Species Differences in Biliary Clearance and Possible Relevance of Hepatic Uptake and Efflux Transporters Involvement

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ABSTRACT

From a search of the available literature, a database of 22 drugs of all charge types and several different therapeutic classes was compiled to compare rat and human biliary clearance data. Dog biliary excretion data were also found for nine of the drugs. For 19 of the 22 drugs (86%), rat unbound biliary clearance values, when normalized for body weight, exceeded those for humans by factors ranging from 9 to over 2500-fold, whereas human/dog differences were much less dramatic. It was possible to define hepatic uptake and efflux transporter involvement for many of the drugs. On the basis of the findings, it is postulated that regardless of the biliary efflux transporters implicated, when drugs do not require active hepatic uptake to access the liver there may be fairly insignificant differences in rat, dog, and human biliary clearance. Conversely, when the organic anion-transporting polypeptide drug transporters are involved, one may expect at least a 10-fold discrepancy in rat to human biliary clearance normalized for body weight and corrected for plasma protein binding.

Introduction

Predicting human pharmacokinetics from preclinical data are a primary goal of Drug Discovery Drug Metabolism and Pharmacokinetic (DMPK) scientists, because therapeutic success can be compromised by poor human pharmacokinetics. Accurate predictions of hepatic metabolic clearance can be made from in vitro data, providing appropriate attention to detail is made (Grime and Riley, 2006; Obach, 2011; Sohlenius-Sternbeck et al., 2012), but other than being eliminated from the body by metabolism, drugs can be eliminated directly into the urine or bile. Both routes are complex and involve passive and active cellular uptake and efflux transport processes. Biliary excretion can involve the hepatic uptake transporters organic anion transporting polypeptide (OATP), organic anion transporter (OAT) and organic cation transporter (OCT), and the canaliculal efflux transporters breast cancer resistance protein (BCRP), P-glycoprotein (P-gp) and multidrug resistance-associated protein 2 (MRP2) (Shitara et al., 2006; Kusuhara and Sugiyama, 2010). Similarly, active renal excretion of drugs can involve several transporters in the basolateral and apical membranes (Brown et al., 2008). Despite the complexity, an effective prediction method based on dog renal clearance after correction for differences in plasma protein binding and kidney blood flow has emerged (Paine et al., 2011). There has not been a wealth of literature on the subject of predicting human biliary clearance of drug candidates, perhaps because of the scarcity of relevant clinical data (Lavé et al., 2009), and as such, in terms of human clearance predictions, biliary stands apart as a largely unresolved problem.

Sandwich-cultured hepatocytes maintain liver-specific functions for several days after cell isolation and exhibit the formation of bile canaliculi and the localization of efflux transporters on the canalicular membrane (LeCluyse et al., 1994). Advancements have been made in using this in vitro technique for predicting biliary clearance (Abe et al., 2009), but the robustness of the method is still to be extensively tested with a wide range of drugs. However, appreciation of the intracellular drug concentrations and calculation of an in vitro biliary intrinsic clearance may see the method emerge as a truly predictive tool (Nakakariya et al., 2012). Nonetheless, preclinical in vivo data retain an extremely important role in facilitating the understanding and contextualization of the risks associated with human pharmacokinetic predictions. A variety of interspecies allometric scaling approaches have been assessed specifically for biliary clearance (Sawada et al., 1984; Mahmood and Sahajwalla, 2002; Mahmood, 2005) but given the low number of drugs used in the analyses, the fact that some of the examples used involved total drug-related material excreted rather than parent drug (Scatina et al., 1989) and that allometry underpredicts human biliary clearance for some drugs but not others (Sawada et al., 1984; Pählman et al., 1998), a more extensive analysis has been required.

Recently a database of 18 drugs having known rat and human biliary clearance was published, representing the most extensive dataset to date (Morris et al., 2012). From this it was evident that, when considering unbound clearance (corrected for plasma protein binding), simple allometry using an exponent of 0.66 gave reasonable human predictions for some drugs, but for others rat overstimulated human biliary clearance by an excess of one order of magnitude. On the basis of a limited number of six drugs, multiple species allometry using unbound biliary clearance data gave good predictions, but it should be reflected on that interspecies allometry may to an extent afford better clinical predictions because of the smoothing out of data from species where there is a large discrepancy with the human data.

