Commentary

Examining Physiologically Based Pharmacokinetic Model Assumptions for Cross-Tissue Similarity of Activity per Unit of Enzyme: The Case Example of Uridine 5’-Diphosphate Glucuronosyltransferase

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ABSTRACT

The default assumption during in vitro in vivo extrapolation (IVIVE) to predict metabolic clearance in physiologically based pharmacokinetics (PBPK) is that protein expression and activity have the same relationship in various tissues. This assumption is examined for uridine 5’-diphosphate glucuronosyltransferases (UGTs), a case example where expression and hence metabolic activity are distributed across various tissues. Our literature analysis presents overwhelming evidence of a greater UGT activity per unit of enzyme (higher kcat) in kidney and intestinal tissues relative to liver (greater than 200-fold for UGT2B7). This analysis is based on application of abundance values reported using similar proteomic techniques and within the same laboratory. Our findings call into question the practice of assuming similar kcat during IVIVE estimations as part of PBPK and call for a systematic assessment of the kcat of various enzymes across different organs. The analysis focused on compiling data for probe substrates that were common for two or more of the studied tissues to allow for reliable comparison of cross-tissue enzyme kinetics; this meant that UGT enzymes included in the study were limited to UGT1A1, 1A3, 1A6, 1A9, and 2B7. Significantly, UGT1A9 (n = 24) and the liver (n = 27) were each found to account for around half of the total dataset; these were found to correlate with hepatic UGT1A9 data found in 15 of the studies, highlighting the need for more research into extrahepatic tissues and other UGT isoforms.

SIGNIFICANCE STATEMENT

During physiologically based pharmacokinetic modeling (in vitro in vivo extrapolation) of drug clearance, the default assumption is that the activity per unit of enzyme is the same in all tissues. The analysis provides preliminary evidence that this may not be the case and that renal and intestinal tissues may have almost 250-fold greater uridine 5’-diphosphate glucuronosyltransferase activity per unit of enzyme than liver tissues.

Introduction

Applications of physiologically based pharmacokinetics (PBPK) over the last 20 years have increased exponentially compared with the rest of pharmacokinetics (El-Khateeb et al., 2021). This has been linked to the ability of PBPK models to extrapolate kinetics beyond the average patient by using fundamental aspects of the biology related to the change in the expression in enzymes between healthy individuals and various patient groups (Howard et al., 2018).

The default assumption during the in vitro in vivo extrapolation (IVIVE) steps of metabolic information for drug clearance during PBPK is that expression mirrors activity regardless of the location of the enzyme. In other words, the activity per unit of enzyme (kcat) is considered to be the same in various tissues. We wished to examine this common assumption for the case example of uridine 5’-diphosphate glucuronosyltransferase (UGT) enzymes. These enzymes are involved in phase II biotransformation of many drugs, and they are currently the second most common route for primary drug metabolism, responsible for the metabolic clearance of 10%–30% of all drugs (Stingl et al., 2014). This proportion is set to increase, as pharmaceutical companies are intentionally designing new drug candidates that go through non-cytochrome P450 (CYP450) pathways to reduce the burden of CYP450-related drug-drug interactions (DDIs) (Achour et al., 2014).

Previous research has identified the liver as the epicenter of xenobiotic metabolic processes, containing the most diverse and abundant population of drug-metabolizing enzymes (Achour et al., 2014). However, some may argue that the contributions of other key metabolic tissues...
involved in drug disposition have been neglected or underestimated. Studies involving extrahepatic metabolism are very limited compared with hepatic metabolism (Scotcher et al., 2016), and to build a clinically realistic model of the human body, the involvement of enzyme kinetics across extrahepatic tissues must be quantified. This is as true of UGTs as of other enzymes. UGT enzyme quantities in the liver do not have a complete set of corresponding expression values in renal and intestinal tissues (Achour et al., 2014; Couto et al., 2020; Al-Majdoub et al., 2021). This highlights the need to generate a reliable dataset for absolute enzyme abundances across the key metabolic organs as a starting point for quantifying tissue-specific enzyme kinetics.

