Efflux Transport Is an Important Determinant of Ethinylestradiol Glucuronide and Ethinylestradiol Sulfate Pharmacokinetics

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ABSTRACT:

17α-Ethinylestradiol (EE) undergoes extensive conjugation to 17α-ethinylestradiol-3-O-glucuronide (EEG) and 17α-ethinylestradiol-3-O-sulfate (EES). Thus, oral contraceptive drug-drug interaction (DDI) studies usually characterize metabolite pharmacokinetics, with changes typically attributed to modulation of metabolism. EE passively diffuses through plasma membranes, but its conjugates are hydrophilic and require active transport. Unlike EE metabolism, EEG and EES transport has not been explored in vivo as a potential mechanism of DDIs. Recent in vitro studies demonstrated that EEG is transported by multidrug resistance-associated protein (MRP) 2 and MRP3 and EES is a breast cancer resistance protein (BCRP) substrate. In the study presented here, pharmacokinetics of EE and conjugates were studied in TR− rats, which lack Mrp2, have marginal hepatic Bcrp expression, and overexpress hepatic Mrp3. EE pharmacokinetics in TR− rats were comparable to wild type; however, EEG and EES systemic exposures were altered markedly. EEG exposure was greatly increased: 20-fold and >100-fold after intravenous and oral EE administration, respectively. In contrast, EES exposure was lower in TR− rats: 65% decreased (intravenously) and 83% decreased (orally). In intestinal and liver perfusions, EE intestinal permeability and metabolism and hepatic clearance were unchanged in TR− rats; however, secretion of EEG into intestinal lumen was halved, EEG was not detected in TR− bile, and EES biliary excretion was 98% decreased. After oral EE administration to Mrp2- and Bcrp-knockout mice, EEG exposure increased 46- and 2-fold, respectively, whereas EES concentrations were decreased modestly. In conclusion, altered efflux transport resulted in major alterations of EEG and EES pharmacokinetics, highlighting transport as a potential site of DDIs with EE conjugates.

Introduction

17α-Ethinylestradiol (EE) is an estrogen used in combination oral contraceptive pills, making it a highly relevant concomitant medication in women of child-bearing potential. As such, EE interaction studies are a common component of the biopharmaceutical package for new chemical entities. EE is extensively metabolized by intestinal and hepatic sulfation via sulfotransferase 1E1, glucuronidation via UDP-glucuronosyltransferase 1A1, and 2-hydroxylation by CYP3A4 and CYP2C9 (Guengerich, 1990; Zhang et al., 2007). Phase II metabolism of EE to 17α-ethinylestradiol-3-O-sulfate (EES) and 17α-ethinylestradiol-3-O-glucuronide (EEG) is extensive (Maggs et al., 1983b); clinical EES exposures exceed that of parent EE by at least an order of magnitude (Back et al., 1980). EES and EEG can undergo enzymatic deconjugation to parent EE by sulfatase and glucuronidase enzymes in vitro; thus, conjugation of EE is theoretically not an irreversible route of elimination, but in vivo in rats and humans extensive deconjugation was not evident (Maggs et al., 1983a,b). Because of high systemic exposure to EE conjugates, at least EES pharmacokinetics are typically studied along with parent EE in oral contraceptive interaction studies.

EE and its conjugates differ vastly in their permeability and transport properties. EE is lipophilic and exhibits high passive membrane permeability (Zhang et al., 2007). EES and EEG are hydrophilic and require active transport into and out of cells (Chu et al., 2004; Han et al., 2010a,b). Alterations in EES or EEG pharmacokinetics have traditionally been attributed to modulation of EE sulfation or glucuronidation by the coadministered drug (Rogers et al., 1987; Zhang et al., 2007). The hypothesis of the studies presented here was that perturbations in intestinal, hepatic, and/or renal transport of EES and EEG will result in altered pharmacokinetics of these metabolite(s), which may in turn affect parent EE pharmacokinetics if appreciable metabolite deconjugation occurs in vivo.