ABBREVIATIONS: BCRP, breast cancer resistance protein; Cp,ss, plasma concentration of drug at steady state; Cp,mid, plasma concentration of drug at the midpoint of the bile collection interval; DMPK, Drug Metabolism and Pharmacokinetic; MRP2, multidrug resistance-associated protein 2; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; P-gp, P-glycoprotein.
Indeed, in a drug discovery setting, the most likely scenario is that a poor prediction of rat clearance from in vitro hepatic metabolic data would precipitate a renal and biliary excretion study if the compound is of sufficient interest as a potential human therapeutic agent. From such information accurate decisions must be made about the suitability of the compound to progress further, and, therefore, understanding rat to human differences in biliary clearance is vital.

To investigate this further, we compiled an even more extensive rat and human biliary clearance data set than that of Morris et al. (2012) for 22 drugs of all charge types and several different therapeutic classes. For nine of the drugs, it was also possible to find data for dog biliary clearance. An extensive literature search was performed to define the hepatic uptake and bile canalicular efflux drug transporters, with the aim of elucidating further the reason for interspecies differences and with the specific intention of helping DMPK scientists make effective decisions with early preclinical data.

**Materials and Methods**

No new data were generated for this work—all data used were obtained from the scientific literature and the references used for this are detailed in Tables 1 and 2.

### TABLE 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Human Cl&lt;sub&gt;bile&lt;/sub&gt;</th>
<th>Human Cl&lt;sub&gt;bile,u&lt;/sub&gt;</th>
<th>Rat Cl&lt;sub&gt;bile&lt;/sub&gt;</th>
<th>Rat Cl&lt;sub&gt;bile,u&lt;/sub&gt;</th>
<th>Dog Cl&lt;sub&gt;bile&lt;/sub&gt;</th>
<th>Dog Cl&lt;sub&gt;bile,u&lt;/sub&gt;</th>
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</thead>
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<td>Doxorubicin</td>
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<td>7.268</td>
<td>3.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.82</td>
<td>1.720&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.059</td>
</tr>
<tr>
<td>Digoxin</td>
<td>1.595&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.279</td>
<td>3.03&lt;sup*e&lt;/sup&gt;</td>
<td>4.329</td>
<td>1.313&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.680</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1.147&lt;sup&gt;g&lt;/sup&gt;</td>
<td>7.169</td>
<td>17.4&lt;sup&gt;*&lt;/sup&gt;</td>
<td>22.38</td>
<td>2.376&lt;sup&gt;h&lt;/sup&gt;</td>
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<td>Cefazolin</td>
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<td>0.007</td>
<td>1.40&lt;sup&gt;j&lt;/sup&gt;</td>
<td>11.24</td>
<td>0.066&lt;sup&gt;k&lt;/sup&gt;</td>
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<td>1.07&lt;sup&gt;o&lt;/sup&gt;</td>
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<td>Cefotetan</td>
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<td>0.320</td>
<td>6.14&lt;sup&gt;q&lt;/sup&gt;</td>
<td>8.771</td>
<td>0.696&lt;sup&gt;r&lt;/sup&gt;</td>
<td>1.141</td>
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<td>Cefixime</td>
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<td>1.00&lt;sup&gt;t&lt;/sup&gt;</td>
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<td>0.82&lt;sup&gt;v&lt;/sup&gt;</td>
<td>12.73</td>
<td>2.5&lt;sup&gt;w&lt;/sup&gt;</td>
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<td>1.000</td>
<td>5.22&lt;sup&gt;y&lt;/sup&gt;</td>
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<td>0.979&lt;sup&gt;z&lt;/sup&gt;</td>
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<td>16.3&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>9.60&lt;sup&gt;ah&lt;/sup&gt;</td>
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<td>0.217&lt;sup&gt;ai&lt;/sup&gt;</td>
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<td>Diclofenac</td>
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<td>0.85&lt;sup&gt;al&lt;/sup&gt;</td>
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<td>Fexofenadine</td>
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<td>8.00&lt;sup&gt;at&lt;/sup&gt;</td>
<td>42.11</td>
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<td>Ciproflaxacin</td>
<td>0.0159&lt;sup&gt;au&lt;/sup&gt;</td>
<td>0.084</td>
<td>3.50&lt;sup&gt;av&lt;/sup&gt;</td>
<td>6.140</td>
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<tr>
<td>Napsagatran</td>
<td>4.357&lt;sup&gt;aw&lt;/sup&gt;</td>
<td>7.922</td>
<td>34.7&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>105.2</td>
<td>32.75&lt;sup&gt;ay&lt;/sup&gt;</td>
<td>62.98</td>
</tr>
</tbody>
</table>

