Short Communication

Glucuronidation of the Antiretroviral Drug Efavirenz by UGT2B7 and an In Vitro Investigation of Drug-Drug Interaction with Zidovudine

Received March 26, 2009; accepted May 28, 2009

ABSTRACT:

The non-nucleoside reverse transcriptase inhibitor efavirenz (EFV) is directly conjugated by the UDP-glucuronosyltransferase (UGT) pathway to form EFV-\(\beta\)-glucuronide (EFV-G), but the enzyme(s) involved has not yet been identified. The glucuronidation of EFV was screened with UGT1A and UGT2B enzymes expressed in a heterologous system, and UGT2B7 was shown to be the only reactive enzyme. The apparent \(K_m\) value of UGT2B7 (21 \(\mu\)M) is similar to the value observed for human liver microsomes (24 \(\mu\)M), whereas the variant allozyme UGT2B7*2 (Tyr268) displayed similar kinetic parameters. Because 3'-azido-3'-deoxythymidine (AZT), one of the most current nucleotide reverse transcriptase inhibitors prescribed in combination with EFV, is also conjugated by UGT2B7, the potential metabolic interaction between EFV and AZT has been studied using human liver microsomes. Glucuronidation of both drugs was inhibited by one another, in a concentration-dependent manner. At \(K_m\) values (25 and 1000 \(\mu\)M for EFV and AZT, respectively), EFV inhibited AZT glucuronidation by 47\%, whereas AZT inhibited EFV glucuronidation by 23\%. With a \(K_i\) value of 17 \(\mu\)M for AZT-glucuronide formation, EFV appears to be one of the most selective and potent competitive inhibitors of AZT glucuronidation in vitro. Moreover, assuming that concentrations of EFV achieved in plasma (\(C_{\text{max}} = 12.9 \mu\)M) are in a range similar to its \(K_i\) value, it was estimated that EFV could produce a theoretical 43\% inhibition of AZT glucuronidation in vivo. We conclude that UGT2B7 has a major role in EFV glucuronidation and that EFV could potentially interfere with the hepatic glucuronidation of AZT.

Efavirenz ([EFV] (Sustiva); Bristol-Myers Squibb Co., Princeton, NJ) is the prefered non-nucleoside reverse transcriptase inhibitor (NNRTI) agent for initial therapy for HIV infection. A typical antiretroviral treatment regimen consists of two nucleoside reverse transcriptase inhibitors (NRTIs) plus a protease inhibitor or an NNRTI. The 2008 IAS-USA guidelines recommend either of two basic three-drug regimens for treatment of antiretroviral-naïve patients: 1) EFV plus two NRTIs; or 2) a ritonavir-boosted protease inhibitor (lopinavir, atazanavir, fosamprenavir, darunavir, or saquinavir) plus two NRTIs [tenofovir and emtricitabine; abacavir and lamivudine; or 3'-azido-3'-deoxythymidine (zidovudine; AZT) and lamivudine] (Hammer et al., 2008). A recent study on HIV-infected adults of Southern Africa further revealed that among patients who began nevirapine-based or EFV-based antiretroviral therapy between January 1998 and September 2004, 1321 of 1822 EFV-treated patients

this work was supported in part by the National Institutes of Health National Institute of Allergy and Infectious Diseases Extramural Activities [Grant AI36219 (to the Center for AIDS Research, Developmental Awards, Case/University Hospitals of Cleveland, Cleveland, OH) (to R.K.M. and C.G.); A.-S.B. was supported by a Canada Graduate Scholarship Doctoral Research Award from the Canada Institutes of Health Research; and C.G. is the holder of a Canada Research Chair in Pharmacogenomics.

Article, publication date, and citation information can be found at http://dmd.aspetjournals.org.


[1] The online version of this article (available at http://dmd.aspetjournals.org) contains supplemental material.

ABBREVIATIONS: EFV, efavirenz; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; AZT, zidovudine; UGT, UDP-glucuronosyltransferase; EFV-G, EFV-\(\beta\)-glucuronide; AZT-G, AZT-glucuronide; HLM, human liver microsomes.

1793
metabolism through the glucuronidation pathway (di Iulio et al., 2009). The enzyme(s) involved in N-glucuronidation pathway of EFV has not yet been characterized.

AZT, one of the most common NRTI prescribed in combination with EFV, is also predominantly metabolized by UGTs, to an inactive 5′-O-glucuronide metabolite (AZT-glucuronide; AZT-G). In humans, this enzymatic reaction is catalyzed exclusively by UGT2B7 (Barbier et al., 2000). The assessment of potential drug interactions at the metabolism level thus becomes essential considering that EFV and AZT are frequently used in combination and that they both undergo glucuronidation.

