al., 2008; Giacomini et al., 2010).

<sup>c</sup> Not calculated; DTG at a concentration of 10 μM inhibited OCT1 by 22%.

<sup>d</sup> n/a = not applicable; transporter not (highly) expressed in the gastrointestinal tract (Hilgendorf et al., 2007).

μM, no discernible inhibition of [3H]Estradiol uptake by DTG was observed for OATP1B1 or OATP1B3 (IC50 > 10 μM; Table 2).

The inhibitory effect of DTG on human OCT1 and OCT2 was investigated by measuring the uptake of [14C]Metformin in HEK293-OCT1 and MDCKII-OCT2 cells, respectively. DTG at 10 μM slightly inhibited OCT1 transport by 22%. However, DTG notably inhibited the renal transporter OCT2 by 91% when tested at a concentration of 25 μM. On the basis of this observation, a study to determine the IC50 value against OCT2 was completed. DTG inhibited OCT2 with an IC50 value of 1.9 μM (Fig. 1; Table 2).

Identification of Enzymes Responsible for the Metabolism of DTG. Using primary human hepatocytes, HLMs, and cDNA-expressed P450 and UGT enzymes, the products of [14C]DTG metabolism and the relative contributions of the enzymes involved were defined. In human hepatocytes and UDPGA-fortified HLMs, the only metabolite of DTG observed was an ether glucuronide (M2). The ether glucuronide formation rate was highest in UGT1A1 incubations, with a much lower rate in UGT1A3 and 1A9 incubations (Fig. 2A). M2 was not detected in human hepatocytes using TaqMan technology. After incubation for 48 h with DTG (1–40 μM), there was no notable induction of CYP1A2, 2B6 or 3A4.

P450 and UGT Inhibition by DTG. The inhibition of human P450 enzymes by DTG was evaluated using HLMs and selective P450 probe substrates (Table 3). DTG inhibited CYP2B6, 2C9, 2C19, 2D6, and 3A4 (nifedipine), but the percentage of inhibition observed (range 20–40%) at the highest concentration tested (100 μM) was weak and insufficient to calculate an IC50. In contrast, DTG inhibited CYP3A4-mediated metabolism ofatorvastatin with a calculated IC50 of 54 μM (Table 3). DTG did not inhibit CYP1A2, 2A6, or 2C8 (IC50 > 100 μM; Table 3). CYP3A4 MDI was observed for nifedipine and atorvastatin with decreases in IC50 values following cofactor preincubation. DTG was not a metabolism-dependent inhibitor of CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, or 2D6 incubations, indicating that CYP3A4 is the primary P450 enzyme involved in the oxidative metabolism of DTG in vitro. The overall metabolic profile for DTG is summarized in Fig. 4.

Discussion

The studies presented herein describe the in vitro disposition and metabolism of DTG and the potential for DTG to be a victim or perpetrator of DDIs via modulation of transporters or metabolizing enzymes. Similar to other antiretroviral agents, DTG will be coadministered in combination with a variety of other drugs. Therefore, assessment of potential DDIs is an essential aspect in the clinical development of DTG.

Based on the limited aqueous solubility and high permeability, DTG is a Biopharmaceutics Drug Disposition Classification System class 2 drug (Benet et al., 2008, 2011), where efflux transporters and metabolizing enzymes are predicted to be important in the drug’s disposition. Indeed, in vitro studies show that DTG undergoes extensive metabolism and is a substrate for Pgp and BCRP, two transporters reported to influence the intestinal absorption and central nervous system penetration of HIV drugs (Kivisto et al., 2004). Although DTG is a substrate for efflux transporters, it exhibits rapid oral absorption (T<sub>max</sub> ~ 2 h following a 50-mg dose) and dose-proportional kinetics.
with low to moderate variability (Min et al., 2010). These data suggest that the high intrinsic passive membrane permeability attenuates any impact efflux transporters may have on DTG’s intestinal absorption in humans. This is further supported by clinical studies that have demonstrated no clinically significant change in DTG pharmacokinetics when coadministered with lopinavir/ritonavir (Song et al., 2011d), which are known Pgp and BCRP inhibitors.