<sup>a</sup> Wilkinson et. al., 1979.
<sup>b</sup> Krishna et al., 1999.
<sup>c</sup> Iguchi et al., 1986.
<sup>d</sup> Hedman et. al., 1991.
<sup>e</sup> Song et al., 1999.
<sup>f</sup> Miyazawa et al., 1990.
<sup>g</sup> Takimura and Lopez-Belio, 1955.
<sup>h</sup> Lam et al., 2006.
<sup>i</sup> Wyman et al., 1988.
<sup>j</sup> Brogard et. al., 1984.
<sup>k</sup> Tsuji et al., 1983.
<sup>l</sup> Yoshihata et al., 1979.
<sup>m</sup> Ratzan et al., 1978.
<sup>n</sup> Wright and Line, 1980.
<sup:o</sup> Morris et al., 2012.
<sup>p</sup> Westphal et al., 1993.
<sup>q</sup> Yasui et al., 1994.
<sup>r</sup> Arvidsson et al., 1982.
<sup>s</sup> Matsui et al., 1984.
<sup>t</sup> Brogard et. al., 1988.
<sup>u</sup> Muraoka et al., 1995.
<sup>v</sup> Matsui et al., 1982.
<sup>w</sup> Kenmerich et al., 1983.
<sup>x</sup> Saikawa et al., 1980.
<sup>y</sup> Brookman et al., 1997.
<sup>z</sup> Yamashiro et al., 2006.
<sup>a</sup> Uchida et al., 1985.
<sup>aa</sup> Mizuji et al., 1987.
<sup>ab</sup> Sasaki et al., 2004.
<sup>ac</sup> Hatanaka et al., 1998.
<sup>ad</sup> Kemmerich et al., 1983.
<sup>ae</sup> Saikawa et al., 1980.
<sup>af</sup> Tanimura et. al., 1986.
<sup>ag</sup> Yoshikawa et al., 1979.
<sup>ah</sup> Jackson et al., 1978.
<sup>ai</sup> Kreek et al., 1980.
<sup>aj</sup> Sutfin et al., 1987.
<sup>al</sup> Tahara et al., 2005 Jaisue et al., 2010.
<sup>am</sup> Tanimura et. al., 1986.
<sup>an</sup> Yamaguchi et al., 2004.
<sup>ao</sup> Klotz and Walker., 1990.
<sup>ap</sup> Peris-Ribera et al., 1991.
For rat and dog data, the methods in the publications all refer to biliary clearance estimated in the same way, as follows: surgically prepared (bile duct cannulated) animals were used and the amount of parent drug eliminated into bile was quantified. Biliary clearance was then calculated from the product of the total systemic clearance and the fraction of dose accounted for in bile as parent drug (Table 3).

For the clinical studies, the literature references detailed the following methods for collecting the bile (see Table 4): in the majority of studies, patients had a temporary bile shunt (T-tube) inserted. The T-tube diverts part of the bile flowing from the liver to a port for external collection. Other studies used duodenal aspiration or drainage, in which biliary secretions were withdrawn from the duodenum. Parent drug was quantified in the bile samples, according to the methods detailed in the individual references, and human biliary clearance was then calculated in one of three ways:

1. From the amount of parent drug in the bile divided by the area under the plasma concentration-time curve (amount in bile/AUC, Table 4);
2. From the ratio of the rate of biliary excretion and the plasma concentration, either steady state or midpoint of the bile collection period (bil. excretion rate/Cp,ss or bil. excretion rate/Cp,mid; Table 4);
3. From the product of the total systemic clearance (CL) and the fraction of dose accounted for in bile as parent drug (CL x % bile, Table 4).

In the source references for the human bile data (Table 1), the biliary clearance was not always calculated (in some of the publications, only the amount of parent drug excreted in to the bile was recorded). In these instances, we calculated the human biliary clearance by method 3 (above) using clearance and bioavailability values obtained from literature (Goodman and Gilman, 1990; Obach et al., 2008).

