Defining the Role of Gut Bacteria in the Metabolism of Deleobuvir: In Vitro and In Vivo Studies

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ABSTRACT

Deleobuvir is a potent inhibitor of the hepatitis C virus nonstructural protein 5B polymerase. In humans, deleobuvir underwent extensive reduction to form CD 6168. This metabolite was not formed in vitro in aerobic incubations with human liver microsomes or cytosol. Anaerobic incubations of deleobuvir with rat and human fecal homogenates produced CD 6168. Using these in vitro formation rates, a retrospective analysis was conducted to assess whether the fecal formation of CD 6168 could account for the in vivo levels of this metabolite. The formation of CD 6168 was also investigated using a pseudo-germ free (pGF) rat model, in which gut microbiota were largely eradicated by antibiotic treatment. Plasma exposure (area under the curve from 0 to ∞) of CD 6168 was approximately 9-fold lower in pGF rats (146 ± 64 ng h/ml) compared with control rats (1,312 ± 649 ng h/ml). Similarly, in pGF rats, lower levels of CD 6168 (1.5% of the deleobuvir dose) were excreted in feces compared with control rats (42% of the deleobuvir dose). In agreement with these findings, in pGF rats, approximately all of the deleobuvir dose was excreted as deleobuvir into feces (105% of dose), whereas only 26% of the deleobuvir dose was excreted as deleobuvir in control rats. These differences in plasma and excretion profiles between pGF and control rats confirm the role of gut bacteria in the formation of CD 6168. These results underline the importance of evaluating metabolism by gut bacteria and highlight experimental approaches for nonclinical assessment of bacterial metabolism in drug development.

Introduction

The gastrointestinal (GI) tract of vertebrates harbors a complex microbial community that provides an essential function for the host (Nordgard et al., 2005). The mucosal surface of the human gut is colonized by approximately 10^{14} bacteria (Suau et al., 1999), with 400 different species (Gorbach, 1996). The composition and distribution of gut bacteria demonstrate high intra-individual and inter-individual variability in humans and is susceptible to changes in composition with age, diet, GI transit time, and disease state. The majority of gut microbiota colonize the colon, where there is very slow motility and low oxidation-reduction potential. This contributes to the fact that 99% of colonic microbiota are obligate anaerobes (Hao and Lee, 2004).

Gut bacteria are responsible for the biotransformation of many endogenous and exogenous molecules, usually involving their breakdown via hydrolysis, de-conjugation, or reduction (Sousa et al., 2008). There are several examples of commercially available drugs that are metabolized by gut bacteria, with extensive reviews provided by Hartalaa (1973) and Sousa et al. (2008). Sousa et al. (2008) suggested that the recent increase in focus on metabolism mediated by gut bacteria correlates with the increase in drugs reaching the market with extended-release formulations or lower permeability and solubility. In addition, drug-drug interactions can be a concern, especially for drugs that affect the composition of the microbiota, which can potentially alter the metabolism of a concomitantly administered substrate for bacterial biotransformation. Drug-drug interactions can also be mediated through metabolites produced by gut bacteria. Sorivudine, an antiviral drug released in the Japanese market in 1993, was withdrawn due to a fatal drug-drug interaction between a gut metabolite of sorivudine and the anticancer drug 5-fluorouracil (Okuda et al., 1998).

There are a number of challenges in evaluating the involvement of gut bacteria in drug metabolism. A drug can be incubated in vitro with intestinal content, fecal samples, or isolated microbes (O’Sullivan, 2000), but there are several shortcomings with these methods. For example, fecal samples may not accurately reflect the actual active proportion of microbes over the entire length of the gut in vivo (Finegold et al., 1983) and it has been suggested that only 25% of intestinal bacteria are cultivable (Bartosch et al., 2004). In vivo evaluation of gut bacterial metabolism in animals is feasible. However, the limitations of large interspecies differences in microbiota composition and distribution have to be taken into consideration (Rowland et al., 1986; Sousa et al., 2008). Pseudo-germ free (pGF) rats can be created by treatment with broad-spectrum antibiotics and have helped in elucidating the role of gut bacteria in the metabolism of drugs (Jin et al., 2010; Lee et al., 2012; Liu et al., 2012; Yoo et al., 2014).