To begin quantifying UGT enzyme kinetics per unit of enzyme, absolute abundance for individual UGT isoforms must be determined as amount of enzyme per milligram of microsomal protein (Crewe et al., 2011). \( V_{\text{max}} \) measured as amount of isofrom-specific probe substrate converted to its metabolite per unit time must also be determined. The enzyme abundance-activity relationship can then be quantified as \( k_{\text{cat}} \). To accurately reflect tissue-specific kinetics, \( k_{\text{cat}} \) must account for tissue-specific enzyme abundances (Robinson, 2015). The common assumption that \( k_{\text{cat}} \) is the same across various tissues has not been examined for UGTs as of other enzymes. UGT enzyme quantities in the liver do not have a complete set of corresponding expression values in renal and intestinal tissues (Achour et al., 2014; Couto et al., 2020; Al-Majdoub et al., 2021).

Where \( V_{\text{max}} \) was not specified for activity, \( K_m \) values (substrate concentration at 1/2 \( V_{\text{max}} \)) were identified for the probe substrates; if the probe concentration (\( \mu \text{M} \)) was found to be significantly above the maximum \( K_m \) value (\( \mu \text{M} \) i.e., \( >2 \)-fold), the assumption was made that the reaction was conducted at \( V_{\text{max}} \) and these data were used to calculate \( k_{\text{cat}} \). On the other hand, if the probe concentration (\( \mu \text{M} \)) was found to be significantly below the minimum \( K_m \) value (\( \mu \text{M} \) i.e., \(<0.5\)-fold), the assumption was made that the activity value was within the intrinsic clearance range and the clearance was used as a supplementary measurement of enzyme activity. Here, the literature was examined to identify reported probe \( K_m \) values, and where this information was not available, reference \( K_m \) values were found (Seo et al., 2014; Miners et al., 2021). We have to acknowledge that our assumption will result in significant errors in the calculation of these parameters depending on how far the substrate concentrations deviate from those related to initial rate [in which case, UGT intrinsic clearance (\( CL_{\text{int, UGT}} = V_{\text{max}}/K_m \)) and \( V_{\text{max}} \). However, the error introduced by this approach will be less that associated with the comparison of kinetic data from studies that used vastly different experimental conditions. Where necessary, intrinsic clearance data were corrected for the microsomal fraction of unbound drug to give unbound intrinsic clearance (\( CL_{\text{int, u}} \) or a closer estimate for in vivo clearance (Hallifax and Houston, 2006; Gao et al., 2008). Once the corrected clearance values had been determined, \( CL_{\text{int, u}} (\mu \text{M/min/mg microsomal protein}) \) values were divided by the abundance (pmol/mg protein) and probe concentrations (\( \mu \text{M} \)) to give \( CL_{\text{int, u}}/\text{per unit enzyme} (\mu \text{M/min/pmol enzyme}) \).

### Results

#### Filtering Data

A total of 19 studies were used in this analysis; 15 of these were relevant for calculating \( k_{\text{cat}} \) (Soars et al., 2001, 2003; Miles et al., 2005; Picard et al., 2005; Al-Jahdari et al., 2006; Shimizu et al., 2007; Benoît-Biancamano et al., 2009; Rowland et al., 2008; Komura and Iwaki, 2011; Liang et al., 2011; Walsky et al., 2012; Gill et al., 2013; Knights et al., 2016; Achour et al., 2017, 2018; Chen et al., 2018), whereas the remaining four were used for calculating \( CL_{\text{int, u}} \) (Cubitt et al., 2009; Gill et al., 2012; Scotcher et al., 2017; Bhatt et al., 2019). Of the total dataset, 29% of the data did not meet the search criteria and were excluded on account of the following: no probe specificity, no availability of abundance data, or not falling into the \( V_{\text{max}} \) or intrinsic clearance range. The data useful in this analysis are summarized in Table 1. Probes were selected based on availability of data across two or (preferably) all of the studied tissues; because data were very limited, it was necessary to focus on availability rather than specificity of probes. Data were available for UGT1A1, 1A6, 1A9, and 2B7 for \( k_{\text{cat}} \) calculations. Only UGT1A6, with probe substrate deferiprone, was comparable across all three tissues (Benoît-Biancamano et al., 2009; Knights et al., 2016); UGT1A1 was comparable across the liver.