The initial objective of our study was to identify the UGT enzyme(s) involved in the metabolism of the active parent drug EFV. Data indicate that UGT2B7 is the only UGT isoform tested producing EFV-G in vitro, displaying glucuronidation activity similar to that observed with human liver microsomes (HLM). Accordingly, we then sought to investigate potential interaction with AZT, primarily in HLM. Results indicate that EFV could potentially interfere with hepatic glucuronidation of AZT.

Materials and Methods

Chemicals and Reagents. AZT (3′-azido-3′-deoxythymidine) and β-glucuronidase from *Escherichia coli* Type VII were obtained from Sigma-Aldrich (St. Louis, MO), and EFV ([S]-6-chloro-4-(cyclopropylmethyln)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one) was obtained from NIH AIDS Research and Reference Reagent Program (Germantown, MD). AZT-G, AZT-d4-G, and EFV-d4-G (racemic) were produced from in vitro enzymatic assays, purified on Stata X cartridges (60 mg; Phenomenex, Torrance, CA), and quantified on an EFV calibration curve following β-glucuronidase (25,000 units/ml) treatment.

High-Performance Liquid Chromatography Tandem Mass Spectrometry. The separation of EFV and AZT was performed onto a Gemini C18 column 3 μm packing material, 100 × 4.6 mm (Phenomenex). Isocratic condition with 90% methanol/10% water/0.5 mM ammonium formate with a flow rate of 0.9 ml/min was used to elute AZT-G, and for EFV-G the isocratic condition was 80% methanol/20% water/0.4 mM ammonium formate with a flow rate of 0.9 ml/min. The glucuronides were quantified by tandem mass spectrometry (model API 3200; Biosystems-Sciex, Concord, ON, Canada). Negative-product multiple reaction monitoring ions pairs were m/z 442 → 124.9 for AZT-G, m/z 445 → 127.9 for AZT-d3-G, m/z 487.4 → 314.1 for EFV-G, and m/z 493.7 → 318.1 for EFV-rac-d4-G. The ion source temperature was set at 550°C. The ion spray voltage, declustering potential, and entrance potential were set at ~4200, ~50, and ~10 V. The collision energy for AZT-G and EFV-G were ~30 and ~25 V, respectively.

Enzymatic and Competition Assays. Human pooled liver microsomes (BD Biosciences, Woburn, MA) and recombinant UGT1A- and UGT2B-HEK293 mesosomal proteins were used in enzymatic assays using a standard procedure (Villeneuve et al., 2003). Initial screening consisted of 16-h incubation at 37°C with 0.2 mM EFV or AZT. Kinetic parameters were then assessed for HLM as well as UGT2B7*1 and *2 allelic variants in the presence of increasing concentrations of EFV (varying from 1 to 75 μM) or AZT (ranging from 100 to 5000 μM) for 1-h incubation at 37°C. Absolute glucuronidation activities were corrected by UGT relative protein content and expressed as relative glucuronidation activities (pmol/min/mg). Eadie-Hofstee plots (V as a function of V/S) were used as visual inspection of fitted functions (V as a function of S) were used to select the best-fit enzyme kinetic model. Kinetic parameter calculations were performed with Sigma Plot 8.0 software assisted by Enzyme Kinetics 1.1 software (SPSS Inc., Chicago, IL). Inhibition constants (K_i) were assessed using AZT and EFV concentrations ranging from 100 to 5000 μM and 5 to 50 μM, respectively. K_i values were estimated using Dixon plots and by fitting the kinetic data into a competitive inhibition model using nonlinear regression analysis with the GraphPad software version 4.01 (GraphPad Software, Inc., San Diego, CA); v = V_max [S]/([S] + K_m/[1 + 1/K_i]). Values are expressed as the mean of at least two experiments performed in triplicate. Results were expressed as mean ± S.D. Statistical significance was calculated by paired Student’s t test. All tests were two sided.

Table 1

<table>
<thead>
<tr>
<th>Microsomes</th>
<th>Apparent K_m (μM)</th>
<th>Relative V_max (% of UGT2B7)</th>
<th>V_max (μmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UGT2B7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1 (His268)</td>
<td>3.5 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>*2 (Tyr268)</td>
<td>4.2 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>Human liver</td>
<td>2.5 ± 0.3</td>
<td>9.7 ± 1.2</td>
<td>0.32 ± 0.04</td>
</tr>
<tr>
<td>AZT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UGT2B7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1 (His268)</td>
<td>11.3 ± 2.2</td>
<td>1.5 ± 0.3</td>
<td>0.44 ± 0.06</td>
</tr>
<tr>
<td>*2 (Tyr268)</td>
<td>9.1 ± 1.2</td>
<td>1.3 ± 0.3</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>Human liver</td>
<td>11.4 ± 2.4</td>
<td>9.2 ± 1.1</td>
<td>0.15 ± 0.05</td>
</tr>
</tbody>
</table>

* P values <0.05 vs. UGT2B7*1.