Transport of EES has been thoroughly characterized in vitro. These studies indicated that EES was a substrate of the efflux transporter BCRP, but not MRPs 1 to 4, bile salt export pump, P-glycoprotein, or multidrug and toxin extrusion 1 protein (Chu et al., 2004; Han et al., 2010a,b). BCRP is localized on the apical membrane of enterocytes, hepatocytes, and the renal proximal tubule (International Transporter Consortium, 2010). BCRP is an efflux pump for sulfate conjugates, which are generally too hydrophilic to passively diffuse out of their sites of formation, mainly hepatocytes and enterocytes (Zamek-Gliszczynski et al., 2006b,d). In addition, EES transport by various uptake transporters was evaluated in vitro; these studies demonstrated EES to be a substrate of organic anion transporting polypeptides 1B1 and 3A4.

ABBREVIATIONS: EE, 17α-ethinylestradiol; EEG, 17α-ethinylestradiol-3-O-glucuronide; EES, 17α-ethinylestradiol-3-O-sulfate; BCRP, breast cancer resistance protein; MRP, multidrug resistance-associated protein; IV, intravenous; PK, pharmacokinetics; AUC, area under the curve; Cl, clearance.
and 2B1, organic anion transporters 3 and 4, and Na(+)-taurocholate cotransporting polypeptide, but not organic anion transporter 1 or organic cation transporter 2 (Han et al., 2010a,b).

In contrast, EEG was transported by the efflux transporters MRP2 and MRP3 in vitro, but it was not a substrate of MRP1 (Chu et al., 2004). MRP2 and MRP3 are located on the apical and basolateral membranes, respectively, of enterocytes and excrete glucuronide conjugates formed in the intestinal wall into the lumen and mesenteric blood (Murakami and Takanou, 2008). Glucuronidation occurs primarily in the liver, where MRP2 and MRP3 are responsible for the subsequent excretion of glucuronide conjugates from the liver into bile and blood, respectively (Zamek-Gliszczynski et al., 2006a,b,c).

Efflux transporters responsible for the excretion of EES and EEG from their sites of formation, hepatocytes and enterocytes, have been identified in vitro. However, the potential effect of modulation of these pathways on the pharmacokinetics of parent EE and its metabolites remains to be elucidated in vivo. In the study presented here, pharmacokinetics of EE and conjugates were studied in TR− rats, which lack Mrp2, have marginal hepatic Bcrp expression, and over-express hepatic Mrp3 (Xiong et al., 2002; Johnson et al., 2006; Yue et al., 2011). TR− rats have been extensively used to study the effect of efflux transport on pharmacokinetics of phase II conjugates (Zamek-Gliszczynski et al., 2006b). In vivo observations in TR− rats were further investigated using liver and intestinal perfusions as well as Mrp2- and Bcrp-knockout mice.

Materials and Methods

Chemicals. EE, estrone-3-sulfate, β-estradiol-17β-3-glucuronide, and taurocholate were purchased from Sigma-Aldrich (St. Louis, MO). EES and EEG were obtained from Steraloids (Newport, RI). D4-EE was purchased from Toronto Research Chemicals Inc. (North York, ON, Canada). All other chemicals were of reagent grade and were readily available from commercial sources.

Animals. Male Wistar wild-type and TR− rats were purchased from Harlan (Indianapolis, IN). For in vivo rat pharmacokinetic experiments, femoral vein and femoral artery cannulations were performed by Harlan before animal shipment to the study site. Male C57BL/6 wild-type, Abcc2(+/−), and Abcc2(−/−) mice were obtained from a proprietary colony maintained for Eli Lilly & Co. by Taconic Farms (Germantown, NY). Abcc2(+/−) and Abcc2(−/−) mice were generated as previously described by Zamek-Gliszczynski et al. (2006d). The Institutional Animal Care and Use Committee at Eli Lilly & Co. approved all animal procedures.

In Vivo Pharmacokinetics. EE was dissolved in microemulsion oil phase and then diluted in deionized water (1:4, v/v) for oral and intravenous (IV) administration (1 ml/kg). Rats received a 0.5 mg/kg EE dose by oral gavage or intravenous injection via femoral vein cannula. Blood samples were collected via the femoral artery cannula at the following times: 0.04 (IV only), 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 h postdose. Mice received a 5 mg/kg EE dose by oral gavage or tail vein injection. Blood samples were collected at the following times: 0.08 (IV only), 0.25 (oral only), 0.5, 1, 2, 3, 4, 6, 8, and 12 h postdose. Three time points were collected per mouse: the first two were retro-orbital bleeds and the third blood draw was by cardiac puncture. As such, the mouse time course is a composite of three groups of mice.