### Materials and Methods

#### Collection of Data

Two electronic databases, Web of Science (https://wok.mimas.ac.uk) and PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), were searched for relevant literature from the years 2000 to 2019 using appropriate keywords (UDP-glucuronosyltransferase, UGT activity). Both UGT abundance and activity studies were searched for glucuronidation data; this involved searching for other key terms in place of ‘activity’ to widen the search scope (glucuronidation, \( k_{\text{cat}} \), metabolism, abundance, concentration, content, quantification, measurement, LC-MS, ELISA, Western blotting). Citation lists within the collected studies were also inspected to identify any further relevant literature. Searches were species and tissue-specific for human intestinal and kidney microsomes; keywords included synonyms for these tissues (gut, renal). The search criteria were repeated for human liver microsomes, focusing on literature using the probe substrates identified in renal and intestinal studies, to compile a database of comparable data. All but one publication included data from ‘adult’ populations; hence only data generated from adult tissue samples were included for analysis.

#### Calculation of Enzyme Activity

The \( k_{\text{cat}} \) values for individual UGT isoforms were calculated using eq. 1, where \( V_{\text{max}} \) represents the maximal metabolic capacity in pmol/min/mg microsomal protein and UGT abundance is tissue-specific for individual isoforms in pmol UGT/mg protein:

\[
k_{\text{cat}}(\text{pmol/min/pmols UGT}) = \frac{V_{\text{max}}(\text{pmol/min/pmols protein})}{\text{UGT abundance (pmol UGT/mg protein)}}
\]

Where \( V_{\text{max}} \) was not specified for activity, \( K_m \) values (substrate concentration at 1/2 \( V_{\text{max}} \)) were identified for the probe substrates; if the probe concentration (\( \mu \text{M} \)) was found to be significantly above the maximum \( K_m \) value (\( \mu \text{M} \) i.e., \( >2 \)-fold), the assumption was made that the reaction was conducted at \( V_{\text{max}} \) and these data were used to calculate \( k_{\text{cat}} \). On the other hand, if the probe concentration (\( \mu \text{M} \)) was found to be significantly below the minimum \( K_m \) value (\( \mu \text{M} \) i.e., \(<0.5\)-fold), the assumption was made that the activity value was within the intrinsic clearance range and the clearance was used as a supplementary measurement of enzyme activity. Here, the literature was examined to identify reported probe \( K_m \) values, and where this information was not available, reference \( K_m \) values were found (Seo et al., 2014; Miners et al., 2021). We have to acknowledge that our assumption will result in significant errors in the calculation of these parameters depending on how far the substrate concentrations deviate from those related to initial rate [in which case, UGT intrinsic clearance (\( CL_{\text{int, UGT}} = V_{\text{max}}/K_m \)) and \( V_{\text{max}} \). However, the error introduced by this approach will be less that associated with the comparison of kinetic data from studies that used vastly different experimental conditions. Where necessary, intrinsic clearance data were corrected for the microsomal fraction of unbound drug to give unbound intrinsic clearance (\( CL_{\text{int, u}} \) or a closer estimate for in vivo clearance (Hallifax and Houston, 2006; Gao et al., 2008). Once the corrected clearance values had been determined, \( CL_{\text{int, u}} (\mu \text{M/min/mg microsomal protein}) \) values were divided by the abundance (pmol/mg protein) and probe concentrations (\( \mu \text{M} \)) to give \( CL_{\text{int, u}}/\text{per unit enzyme} (\mu \text{M/min/pmol enzyme}) \).
and intestine (Komura and Iwaki, 2011); and data for UGT1A9 and 2B7 were comparable across the liver and kidney (Miles et al., 2005; Komura and Iwaki, 2011; Knights et al., 2016). Intrinsic clearance calculations allowed for comparison of UGT1A1 and 1A3 across all three tissues using probe substrates ezetimibe and telmisartan, respectively (Gill et al., 2012); UGT1A9 data were comparable across the liver and kidney (Gill et al., 2012; Scotcher et al., 2017; Bhatt et al., 2019); and data for UGT2B7 were comparable across the liver and intestine (Gill et al., 2012).

**Reference Abundance Values.** The collated reference abundance values (Achour et al., 2014; Couto et al., 2020; Al-Majdoub et al., 2021), presented in Table 2, were used to perform $k_{cat}$ and $CL_{int,u}$ calculations, where activity data did not have corresponding abundance values presented in the literature (Soars et al., 2001, 2003; Miles et al., 2005; Picard et al., 2005; Al-Jahdari et al., 2006; Shimizu et al., 2007; Benoit-Biancamano et al., 2009; Rowland et al., 2008; Cubitt et al., 2009; Komura and Iwaki, 2011; Liang et al., 2011; Gill et al., 2012, 2013; Walsky et al., 2012; Scotcher et al., 2017; Chen et al., 2018).