In a human mass balance study where healthy subjects received 20 mg of [14C]DTG, 31.6% of the total oral dose was excreted in the urine, represented mainly by an ether glucuronide (M2, 18.9% of total dose), N-dealkylation (M1, 3.6% of total dose), and a metabolite formed by oxidation at the benzylic carbon (M3, 3.0% of total dose) (unpublished data). Fecal elimination contributed an additional 3.1%, resulting in approximately 10% of the metabolism of DTG being represented by oxidative products. On the basis of the fraction of the dose renally eliminated and the CLi values determined from in vitro UGT and CYP3A4 studies, the fraction of DTG metabolized by UGT1A1 (fM,UGT1A1) and CYP3A4 (fM,CYP3A4) is estimated to be 0.51 and 0.21, respectively. The fraction of DTG metabolized by UGT1A3 and 1A9 is estimated at 0.028 and 0.055, respectively. It should be noted that the estimates of fM,UGT may be underestimated due to the instability of the ether glucuronide in feces, as the conjugate may hydrolyze and convert back to parent drug under these conditions. Since DTG is a substrate for UGT1A1 and CYP3A4, pharmacokinetic interactions involving coadministration of a UGT1A1 or CYP3A4 inhibitor or inducer could perturb the pharmacokinetics of DTG. Based on the fraction of DTG metabolized, an interaction with UGT1A1 and CYP3A4 inhibitors may be expected if both pathways are fully inhibited (combined fM = 0.72). Ritonavir and atazanavir are HIV PIs that have been coadministered with DTG (Song et al., 2011a).

Both are potent CYP3A4 inhibitors and atazanavir is also a marked UGT1A1 inhibitor. Using a mechanistic static equation (see Materials and Methods) and the estimated fM above, the AUC of DTG was expected to increase by as much as 1.8- to 2.0-fold when coadministered with ritonavir-boosted atazanavir. Indeed, when DTG was co-dosed with 400 mg of atazanavir in a clinical study, there was a 1.9- and 1.5-fold increase in DTG AUC(0-t) and Cmax, respectively (Song et al., 2011a). When dosed with atazanavir/ritonavir (300/100 mg), the DTG AUC(0-t) and Cmax increased 1.62- and 1.34-fold. These data are consistent with UGT1A1 and CYP3A4 being the important pathways of DTG metabolism in vivo, with UGT1A3 and UG1A9 having minor roles in DTG clearance.

In contrast to inhibition of metabolic pathways, metabolic inducers such as rifampin, etravirine, efavirenz, and tipranavir need to be considered, as they are important agents in the treatment of HIV and other infectious diseases. It is also known that strong activators of human pregnane X receptor can lead to increased metabolic activity for UGT1A1, CYP3A4, and transporters (Chen et al., 2012). Induction of these pathways could increase the systemic clearance of DTG, resulting in lower exposures. Both etravirine and efavirenz reduce the AUC of DTG by 71% and 57%, respectively (Song et al., 2011b,c). This interaction with DTG is likely the combined effect of UGT and CYP3A4 induction. This is consistent with etravirine and efavirenz causing modest decreases (20–40%) in the pharmacokinetics of raltegravir, a sensitive UGT substrate (Kassahun et al., 2007; Iwamoto et al., 2008). Support for a role of CYP3A4 induction was demonstrated by coadministration of the potent CYP3A4 inhibitors lopinavir/ritonavir or darunavir/ritonavir that counteracted the decrease in DTG exposure by etravirine (Song et al., 2011c). Alternatively, the decreases in DTG exposure could also involve drug transporters. One interesting possibility is (modest) MRP2 inhibition leading to a partial disruption in the enterohepatic recirculation of DTG; both etravirine and efavirenz are inhibitors of MRP2 (Zembruski et al., 2011b). This has been reported as an explanation for the reduced exposure of mycophenolate mofetil when co-dosed with cyclosporine A (Hesselink et al., 2005).

At clinically relevant concentrations, DTG does not result in drug interactions by inhibition or induction of P450 enzymes or UGT1A1/2B7 inhibition. Although DTG demonstrated weak CYP3A4 MDI of

Fig. 2. Formation of DTG metabolites by recombinant UGT and human P450 enzymes. (A) Formation of DTG glucuronide metabolite (M2) in UDPGA-fortified recombinant human UGT enzyme and HLM incubations. [14C]DTG (16 µM) incubated for 120 min at 1 mg/ml HLM protein. (B) Effect of atazanavir on [14C]DTG glucuronidation in UDPGA-fortified HLMs. [14C]DTG (10 µM) was incubated with HLMs (1 mg/ml protein) for 120 min. (C) M1 and M3 formation in NADPH-fortified human P450 enzyme and HLM incubations. [14C]DTG (5 µM) was incubated for 120 min with 150 pmol/ml P450 enzyme or 2 mg/ml HLMs.
nifedipine and atorvastatin in vitro (approximately 1.6-fold reduction in IC<sub>50</sub>; Table 3), a clinical study demonstrated that DTG had no effect on midazolam’s pharmacokinetics (Min et al., 2010), confirming that DTG does not inhibit or induce CYP3A4 in vivo.