Rat Intestinal Perfusion. All experimental procedures were performed under full anesthesia induced by inhaled isoflurane (1:1:1 v/v/v) for oral and intravenous (IV) administration (1 ml/kg). Rats received a 0.5 mg/kg EE dose by oral gavage or intravenous injection via femoral vein cannula. Blood samples were collected via the femoral artery cannula at the following times: 0.04 (IV only), 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 10 h postdose. Mice received a 5 mg/kg EE dose by oral gavage or tail vein injection. Blood samples were collected at the following times: 0.08 (IV only), 0.25 (oral only), 0.5, 1, 2, 3, 4, 6, 8, and 12 h postdose. Three time points were collected per mouse: the first two were retro-orbital bleeds and the third blood draw was by cardiac puncture. As such, the mouse time course is a composite of three groups of mice.

Results

EE plasma concentration-time profiles in Wistar wild-type and TR− rats after intravenous or oral administration of a 0.5 mg/kg dose
are presented in Fig. 1; the corresponding noncompartmental PK parameters are summarized in Table 1. EE systemic pharmacokinetics in TR− rats were comparable to wild type, as evidenced by superimposable concentration-time profiles and comparable PK parameters. EE clearance was high (5 l/h/kg) and approximately equal to hepatic blood flow (Davies and Morris, 1993). EE was well distributed (volume ~2 l/kg). After intravenous administration, the terminal half-life was fairly short (~1 h). Oral absorption was rapid, with Ttmax ranging between 0.25 and 0.5 h in individual animals; however, bioavailability in rats was very low (~1%). After oral administration, the terminal half-life was significantly higher in TR− rats (1.7 ± 0.5 versus 0.8 ± 0.1 h, p = 0.04); however, this difference is inconsistent with similar EE clearance, volume, intravenous half-life, and oral bioavailability in TR− rats, thus this statistically significant difference is considered physiologically inconsequential.

EEG systemic exposure was strikingly higher in TR− rats; the magnitude of the increase was greater after oral EE administration (Fig. 2; Table 1). After IV administration of EE, EEG exposure was approximately 20-fold and Cmax was approximately equal to hepatic clearance of EE between livers from wild-type and TR− rats; EEG Ttmax was again delayed (0.25 versus 0.875 h). Terminal EEG half-life after oral EE administration was not significantly different but was on average 3- to 4-fold longer in wild-type rats. This observation may be a result of comparing the β-elimination phase in wild-type rats to the α phase in TR− rats.

In contrast to EEG, EES systemic exposure was decreased in TR− rats (Fig. 3; Table 1). After IV administration of EE, EES exposures were 65% lower and Cmax was 89% reduced in TR− rats. After oral administration of EE, EES exposure and Cmax were an order of magnitude lower in TR− rats, although these differences failed to meet statistical significance because of high variability in EES PK parameters in wild-type rats. Unlike EEG, EES Ttmax and half-life were not affected in TR− rats after IV and oral administration of EE. EES Ttmax and half-life values were comparable to parent EE.

Potential differences in intestinal first-pass metabolism and hepatic clearance of EE were studied in perfused jejuna and livers from Wistar wild-type and TR− rats (Tables 2 and 3). Intestinal absorption of EE was not altered in TR− rat jejuna. Approximately half of the EE absorbed from the lumen was metabolized during intestinal transit before reaching mesenteric blood. Likewise, no differences were noted in hepatic clearance of EE between livers from wild-type and TR− rats. In perfused livers, EE clearance was high (28.6 ± 0.3 ml/min), approaching the perfusion rate (30 ml/min). The high hepatic extraction ratio and ~50% first-pass intestinal metabolism are consistent with the high in vivo clearance and very low oral bioavailability in rats.

Despite comparable EE intestinal first-pass metabolism and hepatic clearance, differences were noted in metabolite excretion in intestinal and liver perfusions. The rate of EEG lumenal excretion in jejuna from TR− rats was reduced approximately 50% relative to wild-type rats (Table 2). EES concentrations in lumenal and mesenteric perfusate were below the limit of quantification and are not reported. EEG was not detected in bile from TR− rat livers. Biliary excretion of EES was approximately 98% lower in TR− rats compared to wild-type rats. In addition, EEG half-life was fairly short (~0.5 h) in both wild-type and TR− rats, thus this statistically significant difference is considered physiologically inconsequential.