UGT isoforms included in the analysis were based on availability of common probes and activity across two or more of the tissues identified in the literature. Where activity is not specified as $V_{max}$ or $CL_{int,u}$, if probe concentration is significantly above the $K_m$ value (>2-fold), activity was assumed to be at $V_{max}$. For $k_{cat}$ assessment, probe substrate concentration and $K_m$ have also been recorded. Probe concentration was recorded across all $CL_{int,u}$ enzymes to calculate $CL_{int,u}$ per unit enzyme (see Materials and Methods). Key experimental differences that may have influenced the activities seen across the studies are recorded in Table 5.

To demonstrate the differences in the mean relative expressions and activities of UGT enzymes across the tissues, scatter graphs were generated with the $y$-axis in logarithmic scale ($\log_{10}$), demonstrating the ratio of fold difference of intestinal and renal abundances and activities relative to the liver (Figs. 1 and 2). Data for enzyme activities were segregated for $k_{cat}$ and $CL_{int,u}$ (Fig. 2, A and B). The reference line for the liver crosses the $y$-axis horizontally at 1; values above or below the line represent greater or fewer enzyme expression, respectively, found in the intestine and kidneys than that found in the liver.

**Discussion**

This analysis uniquely reviews a comprehensive list of all significant UGT activity studies conducted using comparable probe substrates for data used for calculating mean $k_{cat}$ (Table 3), where $V_{max}$ was not specified but probe concentration was found to be more than 2-fold greater than the substrate $K_m$ (Knights et al., 2016). Similarly, for $CL_{int,u}$ calculations, where activity data were not specified as $V_{max}$, it was assumed to be in the intrinsic clearance range if probe concentration was found to be less than 0.5-fold of the substrate concentration (Bhatt et al., 2019). Where activity was assumed to be at $V_{max}$ or intrinsic clearance, probe substrate concentration and $K_m$ have also been recorded. Probe concentration was recorded across all $CL_{int,u}$ enzymes to calculate $CL_{int,u}$ per unit enzyme (see Materials and Methods).

**Correlation of UGT Expression and Activity between Different Tissues.** The reported activity values for human liver, intestinal, and kidney microsomes used to calculate $k_{cat}$ and $CL_{int,u}$ are demonstrated in Tables 2 and 4, respectively. Some assumptions for $V_{max}$ were made for data used for calculating mean $k_{cat}$ (Table 3), where $V_{max}$ was not specified but probe concentration was found to be more than 2-fold greater than the substrate $K_m$ (Knights et al., 2016). Similarly, for $CL_{int,u}$ calculations, where activity data were not specified as $V_{max}$, it was assumed to be in the intrinsic clearance range if probe concentration was found to be less than 0.5-fold of the substrate concentration (Bhatt et al., 2019). Where activity was assumed to be at $V_{max}$ or intrinsic clearance, probe substrate concentration and $K_m$ have also been recorded. Probe concentration was recorded across all $CL_{int,u}$ enzymes to calculate $CL_{int,u}$ per unit enzyme (see Materials and Methods). Key experimental differences that may have influenced the activities seen across the studies are recorded in Table 5.

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human liver, intestinal, and kidney microsomal tissues from 2000 to 2019. Nevertheless, it only provides a preliminary database of mean activity values, as \( k_{\text{cat}} \) and \( \text{CL}_{\text{int,u}} \) for comparable UGT isoforms for the three major metabolic organs since the functional assays were not conducted in the same laboratory nor under exactly similar conditions. We were able to map a ratio of fold difference for the intestine and kidney relative to liver for UGT enzyme abundance but more importantly activity per unit of enzyme. The intestinal and kidney abundance data, which we used for calculating activity per unit of enzyme, were all taken from a single laboratory (University of Manchester; Table 2). A meta-analysis conducted by Achour et al. (2014) presented weighted average abundance values for liver from data published between 1980 and 2014 that were measured using liquid chromatography–mass spectrometry (LC-MS) proteomics and that were used for calculating \( k_{\text{cat}} \) values in liver. Mean renal and intestinal (from kidney cortex) activity per unit of enzyme. The intestinal and kidney abundance data, which we used for calculating activity per unit of enzyme, were all taken from a single laboratory (University of Manchester; Table 2). A meta-analysis conducted by Achour et al. (2014) presented weighted average abundance values for liver from data published between 1980 and 2014 that were measured using liquid chromatography–mass spectrometry (LC-MS) proteomics and that were used for calculating \( k_{\text{cat}} \) values in liver.