For the purposes of this present analysis (to assess the cross species differences in biliary clearance normalized for body weight and plasma protein binding and to determine whether definition of the drug transporters involved help in elucidating the differences), the following steps were taken to supply the information in Table 1:

1. The biliary clearance was expressed in ml/min/kg terms, assuming rat, dog, and human body weights of 0.3, 15, and 70 kg, respectively. The body weight normalized biliary clearance (ml/min/kg) was divided by the fraction of each drug unbound in plasma (fup) to obtain the unbound biliary clearance. The following plasma protein binding data (rat, dog, human) were obtained from the scientific literature [where dog fup data were not required for the analysis, a dash (—) is given]: doxorubicin (0.34, 0.34, 0.28), digoxin (0.70, 0.49, 0.7), erythromycin (0.78, 0.53, 0.16), cefazolin (0.12, 0.74, 0.18), cefamandole (0.17, —, 0.25), cephalexin (0.76, —, 0.86), cefotetan (0.70, 0.61, 0.15), cefixime (0.14, —, 0.31), ceftriaxone (0.22, 0.86, 0.075), cefpiramide (0.54, 0.7, 0.04), cefoperazone (0.74, —, 0.07), valsartan (0.03, —, 0.07).

### TABLE 2

<table>
<thead>
<tr>
<th>Rat/human CLbile,u</th>
<th>Dog/Human CLbile,u</th>
<th>Hepatic Uptake Transporter</th>
<th>Biliary Efflux Transporter</th>
<th>Chemotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>1</td>
<td>—</td>
<td>MRP2; P-gp&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>b</td>
</tr>
<tr>
<td>Digoxin</td>
<td>2</td>
<td>—</td>
<td>P-gp&lt;sup&gt;e&lt;/sup&gt;</td>
<td>n</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>3</td>
<td>—</td>
<td>MRP2; P-gp&lt;sup&gt;e&lt;/sup&gt;</td>
<td>b</td>
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<tr>
<td>Cefazolin</td>
<td>1685</td>
<td>13</td>
<td>OATP1B1, 1B&lt;sup&gt;e&lt;/sup&gt;</td>
<td>BCRP&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>Cefamandole</td>
<td>613</td>
<td>—</td>
<td>MRP2, BCRP&lt;sup&gt;e&lt;/sup&gt;</td>
<td>a</td>
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<tr>
<td>Cephalexin</td>
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<td>a</td>
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<tr>
<td>Cefotetan</td>
<td>27</td>
<td>4</td>
<td>—</td>
<td>a</td>
</tr>
<tr>
<td>Cefixime</td>
<td>16</td>
<td>—</td>
<td>—</td>
<td>a</td>
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<tr>
<td>Cefoperazone</td>
<td>10</td>
<td>—</td>
<td>OATP1B1, 1B&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>MRP2, BCRP&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>Valsartan</td>
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<td>—</td>
<td>—</td>
<td>a</td>
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<tr>
<td>Moxalactam</td>
<td>507</td>
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<td>—</td>
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<td>OATP1B1&lt;sup&gt;k&lt;/sup&gt;</td>
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<td>Pravastatin</td>
<td>13</td>
<td>—</td>
<td>OATP1B1&lt;sup&gt;m&lt;/sup&gt;</td>
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<td>Diclofenac</td>
<td>11</td>
<td>—</td>
<td>—</td>
<td>a</td>
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<td>Ranitidine</td>
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<td>—</td>
<td>OCT-1&lt;sup&gt;n&lt;/sup&gt;</td>
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<td>P-gp&lt;sup&gt;h&lt;/sup&gt;</td>
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<td>—</td>
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<td>P-gp&lt;sup&gt;h&lt;/sup&gt;</td>
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<td>Napagatran</td>
<td>13</td>
<td>8</td>
<td>—</td>
<td>z</td>
</tr>
</tbody>
</table>