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**FIG. 1.** EE plasma concentration-time profiles after intravenous (A) or oral (B) administration of a single 0.5 mg/kg EE dose to Wistar wild-type (●, IV; ○, oral) or TR− (▲, IV; △, oral) rats.
tion was rapid (T\textsubscript{max} = 0.25–0.5 h) and bioavailability was low (5–9%); mouse IV clearance of EE was high (2.2–3.4 l/h/kg). Knockout mice were comparable to wild type, although significance of a 5 mg/kg EE dose are presented in Fig. 4. EE pharmacokinetics in Mrp2-knockout mice, (Table 3).

livers with a corresponding 97% reduction in EES biliary clearance (Table 3).

Composite plasma concentration-time profiles (three groups of five mice, n = 5 per time point) of EE, EEG, and EES in C57BL/6 wild-type, Mrp2-, and Bcrp-knockout mice after oral administration of a 5 mg/kg EE dose are presented in Fig. 4. EE pharmacokinetics in knockout mice were comparable to wild type, although significance testing was not performed on composite parameters. EE oral absorption was rapid (T\textsubscript{max} = 0.25–0.5 h) and bioavailability was low (5–9%); mouse IV clearance of EE was high (2.2–3.4 l/h/kg). Knockout-to-wild-type mouse exposure ratios of EEG and EES after oral or intravenous EE administration are summarized in Table 4. EEG concentrations were markedly elevated in Mrp2-knockout mice (Chu et al., 2004; Han et al., 2010a,b). However, the extent to which transport determines the in vivo pharmacokinetics of EE and its conjugates has not been directly evaluated. In the studies presented here, the in vivo importance of Mrp2, Mrp3, and Bcrp, which were identified in vitro as efflux transporters of EEG or EES, was studied in relevant mutant rats and knockout mice (Chu et al., 2004; Han et al., 2010a,b). These studies demonstrated that EEG and EES systemic pharmacokinetics can be markedly affected by efflux transport modulation. The observation of increased EEG systemic exposures in TR\textsuperscript{−} rats, which lack Mrp2, have marginal Bcrp expression, and overexpress hepatic Mrp3 (Xiong et al., 2002; Johnson et al., 2006; Yue et al., 2011), is consistent with numerous previous studies of glucuronide conjugates in this animal model (Zamek-Gliszczynski et al., 2006b); however, the large magnitude of the increase (>100-fold) was surprising. Mrp2 was necessary for EES biliary excretion and was also important in its secretion into the intestinal lumen. Because hepatic basolateral Mrp3 expression is increased an order of magnitude in

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**Discussion**

Previous in vitro studies demonstrated that unlike highly permeable parent EE, EEG and EES require active transport for uptake and excretion from cells (Chu et al., 2004; Zhang et al., 2007; Han et al., 2010a,b). However, the extent to which transport determines the in

\begin{table}[h!]
\centering
\caption{Intestinal perfusion parameters for EE, EEG, and EES}
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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild-Type Rat Jejunum</th>
<th>TR\textsuperscript{−} Rat Jejunum</th>
</tr>
</thead>
<tbody>
<tr>
<td>EE jejunal metabolism (%)</td>
<td>60 ± 10</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>EE jejunal permeability (10\textsuperscript{−6} cm/s)</td>
<td>40.8 ± 5.6</td>
<td>40.8 ± 5.2</td>
</tr>
<tr>
<td>EE influx (jejunum) (ng/min)</td>
<td>6.20 ± 1.61</td>
<td>4.43 ± 0.29</td>
</tr>
<tr>
<td>EE efflux (blood) (ng/min)</td>
<td>2.43 ± 0.47</td>
<td>2.49 ± 0.21</td>
</tr>
<tr>
<td>EEG efflux (lumen) (ng-eq/min)</td>
<td>0.90 ± 0.19</td>
<td>0.48 ± 0.10*</td>
</tr>
<tr>
<td>EES efflux (blood) (ng-eq/min)</td>
<td>1.96 ± 0.31</td>
<td>1.32 ± 0.33</td>
</tr>
<tr>
<td>EES efflux (lumen) (ng-eq/min)</td>
<td>N.D.</td>
<td>N.D.</td>
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N.D., not detected.
*p < 0.05.