ABBREVIATIONS: ADME, absorption, distribution, metabolism, and excretion; AUC, area under the curve; GI, gastrointestinal; HLC, human liver cytosol; HLM, human liver microsomes; IVIVE, in vitro–in vivo extrapolation; LC-MS/MS, liquid chromatography–tandem mass spectrometry; m/z, mass to charge ratio; pGF, pseudogerm free.
Deleobuvir is a potent inhibitor of the hepatitis C virus nonstructural protein 5B polymerase. In a human phase Ia study in healthy male volunteers, deleobuvir was found to undergo extensive reduction to form a major circulating metabolite, CD 6168 (Fig. 1), which was confirmed later in a human absorption, distribution, metabolism, and excretion (ADME) study (Chen et al., 2015). In 14C-deleobuvir ADME studies in rats, CD 6168 was also found in the feces and accounted for approximately 43% of the administered dose (data on file, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT). Interestingly, in bile-cannulated rats, CD 6168 represented only 3% of the radioactive dose recovered in the bile. This observation, together with the fact that CD 6168 is a reduction product of the parent molecule, suggested that gut bacteria might be involved in the formation of CD 6168.

As previously mentioned, methodologies to evaluate gut bacteria metabolism are associated with several caveats. Therefore, the studies reported herein use a combination of in vitro anaerobic incubations with rat and human feces and an in vivo antibiotic-treated (pseudogerm free) rat model to confirm the role of gut bacteria in the formation of CD 6168 from deleobuvir. In addition, since in vitro studies for gut bacterial metabolism generally only provide a qualitative answer for the extent of metabolism observed in vivo, we have attempted to use a scaling approach to provide a more quantitative result. The advantages and limitations of these approaches are discussed.

Materials and Methods

Chemicals, Reagents, and Other Materials

Deleobuvir, CD 6168, 13C6-deleobuvir (label on the benzimidazole ring), and 13C6-CD 6168 (label on the benzimidazole ring) were synthesized at Boehringer Ingelheim Pharmaceuticals, Inc. Hesperidin, hesperetin, streptomycin, neomycin, D-(-)-glucose, NADH, and NADPH were purchased from Sigma-Aldrich (St. Louis, MO). d5-Hesperetin was purchased from Toronto Research Chemicals (Toronto, ON, Canada). Pooled human liver microsomes and human liver cytosol were purchased from Bioreclamation (Westbury, NY). Blank rat plasma was obtained from Bioreclamation (Westbury, NY). β-glucuronidase (140 U/ml) was purchased from Roche Applied Science (Penzberg, Upper Bavaria, Germany). All other reagents and solvents were of analytical grade or higher purity and obtained from commercial suppliers.

Incubation of Deleobuvir with Human Liver Microsomes or Human Liver Cytosol

Deleobuvir (0.1, 1, and 10 μM) was incubated with human liver microsomes (HLMs) or human liver cytosol (HLC) at protein concentrations of 1 mg/ml in 50 mM of potassium phosphate buffer (pH 7.4) at 37°C. After 5 minutes of preincubation, reaction was initiated by the addition of 2 mM NADPH, NADH, or buffer. Reactions were terminated at 0, 5, 10, 15, 30, 60, 90, and 120 minutes by removing an aliquot of incubate and adding a quench solution containing 40% acetonitrile, with 13C6-deleobuvir and 13C6-CD 6168 as internal standards. Samples were analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS), monitoring selective ions for deleobuvir and CD 6168.

Incubation of Deleobuvir with Rat or Human Feces

Individual fecal samples from three untreated male rats and also two healthy male human volunteers were collected and immediately transferred to an anaerobic chamber with an oxygen < 5 ppm atmosphere (Coy Laboratory Products, Inc., Grass Lake, MI). All of the in vitro processes up to analysis by LC-MS/MS were carried out under anaerobic conditions. Fecal samples were mixed with Dulbecco’s phosphate-buffered saline containing 20 mM of glucose to obtain a concentration of 0.05 g/ml (weight of fecal sample/volume of buffer). Samples were then homogenized and centrifuged at 500 rpm for 5 minutes at 4°C to remove debris. Processed aliquots were preincubated for 5 minutes at 37°C, and reactions were initiated by the addition of deleobuvir (100 μM, final concentration). Reactions were terminated at 0, 5, 10, 20, 30, 60, 90, and 120 minutes by removing an aliquot of incubate and adding a quench solution containing 80% acetonitrile, with 13C6-CD 6168 as an internal standard. Sealed samples were removed from the anaerobic chamber and analyzed by LC-MS/MS for levels of CD 6168.