Results and Discussion

Glucuronidation of EFV and AZT by Heterologous UGTs: A Main Role for UGT2B7. Assays with HLM demonstrated the formation of a single glucuronic acid conjugate for both drugs, EFV-G and AZT-G. Incubations with recombinant human UGT1A and UGT2B enzymes revealed that UGT2B7 is the only heterologous enzyme capable of producing EFV-G and AZT-G (Supplemental Fig. S1). The recombinant human UGT1A3, UGT1A8, UGT1A9, and UGT1A10 are also able to form EFV-G but with an apparent velocity at least 48-fold lower than UGT2B7. Hence, these data provide the first experimental evidence for a major role of UGT2B7 in EFV glucuronidation.

Kinetic analyses were then performed to compare human liver and UGT2B7 microsomes for the glucuronidation of EFV and AZT, and all kinetics were well described by the Michaelis-Menten equation (Table 1; Supplemental Fig. S2). Apparent K_m values for UGT2B7 and HLM are similar for EFV-G formation (24 and 21 μM for HLM and UGT2B7, respectively), which emphasizes the importance of UGT2B7 in EFV glucuronidation. The variants UGT2B7*1 (His268) and UGT2B7*2 (Tyr268) also displayed similar K_m values, and we observed no statistically different apparent velocities when normalized by UGT protein content. The codon 268 polymorphism was already shown not to have a functional significance for other drugs such as morphine and epirubicin (Coffman et al., 1998; Innocenti et al., 2001). Whether other coding polymorphisms result in impaired catalytic activity toward EFV is still unknown, whereas several promoter variants were reported to alter expression of UGT2B7 (Duguay et al., 2004). Kinetics for AZT were comparable to previous reports (Barbier et al., 2000; Uchaipichat et al., 2008), and a 2.3-fold reduced apparent velocity of AZT-G formation was observed for UGT2B7*2 compared with UGT2B7*1 (Supplemental Fig. S2), in accordance with the previous study of Barbier et al. (2000).
Evidence of a Potential EFV-AZT Interaction. Because UGT2B7 is primarily involved in conjugation of EFV and AZT, frequently coadministered in HIV patients, we investigated whether these drugs may interfere with each other’s conjugation in HLM. A marked decrease in the AZT-G formation by EFV was shown to be dose dependent. At 5000 μM AZT, the mean of AZT glucuronidation was reduced to 21% of the control rate by 25 μM EFV, and this reduction reached 57% for 100 μM AZT. At K_i values of both drugs (25 and 1000 μM for EFV and AZT, respectively), EFV inhibited AZT glucuronidation by 47%, whereas AZT inhibited EFV glucuronidation by 23%. The apparent K_i values estimated from Dixon plots were 2700 μM and 47,000 μM for inhibition of EFV glucuronidation by AZT and 17 μM for inhibition of AZT glucuronidation by EFV (Fig. 1).

Using the simultaneous nonlinear regression method, a K_i of 3902 ± 211 μM was derived for AZT on EFV glucuronidation and of 16.8 ± 1.2 μM for EFV on AZT glucuronidation, which is consistent with the K_i values independently determined from the competition study. For the inhibition of AZT upon EFV-G, K_i > K_m indicate that the affinity of UGT2B7 for EFV is greater than for AZT, in accordance with the kinetic described above. It is noteworthy that previous K_i values for AZT glucuronidation inhibition ranged from 38 μM [ethinylestradiol (Herber et al., 1992)] to 47 000 μM [sulfisoxazole (Resetar et al., 1991)]. Moreover, kinetic experiments in the absence of bovine serum albumin as performed herein, overestimate K_m and underestimate true K_i, particularly for substrates of UGT2B7 (Rowland et al., 2007).

Based on these observations, EFV would then appear to be in the lowest range of competitive inhibitors for AZT glucuronidation in vitro.