— Indicates that no literature reference was found.
* Fujisawa et al., 2011.
* Hiddenma et al., 2003.
* Koihe et al., 1997.
* Bi et al., 2006.
* Harhilan et al., 2009.
* Nakakariya et al., 2008a.
* Kato et al., 2008a.
* Yamaguchi et al., 2011.
* Nakakariya et al., 2008b.
* Yamashiro et al., 2006.
* Gui et al., 2010.
* Choi et al., 2009.
* Kusuhara and Sugiyama, 2010.
* Watanabe et al., 2009.
* Bourdet et al., 2005.
* Huang et al., 2005.
* van Zanden et al., 2005.
* Watanabe et al., 1995.
* Rodriguez et al., 2004.
* Matsumoto et al., 2008.
* Cvetkovic et al., 1999.
0.04), moxalactam (0.51, 0.39), methotrexate (0.77, 0.63, 0.37), pravastatin (0.35, —, 0.5), diclofenac (0.009, —, 0.005), ranitidine (0.9, —, 0.95), vincristine (0.39, —, 0.4), methadone (0.36, —, —, 0.21), fexofenadine (0.19, —, —, 0.35), ciprofloxacin (0.57, —, —, 0.7), and napsagatran (0.33, 0.52, 0.55).

2. A literature search was performed to identify the human hepatic uptake and canalicular efflux drug transporters implicated in the in vivo biliary clearance. The references are given in Table 2.

**Results**

In the analysis presented here, rat, dog, and human differences in biliary clearance were explored by assembling, from the scientific literature, the most extensive database available to date. Additional definition of the uptake and efflux drug transporters involved was incorporated, with the intention of providing greater insight into the likely extent of cross-species differences. From this, it is hoped that more enlightened early decision making may be possible in a drug discovery setting.

As with previous literature analyses, to overcome species differences in plasma protein binding, unbound biliary clearances were compared. In the analysis presented here, biliary clearance values were also normalized for body weight and presented in units of ml/min/kg body weight to allow a simpler cross-species comparison. Comparing rat, dog, and human hepatic blood flow values of 72, 55, and 20 ml/kg/min, respectively, the following calculation was performed to normalize the data:

**TABLE 3**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Admin. Route (rat)</th>
<th>% Dose Excreted into Bile (Rat)</th>
<th>Admin. Route (Dog)</th>
<th>% Dose Excreted into Bile (Dog)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>i.v.</td>
<td>17</td>
<td>i.v.</td>
<td>10</td>
</tr>
<tr>
<td>Digoxin</td>
<td>i.v.</td>
<td>31</td>
<td>i.v.</td>
<td>7</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>i.v.</td>
<td>30</td>
<td>i.v.</td>
<td>5</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>i.v.</td>
<td>30</td>
<td>i.v.</td>
<td>2</td>
</tr>
<tr>
<td>Cefamandole</td>
<td>i.v.</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalexin</td>
<td>i.v.</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefotetan</td>
<td>i.v.</td>
<td>48</td>
<td>i.v.</td>
<td>17</td>
</tr>
<tr>
<td>Cefixime</td>
<td>i.v.</td>
<td>41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>i.v.</td>
<td>62</td>
<td>i.v.</td>
<td>63</td>
</tr>
<tr>
<td>Cefpiramide</td>
<td>i.v.</td>
<td>58</td>
<td>i.v.</td>
<td>19</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>i.v.</td>
<td>86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valsartan</td>
<td>i.v.</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moxalactam</td>
<td>i.v.</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methotrexate</td>
<td>i.v.</td>
<td>64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pravastatin</td>
<td>i.v.</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>i.v.</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ranitidine</td>
<td>i.v.</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vincristine</td>
<td>i.v.</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methadone</td>
<td>i.v.</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>i.v.</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fexofenadine</td>
<td>i.v.</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Napsagatran</td>
<td>i.v.</td>
<td>61</td>
<td>i.v.</td>
<td>97</td>
</tr>
</tbody>
</table>

AUC, area under the plasma drug concentration-time profile; bil. excr. rate/Cp,ss or bil. excr. rate/Cp,mid, ratio of biliary excretion rate and the steady state plasma concentration or mid-point plasma concentration during the bile collection period; Duodenal asp., duodenal aspiration; Duodenal perf., duodenal perfusion; F, bioavailability; Fecal ext., fecal extraction; %bile, percentage of parent drug accounted for in bile; — indicates that the precise details of the procedure were not available in the literature.