### TABLE 4

<table>
<thead>
<tr>
<th>Enzyme/Identified Probe</th>
<th>Probe Conc. (mM)</th>
<th>( K_m ) (mM)</th>
<th>Liver Activity</th>
<th>Liver ( \text{CL}_{\text{int,u}} ) (pmol/mg protein)</th>
<th>Intestine Activity</th>
<th>Intestine ( \text{CL}_{\text{int,u}} ) (pmol/mg protein)</th>
<th>Kidney Activity</th>
<th>Kidney ( \text{CL}_{\text{int,u}} ) (pmol/mg protein)</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT1A1 Ecteinascidin</td>
<td>1</td>
<td>—</td>
<td>5180</td>
<td>126</td>
<td>1160</td>
<td>620</td>
<td>495</td>
<td>3540</td>
<td>(Gill et al., 2012)</td>
</tr>
<tr>
<td>UGT1A3 Telmisartan</td>
<td>1</td>
<td>—</td>
<td>395</td>
<td>12.6</td>
<td>91.4</td>
<td>80.2</td>
<td>34.3</td>
<td>245</td>
<td>(Gill et al., 2012)</td>
</tr>
<tr>
<td>UGT1A9 Propofol</td>
<td>20</td>
<td>98–127</td>
<td>603</td>
<td>1.67</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>(Bhatt et al., 2019)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>—</td>
<td>201</td>
<td>1.26</td>
<td>1020</td>
<td>78.1</td>
<td>—</td>
<td>—</td>
<td>(Gill et al., 2012)</td>
</tr>
<tr>
<td>UGT1A9 MPA</td>
<td>1</td>
<td>—</td>
<td>233</td>
<td>7.28</td>
<td>—</td>
<td>—</td>
<td>1370</td>
<td>523</td>
<td>(Gill et al., 2012)</td>
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<tr>
<td>UGT2B7 Naloxone</td>
<td>10</td>
<td>423–870</td>
<td>59.6</td>
<td>0.163</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>(Bhatt et al., 2019)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>—</td>
<td>17.4</td>
<td>0.200</td>
<td>21.4</td>
<td>16.0</td>
<td>—</td>
<td>—</td>
<td>(Cubitt et al., 2009)</td>
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<tr>
<td></td>
<td>1</td>
<td>—</td>
<td>55.6</td>
<td>0.639</td>
<td>14.2</td>
<td>10.6</td>
<td>52.7</td>
<td>34.7</td>
<td>(Gill et al., 2012)</td>
</tr>
</tbody>
</table>

**Notes:**
- *Probe concentration used to calculate \( \text{CL}_{\text{int,u}} \) per unit enzyme (see Materials and Methods).*
- *Where activity is not specified as \( \text{V}_{\text{max}} \) or \( \text{CL}_{\text{int,u}} \), if probe concentration was significantly below the \( K_m \) value (<0.5-fold), activity was assumed to be in the intrinsic clearance range.*
- *Activity after correction for fraction drug unbound in the microsomal incubation (\( f_{\text{mo}} \); Halifax and Houston, 2006; Gao et al., 2008).*
- *—, No data.*