At steady-state, after an AZT oral dose of 200 mg twice daily, the maximum concentration achieved in plasma is approximately 1.2 μg/ml (4.5 μM) with a relatively short half-life (0.5–3 h) (Hoetelmans, 1999). For EFV, oral administration of 600 mg daily has been reported to produce mean steady-state C_max of 12.9 μM, drug concentrations ranging from 125 to 15,230 μg/l (median 2188 μg/l, 0.4–48 μM), and a half-life of >40 h (Marzolini et al., 2001). Because AZT plasma concentration is far less than its K_i value of 3902 μM, an interaction on EFV glucuronidation in the presence of AZT is unlikely. Conversely, considering that the pharmacological drug concentration of EFV and its apparent K_i (16.8 μM; assessed herein) are in the same range (ratio [I]/K_i value of 0.77) (Tucker et al., 2001), an inhibition of EFV on UGT2B7-mediated AZT glucuronidation is possible, but it remains to be demonstrated in vivo. According to in vitro data, we estimated that the competitive inhibitor EFV could reduce AZT glucuronidation by approximately 43% at steady-state maximum concentrations (see Materials and Methods). The magnitude of the potential EFV inhibition of AZT hepatic clearance was predicted based on the increase in the AUC ratio at the maximum inhibitor concentration in plasma (|EFV|_max = 12.9 μM) and average inhibitor concentration in plasma (|EFV|_ave = 5.6 μM and |EFV|_ave = 12.9 μM, so |EFV|_ave = (5.6 + 12.9)/2 = 9.25 μM (EFV)). Because glucuronidation is mainly responsible for hepatic clearance of AZT, f_m (the fraction of the metabolic process subject to inhibition) was taken as 1. The predicted in vivo AUC ratio of AZT is 1.75 at |EFV|_max and 1.54 at |EFV|_ave, meaning that EFV could perhaps increase AZT AUC upon concurrent use. The maximum unbound EFV (inhibitor) concentration in plasma was also used to predict the magnitude of the interaction between EFV and AZT in vivo. However, because EFV is highly bound (approximately 99.5–99.75%) to human plasma proteins, only calculations that incorporated the total EFV concentration predicted a clinically significant interaction. This prediction is only a rough estimate of the potential drug interactions in vivo given that several factors might influence drug interaction in this context, such as the presence of compensatory pathways, extrahepatic metabolism, or extensive binding to plasma proteins. Drugs that are at the greatest risk of having a high (>5) AUCi/AUC ratio are high extraction drugs metabolized by a single enzyme, simultaneously presented to the liver along with an inhibitor in which the ratio of liver concentration ([I]/K_i) is high (Williams et al., 2004). AZT is a good example, with a high hepatic extraction and a metabolism catalyzed specifically by UGT2B7. Therefore, the potential for a high AUCi/AUC ratio would be elevated, given a high [I]/K_i ratio of a coadministered inhibitor. We showed that EFV has a strong in vitro inhibitory effect upon UGT2B7-mediated AZT glucuronidation, making the EFV-AZT interaction possible. Moreover, in spite of the high glucuronidation capacity of UGT2B7, there is literature evidence that shows that the glucuronidation activity of this enzyme can be limited by drug-drug interaction. An example is the observed 2-fold increase in AUC of AZT with concomitant valproic acid.
acid administration (Lertora et al., 1994). Moreover, an increase of 225% in the AZT drug plasma concentration was reported with the coadministration of EFV (U.S. prescribing information, http://packageinserts.bms.com/pi/pi_sustiva.pdf).

To the best of our knowledge, the clinical significance of variation in the EFV glucuronidation pathway has not yet been studied. Conversely, the importance of polymorphisms in the gene that encodes the hepatic enzyme primarily responsible for EFV oxidation (CYP2B6) has been the subject of intense research (for a review, see Rodríguez-Novoa et al., 2006). As an example, a study observed that carriers of a CYP2B6 polymorphism (the variant G516T) have greater plasma EFV exposure during the first 24 weeks of antiretroviral therapy, and they experienced frequent central nervous system-related side effects during the first week of treatment (Rodríguez-Novoa et al., 2005).

Moreover, a more recent investigation also concludes that dosage adjustment in accordance with the type of polymorphism (CYP2B6, CYP2B6 CYP3A4) is required to maintain EFV within the therapeutic target levels (Arab-Alameddine et al., 2009). In addition to cytochrome P450s, other polymorphic enzymes such as UGT2B7 may contribute to interindividual variability of EFV plasma levels.

ACKNOWLEDGMENTS. We thank Lyne Villeneuve for technical assistance.

Pharmacogenomics Laboratory, Centre Hospitalier Universitaire de Québec Research Center and Faculty of Pharmacy, Laval University, Quebec, Canada (A.-S.B., P.C., M.H., C.G.) and Center for Global Health and Diseases, Case Western Reserve University School of Medicine, Cleveland, Ohio (P.A.Z. and R.K.M.)

References


Address correspondence to: Dr. Chantal Guillemette, Pharmacogenomics Laboratory, CHUQ Research Center, 2705, boul. Laurier, T3-48, G1V 4G2, Québec, Canada. E-mail: chantal.guillemette@crchul.ulaval.ca