**TABLE 4**

Experimental human biliary excretion information used in the analysis.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Calculation Method</th>
<th>% Parent Drug in Bile</th>
<th>Admin. Route</th>
<th>Method</th>
<th>Collection Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>CL × % bile</td>
<td>14</td>
<td>i.v.</td>
<td>T-tube</td>
<td>24</td>
</tr>
<tr>
<td>Digoxin</td>
<td>bil. excr. rate/Cp,mid</td>
<td>41</td>
<td>i.v</td>
<td>Duodenal perf.</td>
<td>8</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>CL × % bile</td>
<td>13</td>
<td>i.v.</td>
<td>Duodenal asp.</td>
<td>20</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>CL × % bile</td>
<td>0.1</td>
<td>i.v.</td>
<td>T-tube</td>
<td>6</td>
</tr>
<tr>
<td>Cefamandole</td>
<td>CL × % bile</td>
<td>0.4</td>
<td>i.v.</td>
<td>T-tube</td>
<td>6</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cefotetan</td>
<td>CL × % bile</td>
<td>11</td>
<td>i.v.</td>
<td>Duodenal perf.</td>
<td>7</td>
</tr>
<tr>
<td>Cefixime</td>
<td>Amount in bile/AUC</td>
<td>11</td>
<td>p.o</td>
<td>T-tube</td>
<td>24</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>bil. excr. rate/Cp,ss</td>
<td>30</td>
<td>i.v.</td>
<td>Duodenal perf.</td>
<td>6-8</td>
</tr>
<tr>
<td>Cefpiramide</td>
<td>Amount in bile/AUC</td>
<td>28</td>
<td>i.v.</td>
<td>T-tube</td>
<td>24</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>CL × % bile</td>
<td>12</td>
<td>i.v.</td>
<td>T-tube</td>
<td>24</td>
</tr>
<tr>
<td>Valsartan</td>
<td>CL × % bile</td>
<td>88</td>
<td>i.v.</td>
<td>T-tube</td>
<td>24</td>
</tr>
<tr>
<td>Moxalactam</td>
<td>CL × % bile</td>
<td>0.5</td>
<td>i.v.</td>
<td>T-tube</td>
<td>8</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>CL × % bile</td>
<td>10</td>
<td>i.v.</td>
<td>T-tube</td>
<td>24</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>CL × % bile</td>
<td>23</td>
<td>i.v.</td>
<td>T-tube</td>
<td>24</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>CL × % bile</td>
<td>1</td>
<td>i.v.</td>
<td>T-tube</td>
<td>8</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>CL × % bile</td>
<td>1.5</td>
<td>i.v.</td>
<td>T-tube</td>
<td>5</td>
</tr>
<tr>
<td>Vincristine</td>
<td>CL × % bile</td>
<td>10</td>
<td>i.v.</td>
<td>T-tube</td>
<td>72</td>
</tr>
<tr>
<td>Methadone</td>
<td>CL/F × % bile</td>
<td>0.06</td>
<td>p.o</td>
<td>T-tube</td>
<td>24</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>CL/F × % bile</td>
<td>0.5</td>
<td>p.o</td>
<td>T-tube</td>
<td>24</td>
</tr>
<tr>
<td>Fexofenadine</td>
<td>CL/F × % bile</td>
<td>23</td>
<td>p.o</td>
<td>Fecal ext.</td>
<td>12</td>
</tr>
<tr>
<td>Napsagatran</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
min/kg (McEntee et al., 1996; Barter et al., 2007; Taylor et al., 2007), one may expect a 3.6-fold difference between rat and human and a 2.8-fold difference between dog and human hepatic clearance in the absence of any mechanistic differences at the level of drug transport and metabolism. In this context, the most striking thing about the data set (Fig. 1; Table 1) is that for 19 of the drugs (86%), rat unbound biliary clearance values exceed those for humans by factors ranging from 9- to over 2500-fold.

**Discussion**

Of the 19 drugs with a rat/human unbound biliary clearance ratio of approximately one order of magnitude or greater, it was possible to find literature references for hepatic uptake transporter involvement for nine (Fig. 2A; Table 2). However, because four of those drugs with undefined hepatic uptake mechanisms (cefixime, cefamandole, cefotetan, and cefpiramide) have similar properties to ceftriaxone, cefazolin, cephalixin, and cefoperazone (structurally similar acidic drugs of the same antibiotic class with low logD<sub>7.4</sub> values and similar biliary efflux transporter substrate recognition) it is perhaps not unreasonable to speculate that the uptake transporters may also be similar—in other words the organic anion transporting polypeptides may be involved. However, it should be acknowledged that the most lipophilic of these, ceftriaxone, is a substrate for OATP1B3 (Yamaguchi et al., 2011) but is not apparently a substrate for rat oatp1a4 (Nakakariya et al., 2008a). Biliary efflux transporter involvement could be defined for the majority of the 22 drugs, but no obvious relationship between the transporter definition and the rat/human discrepancy in unbound biliary clearance was apparent (Fig. 2B; Table 2).