**Fig. 2.** EEG plasma concentration-time profiles after intravenous (A) or oral (B) administration of a single 0.5 mg/kg EE dose to Wistar wild-type (○, IV; △, oral) or TR\textsuperscript{−} (▲, IV; △, oral) rats.

**Fig. 3.** EES plasma concentration-time profiles after intravenous (A) or oral (B) administration of a single 0.5 mg/kg EE dose to Wistar wild-type (○, IV; △, oral) or TR\textsuperscript{−} (▲, IV; △, oral) rats.
TR⁻ rats (Xiong et al., 2002), EEG is efficiently excreted into blood instead of bile. In intestinal perfusions, mesenteric excretion of EEG was not increased despite decreased luminal secretion, which is consistent with 2- to 4-fold down-regulation of intestinal Mrp3 in TR⁻ rats (Johnston et al., 2006). It is interesting to note that the magnitude of the increase in EEG exposure in TR⁻ rats (112-fold) was approximately equal to the product of the increase in Mrp2- (46-fold) and Bcrp-knockout (2.2-fold) mice. Substrate interactions of EEG with BCRP have not been evaluated to date, and BCRP, which is colocalized with MRP2 in the intestine and the liver, excreted some glucuronide conjugates from their site(s) of formation (Zamek-Gliszczynski et al., 2006d; International Transporter Consortium, 2010). The increased EEG exposure in Bcrp-knockout mice suggests that it is also transported by Bcrp, although its in vivo contribution was far less than that of Mrp2/3.

In contrast to EEG, EES systemic exposure was decreased (up to an order of magnitude) in TR⁻ rats. EES is a BCRP substrate (Han et al., 2010b), so these alterations are primarily attributed to the greatly decreased Bcrp expression and function in TR⁻ rats (Yue et al., 2011). In the studies presented here, EES biliary excretion in TR⁻ rat liver perfusions was negligible. The decrease in EES exposure was most notable after oral EE administration, suggesting that this is primarily a first-pass effect requiring intestinal contribution. Likewise in mice, the reduction was noted only after oral administration. Although Han et al. (2010b) and Chu et al. (2004) concluded that EES is not a substrate of MRP2, the Chu et al. (2004) study demonstrated a very low level of MRP2 transport activity, although it did not meet the prespecified substrate criteria. The MRP2-knockout mouse data presented here and the fact that the decrease in EES exposure was greater in TR⁻ rats than either Mrp2- or Bcrp-knockout mice alone suggest that EES may be a poor MRP2 substrate and that this transporter by itself has a very modest influence on EES pharmacokinetics. However, when Bcrp is largely absent in addition to the absence of Mrp2, the effect of the normally low contribution of Mrp2 becomes accentuated per fraction-excreted principles (Zamek-Gliszczynski et al., 2009; Kodaira et al., 2010). It is interesting to note that after intravenous EE administration to Bcrp-knockout mice, EES exposure was increased (3.3-fold) despite a decreased $C_{\text{max}}$. The discrepancy with oral data suggests that the noted decrease in EES concentrations is primarily a first-pass effect and that Bcrp may be important in vivo to EES renal clearance (Han et al., 2010a).

Despite large changes in EEG and EES exposure (1–2 orders of magnitude), parent EE pharmacokinetics were not altered in TR⁻ rats and in Mrp2- and Bcrp-knockout mice. Because EES and EEG can undergo enzymatic deconjugation to parent EE in vitro (Maggs et al., 1983b), it was hypothesized that changes in EEG and/or EES pharmacokinetics may result in altered parent EE exposure. Unchanged systemic EE pharmacokinetics during pronounced changes in conjugate exposure, as well as unaltered EE intestinal permeability, hepatic extraction, and parent biliary excretion in TR⁻ rats, do not support the above hypothesis and indicate that the deconjugation of EEG and EES in vivo is not extensive enough to affect EE systemic pharmacokinetics.
Transporter-knockout and mutant animal models are useful in evaluating the contribution of in vitro transport findings to in vivo pharmacokinetics, because specific and potent inhibitors, which elicit extensive inhibition of a single transport pathway, have not been identified (Zamek-Gliszczynski et al., 2009; International Transporter Consortium, 2010). However, differences were noted in EE pharmacokinetics between rodents and humans. In humans, EE clearance after intravenous administration was moderate, approximately 23% of hepatic blood flow (Humpel et al., 1979; Davies and Morris, 1993); in rodents EE clearance was high, approaching hepatic blood flow. Human oral bioavailability was high and variable, 20 to 65% (Zhang et al., 2007), whereas bioavailability in the rodent studies presented here was low (~1–9%). As expected from allometric principles, the rodent half-life of approximately 1 to 2 h was shorter than the average human half-life of approximately 17 h (Zhang et al., 2007). Clinically, EEG concentrations are far lower than parent EE, whereas clinical EES exposures exceed parent by at least an order of magnitude (Back et al., 1980). In rats, EEG exposures exceeded parent EE, whereas EES exposures were comparable to parent. In mice, EEG and EES exposures were lower than EE. Despite these species differences, the metabolite-to-parent ratios were higher in all cases after oral EE administration, which is consistent with extensive first-pass conjugation in humans (Zhang et al., 2007). Although knockout models are useful in hypothesis testing, they are not directly representative of EE disposition in humans.