Pseudo-germ Free Rat Study

This pharmacokinetic study was conducted in accordance with guidelines from the Institutional Animal Care and Use Committee. Twenty male Sprague-Dawley rats, approximately 320-380 g, were used for this study. Rats were fasted overnight until 4 hours after dosing with water available ad libitum. A cross-over design was used. Briefly, the drug was administered to control rats

![Fig. 1. Chemical structures of deleobuvir and CD 6168](image-url)

![Fig. 2. Formation of CD 6168 over time in (A) rat fecal samples, with an average of three male rats, r² = 0.99, and (B) human fecal samples (closed circles for subject I, r² = 0.97, and open circles for subject II, r² = 0.90).](image-url)
Gastric Emptying. Determining the rate of emptying of radioactively labeled particles of 2.8 mm in diameter (Majzoub et al., 2001) is the protocol used. The test was performed 12 hours after feeding (i.e., at 8 a.m.), and the results were expressed as percentage of the meal remaining in the stomach. The rate of gastric emptying was calculated using the formula:

\[
\text{Rate of emptying} = \frac{\text{Initial meal mass} - \text{Remaining meal mass}}{\text{Remaining meal mass}} \times 100
\]

The study was conducted in a fasting state, and the meal was administered to the rats (de Zwart et al., 1999) and humans (Rowland et al., 1986). The average transit time was 6 hours for rats (de Zwart et al., 1999) and 24 hours for humans (Wilson, 2000).

**Results**

In Vivo Metabolism of Deleobuvir by HLM or HLC. Deleobuvir (0.1, 1, and 10 \(\mu\)M) was stable with both HLM and HLC up to 120 minutes of incubation in the presence and absence of NADPH and NADH (data not shown). No quantifiable levels of CD 6168 were detected (lower limit of quantification was 7.8 nM, which would represent 0.078% conversion of 10 \(\mu\)M deleobuvir).

In Vitro Metabolism of Deleobuvir with Rat or Human Fecal Homogenates. Preliminary studies were conducted to establish linearity with respect to time and deleobuvir concentration up to 100 \(\mu\)M for the formation of CD 6168 (data not shown). Incubation of}

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Pharmacokinetic parameters of hesperetin in plasma after oral administration of 50 mg/kg of hesperidin to control rats (closed circles) and pGF rats (open circles).</th>
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<tbody>
<tr>
<td>Group</td>
<td>(t_{\text{max}})</td>
</tr>
<tr>
<td>---------</td>
<td>------------------</td>
</tr>
<tr>
<td>Control (in house)</td>
<td>6–8(^a)</td>
</tr>
<tr>
<td>Control (Jin et al., 2010)</td>
<td>8</td>
</tr>
<tr>
<td>pGF (in house)</td>
<td>4–6(^a)</td>
</tr>
<tr>
<td>pGF (Jin et al., 2010)</td>
<td>6.5</td>
</tr>
</tbody>
</table>

*Expressed as a range.
deleobuvir (100 μM) with rat fecal homogenate generated CD 6168 at a rate of 3.18 ± 2.05 nmol/min per g of fecal content (average of three animals) (Fig. 2A). With human fecal homogenate, under similar incubation conditions, the formation rates of CD 6168 were 1.95 and 0.184 nmol/min per g of fecal content for subject I and subject II, respectively (Fig. 2B).

Validation of Pseudo-germ Free Rat Model. The mean plasma concentration-time profiles of total hesperetin (hesperetin and hydrolyzed hesperetin-glucuronide) after administration of 50 mg/kg hesperidin to control and pGF rats are illustrated in Fig. 3. As shown in Table 1, \( t_{\text{max}} \) values ranged from 6 to 8 hours for control rats and 4 to 8 hours for pGF rats. The area under the curve (AUC) from 0 to ∞ values of hesperetin were 5-fold higher in the control rats (3,454 ± 760 ng·h/ml) compared with the pGF rats (685 ± 368 ng·h/ml).

The cumulative amounts of hesperidin and hesperetin excreted in feces up to 48 hours postdose are shown in Fig. 4. Slightly higher levels of hesperetin were excreted by pGF rats (12 ± 5% of dose) compared with control rats (7.8 ± 3.4% of dose). This finding was also reflected in the lower levels of hesperetin excreted by pGF rats (0.9 ± 0.3% of parent dose) compared with control rats (2.4 ± 1.6% of parent dose). The excretion of both hesperidin and hesperetin in pGF rats was statistically significant \( (P < 0.05) \) compared with control.