The DTG IC<sub>50</sub> values for all transporters examined were &gt;30 μM except for OCT2 (discussed below). These IC<sub>50</sub> values are higher than the peak plasma concentration of DTG following a 50-mg daily dose (3.45 μg/ml, ~8 μM) (Song et al., 2010), supporting the overall low potential for transporter-mediated drug interactions ([I<sub>1</sub>IC<sub>50</sub>] (Table 2) (Zhang et al., 2008; Giacomini et al., 2010). Further, the overall drug interaction risk is even lower due to the high plasma protein binding (&gt;99%) of DTG (unpublished data). Finally, calculation of inhibition of efflux transporters in the intestine following a 50-mg dose supports a limited risk of DDIs as the [I<sub>2</sub>/IC<sub>50</sub>] values for both Pgp and MRP2 are &lt;5 (Table 2) (Zhang et al., 2008; Giacomini et al., 2010). However, based on the [I<sub>2</sub>/IC<sub>50</sub>] value for BCRP likely being close to 10, there could be a small risk of inhibition of this transporter by DTG. Our findings are consistent with in vitro data reported by Lepist et al. (2011) indicating that DTG is a weak or noninhibitor of Pgp (IC<sub>50</sub> &gt; 90 μM), MRP2 (IC<sub>50</sub> &gt; 90 μM), and BCRP (IC<sub>50</sub> = 67 μM). Overall, the data support a low potential for DTG to cause clinically significant DDIs with other drugs such as simvastatin, rosuvastatin, and atorvastatin (OATP, BCRP, and P450 substrates), and metformin (OCT1/2 substrate). A clinical study with tenofovir, an OAT and MRP4 substrate (Ray et al., 2006), demonstrated no interaction of DTG on tenofovir pharmacokinetics (Song et al., 2010), suggesting that DTG is not an inhibitor of these transporters in vivo.

In contrast to the other transporters discussed, DTG causes notable in vitro inhibition of the renal transporter OCT2 (IC<sub>50</sub> = 1.9 μM). As this value is 4-fold lower than the C<sub>max</sub> of DTG ([I<sub>1</sub>/IC<sub>50</sub>] = 4.2), a potential interaction exists for compounds that undergo renal secretion via OCT2, such as circulating serum creatinine (a product of muscle turnover) and the anti-arrhythmic drug dofetilide. The in vitro OCT2 inhibition data are consistent with the mild 10–14% decreases in serum creatinine clearance observed in patients administered DTG (Koteff et al., 2011; van Lunzen et al., 2012). These mild, reversible changes are similar to other clinical observations with OCT/MATE inhibitors such as dronedarone, AZD0837, and DX-619 (Sarapa et al., 2007; Tschuppert et al., 2007; Schutzer et al., 2010; Imamura et al., 2011). A clinical study confirmed that the changes in creatinine were not due to DTG altering renal blood flow or renal clearance; therefore,

![Fig. 3. Michaelis-Menten kinetic plots for DTG glucuronidation. HLMs (A) and human (B) UGT1A1 enzyme incubations were conducted for 120 min with 1 mg/ml microsomal protein. Single incubations were run for 25 and 50 μM concentrations of DTG in UGT1A1 incubations only.](image-url)

![Fig. 4. Metabolic scheme of DTG.](image-url)
the changes in creatinine serum levels are likely due to the inhibition of the OCT2 transporter (Schutzer et al., 2010; Koteff et al., 2011; Kusuhara et al., 2011). Caution with concomitant dosing of DTG and dofetilide, a narrow therapeutic index class III antiarrhythmic agent, is warranted, as OCT2 inhibition can significantly reduce its renal clearance as shown by a clinical interaction with cimetidine (Roukoz and Saliba, 2007). However, there is less concern for other OCT substrates, such as metformin, as these drugs have larger therapeutic windows compared with dofetilide. In addition, the lack of significant inhibition of OCT1 allows transport of metformin to the liver, its site of action, to maintain its pharmacological effect.

In conclusion, these in vitro studies reveal the low propensity of DTG to cause drug interactions through metabolic or transporter pathways, consistent with the lack of clinical interactions observed to date with DTG. Further, these studies provide a mechanistic explanation for the DDIs observed with DTG when coadministered with CYP, UGT, and transporter inhibitors and/or inducers. As a result of the multiple clearance pathways of DTG, few clinically significant alterations in DTGs pharmacokinetics have been observed that require a dose adjustment. This is consistent with the current safety profile of DTG in humans.

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In Vitro Investigations with Dolutegravir