A key skepticism associated with the use of knockout animal models involves the compensatory changes in metabolism and transport (International Transporter Consortium, 2010). However, TR- rats and the Mrp2- and Bcrp-knockout mice used in the studies presented here were thoroughly characterized with respect to their glucuronidation, sulfation, sulfatase, and uptake and efflux transport properties at the protein expression and functional levels. Similar or modestly increased hepatic glucuronidation and sulfation activities have been demonstrated with various substrates in TR- rats, although increased UDP-glucuronyltransferase (no change in intestine, no change to <4-fold in liver, and 5-fold in kidney) and sulfotransferase (<2-fold) protein expression has been reported in TR- rats (Xiong et al., 2000; Zamek-Gliszczynski et al., 2005, 2006a, 2008; Johnson et al., 2006). Glucuronidation and sulfation activities are notably not decreased in TR- rats. Hepatic sulfatase activity is also maintained in TR- rats (Zamek-Gliszczynski et al., 2006a). Except for Mrp3, which is up-regulated an order of magnitude, and Bcrp, the expression and function of which are marginal, no other major alterations in hepatic uptake or efflux transport have been reported (Zamek-Gliszczynski et al., 2003, 2006a; Hoffmann et al., 2005; Johnson et al., 2006; Yue et al., 2011). In TR- rats, renal Mrp3 protein expression is increased 3-fold, but intestinal Mrp3 is decreased 2- to 4-fold (Johnson et al., 2006). In the Mrp2- and Bcrp-knockout mice used in these studies, the hepatic sulfation/desulfation and glucuronidation activity for several substrates were maintained (Zamek-Gliszczynski et al., 2006d). Mrp2 and Mrp3 expression is not altered in the Bcrp-knockout mice (Nezasa et al., 2006). Likewise, Bcrp expression was not affected in the Mrp2-knockout mice; however, Mrp3 expression and function were increased 1.6- to 3-fold (Nezasa et al., 2006).

In conclusion, modulation of efflux transport resulted in major alterations in EEG and EES pharmacokinetics, highlighting transport as an important determinant of EE conjugate disposition and a potential mechanism of drug-drug interactions with these metabolites. The marked changes in EEG and EES exposure were not accompanied by altered EE systemic pharmacokinetics. The studies presented here underscore the need for interpretation of altered EEG or EES exposures with consideration of transport in addition to metabolism. Oral contraceptive interaction studies should routinely examine EEG pharmacokinetics in addition to EE and EES, especially with Mrp- and Bcrp-modulator drugs, because although EEG concentrations may be low in naive subjects, they could be increased orders of magnitude secondary to Mrp2 or Bcrp inhibition and/or Mrp3 induction. Reduced EES exposure may not be the result of sulfation inhibition, but it can also be observed secondary to the inhibition of EES efflux transport. Interpretation of future clinical drug interaction studies with consideration of transport will be enabled as in vitro evaluation of transport becomes routine in drug development (http://www.ema.europa.eu) (International Transporter Consortium, 2010).

Authorship Contributions

Participated in research design: Zamek-Gliszczynski, Day, Hillgren, and Phillips.

Conducted experiments: Zamek-Gliszczynski and Day.

Performed data analysis: Zamek-Gliszczynski and Day.

Wrote or contributed to the writing of the manuscript: Zamek-Gliszczynski.

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