Metabolism of Deleobuvir in Pseudo-germ Free Rats. The mean plasma concentration-time profiles of deleobuvir and CD 6168 are presented in Fig. 5, A and B, respectively, following the administration of 10 mg/kg deleobuvir to control and pGF rats. Pharmacokinetic parameters are summarized in Table 2. Deleobuvir was rapidly absorbed in both control and pGF rats, with \( t_{\text{max}} \) values of 1 hour for both groups. Comparable plasma exposure (AUC) was observed for deleobuvir in control rats (63,438 ± 18,181 ng·h/ml) and pGF rats (66,683 ± 21,253 ng·h/ml). In addition, although plasma levels of CD 6168 were relatively low compared with the parent (representing 0.7% of parent \( C_{\text{max}} \) and 2.1% of parent AUC), levels of CD 6168 were much higher in control rats (\( C_{\text{max}} \) of 114 ± 65 ng/ml and AUC of 1,312 ± 649 ng·h/ml) compared with pGF rats (\( C_{\text{max}} \) of 15.5 ± 7.7 ng/ml and AUC of 146 ± 64 ng·h/ml). The cumulative amounts of deleobuvir and CD 6168 excreted in feces over 48 hours in both control and pGF rats are presented in Fig. 6A (deleobuvir) and Fig. 6B (CD 6168). Substantially higher levels of deleobuvir were excreted in the feces of pGF rats (105 ± 21% of dose) compared with control rats (26 ± 15% of dose). In addition, CD 6168 accounted for 1.5 ± 1.3% of the deleobuvir dose in pGF rats compared with 42 ± 8% in control rats.

In Vitro to In Vivo Extrapolation Based on Fecal Incubations. Based on in vitro formation rates and the assumptions inherent in these calculations (as outlined in Materials and Methods and Discussion), the total amount of CD 6168 estimated to be formed from deleobuvir (using eq. 1) was 2.18 ± 1.40 mg in rats and 201 mg (subject I) and 19.0 mg (subject II) in humans. These numbers were compared with in vivo levels of CD 6168 excreted into feces, which are summarized in Table 3. For rats, the amount of CD 6168 excreted into feces was 1.5 ± 0.3 mg (data derived from control rats in the pseudo-germ free rat study). For humans, the amount of CD 6168 excreted into feces was 280 mg (35% of an 800-mg dose) (Chen et al., 2015).

Discussion

The overall role of the gut microbiome in health and disease is increasingly being appreciated (Owyang and Wu, 2014; Ursell et al., 2014; C. D. Klaassen and J. Y. Cui, submitted manuscript), as is the contribution of gut bacteria to the metabolism of drugs (Sousa et al., 2008). Our own experience with BILR 355 (Li et al., 2012) and

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Analyte</th>
<th>( t_{\text{max}} )</th>
<th>( t_{1/2} )</th>
<th>( C_{\text{max}} )</th>
<th>AUC&lt;sub&gt;0-( t )&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>hours</td>
<td>hours</td>
<td>ng/ml</td>
<td>ng·h/ml</td>
</tr>
<tr>
<td>Control</td>
<td>Deleobuvir</td>
<td>1</td>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15,784 ± 4,533</td>
<td>63,438 ± 18,181</td>
</tr>
<tr>
<td>pGF</td>
<td></td>
<td>1</td>
<td>5 ± 1</td>
<td>18,718 ± 9,840</td>
<td>66,683 ± 21,253</td>
</tr>
<tr>
<td>Control</td>
<td>CD 6168</td>
<td>6.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N.D.</td>
<td>114 ± 65</td>
<td>1,312 ± 649</td>
</tr>
<tr>
<td>pGF</td>
<td>N.D.</td>
<td>N.D.</td>
<td>15.5 ± 7.7</td>
<td>146 ± 64</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>S.D. lower than 1.  
<sup>b</sup>Expressed as a range.

<sup>n.d.</sup> not determined.
deleobuvir, reported here, has also underlined an important role of gut bacteria in the presystemic conversion of the parent drug.

For deleobuvir, the substantial presence of a reduced metabolite, CD 6168, in rat feces but not in bile in a rat 14C-ADME study, suggested that CD 6168 was likely formed by gut bacteria. Although reductive reactions can be carried out by other drug metabolizing enzymes, including cytochrome P450 (Guengerich, 2001), these are relatively rare. In addition, in vitro incubations of deleobuvir with human liver microsomes and cytosol did not generate CD 6168. Anaerobic incubations with deleobuvir using rat and human fecal homogenates demonstrated a substantial formation of CD 6168. The enzymes responsible for reduction can be sensitive to the presence of oxygen, and as such, it was important to adopt appropriate in vitro conditions. An anaerobic chamber was instrumental to these studies (Finegold et al., 1983; Kang et al., 2013).