Methadone, cefazolin, and moxalactam (0.001, 0.001, and 0.003 ml/min/kg) had by far the lowest measured total human biliary clearance values, and the rat/human unbound biliary clearance differences were calculated to range from approximately 500 to 2500. With the human biliary clearance values being so low and patient numbers being limited (one, five, and six for the three drugs, respectively), the absolute values should be treated with caution. Methadone is of particular concern because it has the highest estimated rat/human discrepancy and yet is a basic drug of moderate to low lipophilicity. It is therefore unlikely to be an OATP substrate, and the human biliary clearance value we found in the literature (Kreek et al., 1980) may not be a true representation in a wider patient group.

Interestingly, for the only three drugs in which unbound rat, human, and dog biliary clearance values were found to be approximately equal (in ml/min/kg), namely erythromycin, doxorubicin, and digoxin (Table 1; Fig. 2A), active uptake into the liver has been reported to be at best very slow or only partially responsible for the permeability of the sinusoidal membrane or indeed nonexistent (Hilmer et al., 2004; Sun et al., 2004; Bi et al., 2006; Taub et al., 2011; Yabe et al., 2011). Therefore, although there are dramatic species differences in the expression and activity of canalicular transporters that may be up to 10-fold for rat/human Mrp2/MRP2 (Ishizuka et al., 1999; Li et al., 2009), the fact that erythromycin, doxorubicin, and digoxin are substrates for P-gp or MRP2, along with many of the other drugs for
which rat biliary clearance is considerably greater than for humans, suggests that the key determinant in the biliary clearance species difference may indeed be the uptake step from the blood into the liver. It is not a new suggestion that sinusoidal active uptake from the blood into the liver may often be the rate limiting step for drugs cleared by biliary excretion (Yamazaki et al., 1996) and a similar inference comes from a recent study of 123 compounds showing significant excretion into rat bile and having considerable overlap in the physicochemical space occupied by human OATP and rat oatp substrates (Varma et al., 2012). Given this, the observation that rat biliary clearance exceeds that of human once normalized for body weight is perhaps not surprising, because functionally rat hepatic uptake transporters are more efficient than their human counterparts, whereas dog hepatic uptake rates appear similar or lower than for humans when one compares hepatocyte uptake data for the same actively transported drugs (Soars et al., 2007; Grime et al., 2008; Gardiner and Payne, 2011; Wilby et al., 2011).

On the basis of these observations, we postulate that when drugs do not require active hepatic uptake to access the liver, there may be fairly insignificant differences in rat, dog, and human biliary clearance once normalized for body weight and plasma protein binding differences. Conversely, when the organic anion-transporting polypeptide drug transporters are involved, one may expect at least a 10-fold discrepancy in rat to human biliary clearance (Fig. 2A). Additionally, on the basis of very limited dog to human findings, one may expect very little dog/human discrepancy in biliary clearance regardless of the processes involved. Ultimately this may infer that in vitro hepatocyte active uptake intrinsic clearance data may be used to predict total hepatic clearance without the need to define the final fate of the drug, be it metabolized or excreted in the bile. This certainly appears to be the case for pravastatin (Soars et al., 2007). Although this is only an emerging perspective based on an analysis of the available literature, we believe it to be a useful indicator to Drug Discovery DMPK scientists handling rat biliary clearance data in a preclinical setting when rapid but educated decisions are required.

Toward drug candidate selection at the preclinical/clinical development interface, biliary clearance data from multiple species will of course help in minimizing the risk of a poor human prediction (Morris et al., 2012) and a pharmacokinetic modeling/simulation approach, taking into account all the relevant processes and in vitro data to facilitate more precise predictions of human biliary clearance should be a strong consideration (Kusuhara and Sugiyama 2010; Swift et al., 2010). Future work to further understanding should certainly involve a detailed package of in vitro work with the drugs discussed here to define the active uptake intrinsic clearance values in rat, dog, and human hepatocytes and to more fully define the transporter involved a detailed package of in vitro work with the drugs discussed should be a strong consideration (Kusuhara and Sugiyama 2010; Swift et al., 2010).

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