To assess whether the plasma levels of CD 6168 could arise from bacterial biotransformation, the in vitro formation rate of CD 6168 was used to extrapolate the amount of CD 6168 that could be formed in vivo, with a number of assumptions (see below), and which was compared with levels of CD 6168 in the human 14C-ADME and rat studies (Table 3). It should be noted that the in vitro deleobuvir concentrations (100 μM) were limited by solubility and in vivo gut concentrations could be higher. An 800-mg dose of deleobuvir in humans could theoretically achieve gut concentrations of almost 5000 μM (800 mg in 250 ml). In rats, a 10-mg/kg dose would translate to ~1700 μM (~3.5 mg in a 3.2-ml gut volume) (McConnell et al., 2008). In rats, the average total amount of CD 6168 formed was estimated to be 2.18 ± 1.40 mg based on in vitro incubations compared with 1.5 ± 0.3 mg excreted in feces (Table 3), which is only a 1.5-fold difference between the predicted and observed amounts. In humans, based on in vitro incubations, 202 mg (subject I) and 19.1 mg (subject II) of CD 6168 were estimated to be formed in vivo. In the human 14C-ADME study (Chen et al., 2015), a single oral dose of 14C-deleobuvir (800 mg) resulted in 280 mg of CD 6168 being recovered in feces (35% of the dose of deleobuvir). There was negligible excretion in urine (< 1% of dose). In human feces, significant amounts (~70% of CD 6168) of oxidative metabolites that may originate from CD 6168 were also found (Chen et al., 2015). Such a secondary metabolism was significantly lower in rats (data on file). As such, in humans, the actual amount of CD 6168 being generated could be up to 1.7-fold higher than nominally found in feces. Anaerobic in vitro fecal incubations will only generate CD 6168 since the oxidative enzymes that can generate secondary metabolites of CD 6168 will not be active. The secondary metabolism of CD 6168 in vivo likely contributes to the underestimation of the total amount of CD 6168 predicted from in vitro experiments.

There are several caveats associated with in vitro–in vivo extrapolation (IVIVE) of gut bacterial metabolism. There is a large variability associated with the fecal content/fecal weight and GI transit time (Kelsay et al., 1978; C. D. Klaassen and J. Y. Cui, submitted manuscript). In addition, the composition of gut bacteria in fecal homogenate may not reflect the abundance and distribution of bacteria in the GI tract (Finegold et al., 1983). An alternative approach is to consider bacterial load (colony forming unit/grams of feces) and the total number of bacteria in the GI tract. However, these numbers are not readily available and, at best, are approximations. This highlights the necessity to better characterize the gut microbiome. In this study, the two volunteers generated very different levels of CD 6168, which emphasizes a challenge with this approach. Clearly, due to inherent variability in gut bacterial metabolism, several human fecal donor samples should be tested for a more robust measure of IVIVE. Interspecies differences in CD 6168 oxidation adds additional challenges for a cross-species IVIVE using a single approach. However, for both the human and rat, the goal was to determine whether bacterial

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**TABLE 3**

<table>
<thead>
<tr>
<th>Species</th>
<th>Rate of Formation</th>
<th>Estimated Formation of CD 6168 Based on In Vitro data</th>
<th>For Comparison (In Vivo)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per gram</td>
<td>mg</td>
<td>Deleobuvir Dose</td>
</tr>
<tr>
<td>Rat</td>
<td>3.18 ± 2.05b</td>
<td>2.18 ± 1.40</td>
<td>3.6</td>
</tr>
<tr>
<td>Human</td>
<td>1.95 (subject I)</td>
<td>202 (subject I)</td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>0.184 (subject II)</td>
<td>19.1 (subject II)</td>
<td></td>
</tr>
</tbody>
</table>

*Using IVIVE approach.

b = 3.

Secondary metabolism is not accounted for.
biotransformation could possibly account for large circulating levels of CD 6168. Considering the limitations of this IVIVE, we believe that this goal was achieved, although generating an accurate IVIVE for such mechanisms needs further work in the field of microbiome.

A similar methodology was used to investigate the metabolism of BLIR 355 by gut bacteria, forming BLIR 402 (Li et al., 2012). The in vitro formation rate of BLIR 402 in human fecal homogenates (9.2 nmol/min per g of feces), with the approach outlined here, was used to estimate the in vivo formation of BLIR 402. The turnover of BLIR 355 to BLIR 402 was fast at 0.430 mg/min and confirmed the observation that BLIR 355 was present in only trace amounts in feces in vivo, and the majority of fecal radioactivity was accounted for by BLIR 402 and its downstream metabolites (Li et al., 2012).

A pGF rat model was also investigated to assess the importance of gut bacterial biotransformation in the disposition of deleobuvir. There can be substantial species differences in specificity and substrate selectivity for drug metabolizing enzymes between rats and humans (Martignoni et al., 2006). Similarly, species differences in gut microbiota (Rowland et al., 1986) make interspecies extrapolations difficult. Germ-free rats have been used to evaluate the role of bacterial microbiota in the metabolism of several compounds, including hesperidin, acacetaminophen, and merigliferin (Jin et al., 2010; Lee et al., 2012; Liu et al., 2012). There are significant challenges in the maintenance and use of germ-free animals (Gordon and Pesti, 1971). Pretreatment of animals with antibiotics can provide a temporary, almost complete microbe-free animal model (Sousa et al., 2008; Kang et al., 2013). This simpler pGF rat model was validated in our studies using a known probe for gut bacterial metabolism, hesperidin. The pharmacokinetic parameters obtained for hesperidin in this study (Table 1) were comparable to data reported by Jin et al. (2010). The pGF rats excreted significantly higher levels of hesperidin compared with control rats, consistent with the lack of conversion of hesperidin to hesperitin by gut bacteria (Fig. 4). Similarly, the plasma exposure of the metabolite, hesperitin, was significantly higher in control rats compared with pGF rats (Fig. 3). The flavonoid backbone of hesperitin is further degraded by gut bacteria into numerous phenolic and carboxylic acid products (Garg et al., 2001), which explains the low levels of hesperitin in the feces of pGF and control rats (< 3% of dose).

Although formation of CD 6168 in rats compared with humans is a relatively less abundant metabolic pathway, these findings in pGF rats support a key role of gut bacteria in the formation of CD 6168 from deleobuvir. CD 6168 exposure levels were significantly lower in pGF rats, with an average AUC of 146 ± 64 ng·h/ml (Fig. 5B; Table 2) compared with control rats (1,312 ± 649 ng·h/ml). About 1.5 mg (42% of dose) of CD 6168 was excreted in the feces of control rats, but a mere 0.06 mg (1.51% of dose) was found in the feces of pGF rats. Conversely, significantly higher levels of deleobuvir were excreted into the feces in pGF rats (105% of dose) compared with control rats (26% of dose), confirming the role of gut bacteria in the biotransformation of deleobuvir. For deleobuvir, the plasma exposure levels were similar in both rat groups but significantly different in the amount excreted into feces. The amount of deleobuvir excreted into feces is a combination of unabsorbed drug (60% bioavailability) with the amount excreted into bile as deleobuvir and deleobuvir-glucuronide.

Deleobuvir as well as CD 6168 were rapidly absorbed, with a tmax of approximately 3 hours. Both exhibited similar pharmacokinetics and high variability (Chen et al., 2015), but there was no clear inverse correlation of deleobuvir and CD 6168 exposure in vivo. This suggests that although the variability in gut bacteria between humans may account for some of the variability in plasma exposure, other aspects of differential cleavage between the two compounds, i.e., further metabolism of CD 6168 versus direct glucuronidation of deleobuvir, may also play a role.

Overall, using appropriate in vitro and in vivo tools of gut bacterial metabolism offers experimentally viable approaches to identify a role for gut bacteria in the metabolism of a drug. Attempts at IVIVE, taking the rate of metabolite formation in feces and scaling to the amount of gut bacterial metabolite formed in vivo, may offer a way to assess the contribution of gut bacteria to the overall biotransformation, with awareness of interindividual variability and the complex nature of the GI tract.

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Authorship Contributions

Participated in research design: McCabe, Sane, Tweedie, Johnstone, Li.
Conducted experiments: Whitcher-Johnstone, Xu, McCabe, King, Li, Keith-Luzzi.
Wrote or contributed to the writing of the manuscript: McCabe, Sane, Tweedie, Li.

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