### TABLE 5

<table>
<thead>
<tr>
<th>Literature</th>
<th>UDPGA (mM)*</th>
<th>Alamethicin*</th>
<th>MgCl2 (mM)</th>
<th>BSA (%)</th>
<th>Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Achour et al., 2018)</td>
<td>5</td>
<td>10(\mu)g/ml</td>
<td>5</td>
<td>—</td>
<td>100mM Tris-HCl, pH 7.5</td>
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<tr>
<td>(Al-Jahdari et al., 2006)</td>
<td>3</td>
<td>50(\mu)g/ml</td>
<td>8</td>
<td>—</td>
<td>50mM Tris-HCl, pH 7.6</td>
</tr>
<tr>
<td>(Benoit-Biancamano et al., 2009)</td>
<td>2</td>
<td>—</td>
<td>10</td>
<td>—</td>
<td>50mM Tris-HCl, pH 7.7</td>
</tr>
<tr>
<td>(Bhatt et al., 2019)</td>
<td>2.5</td>
<td>100(\mu)g/ml</td>
<td>—</td>
<td>0.01</td>
<td>100mM phosphate, pH 7.4</td>
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<tr>
<td>(Chen et al., 2018)</td>
<td>—</td>
<td>25(\mu)g/ml</td>
<td>5</td>
<td>—</td>
<td>50mM Tris-HCl, pH 7.4</td>
</tr>
<tr>
<td>(Cubitt et al., 2009)</td>
<td>5</td>
<td>50(\mu)g/ml</td>
<td>10</td>
<td>—</td>
<td>100mM phosphate, pH 7.1</td>
</tr>
<tr>
<td>(Gill et al., 2012)</td>
<td>5</td>
<td>50(\mu)g/ml protein</td>
<td>3.45</td>
<td>1 to 2'</td>
<td>100mM phosphate, pH 7.1</td>
</tr>
<tr>
<td>(Gill et al., 2013)</td>
<td>5</td>
<td>50(\mu)g/ml</td>
<td>3.45</td>
<td>—</td>
<td>100mM phosphate, pH 7.1</td>
</tr>
<tr>
<td>(Knights et al., 2016)</td>
<td>5</td>
<td>50(\mu)g/ml protein</td>
<td>4</td>
<td>—</td>
<td>100mM phosphate, pH 7.4</td>
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<tr>
<td>(Komura and Iwaki, 2011)</td>
<td>2</td>
<td>50(\mu)g/ml protein</td>
<td>10</td>
<td>—</td>
<td>100mM Tris-HCl, pH 7.4</td>
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<tr>
<td>(Liaw et al., 2011)</td>
<td>5</td>
<td>25(\mu)g/ml</td>
<td>5</td>
<td>—</td>
<td>50mM Tris-HCl, pH 7.4</td>
</tr>
<tr>
<td>(Miles et al., 2005)</td>
<td>3</td>
<td>50(\mu)g/ml protein</td>
<td>10</td>
<td>—</td>
<td>75mM Tris-HCl, pH 7.45</td>
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<tr>
<td>(Picard et al., 2005)</td>
<td>2</td>
<td>—</td>
<td>10</td>
<td>—</td>
<td>100mM Tris-HCl, pH 7.4</td>
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<tr>
<td>(Rowland et al., 2008)</td>
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<td>50(\mu)g/ml protein</td>
<td>4</td>
<td>—</td>
<td>100mM phosphate, pH 7.4</td>
</tr>
<tr>
<td>(Scotcher et al., 2017)</td>
<td>5</td>
<td>50(\mu)g/ml protein</td>
<td>3.45</td>
<td>1</td>
<td>100mM phosphate, pH 7.4</td>
</tr>
<tr>
<td>(Shimizu et al., 2007)</td>
<td>3</td>
<td>50(\mu)g/ml</td>
<td>10</td>
<td>—</td>
<td>50mM Tris-HCl, pH 7.4</td>
</tr>
<tr>
<td>(Soars et al., 2001)</td>
<td>5</td>
<td>—</td>
<td>10</td>
<td>—</td>
<td>100mM Tris-maleate, pH 7.4</td>
</tr>
<tr>
<td>(Soars et al., 2003)</td>
<td>5</td>
<td>50(\mu)g/ml</td>
<td>1</td>
<td>—</td>
<td>100mM phosphate, pH 7.1</td>
</tr>
<tr>
<td>(Walsky et al., 2012)</td>
<td>5</td>
<td>10(\mu)g/ml</td>
<td>5</td>
<td>—</td>
<td>100mM Tris-HCl, pH 7.5</td>
</tr>
</tbody>
</table>

**Notes:**
- *Minimum 5\(\mu\)M required for reliable glucuronidation activities for \( k_{\text{cat}} \) and \( \text{CL}_{\text{int,u}} \) calculations (Miners et al., 2021).*
- *Factors influencing measured \( \text{V}_{\text{max}} \) specifically (Miners et al., 2021).*
- *Reduces substrate Km, impacting on measured \( \text{CL}_{\text{int,u}} \) (Miners et al., 2021).*
- *Increases glucuronidation activities, reflected as greater mean \( \text{CL}_{\text{int,u}} \) (Budée et al., 2019).*
- *UGT1A1, 2% BSA; UGT1A3, 1% BSA.*
- *—, No data.*
and renal tissues as both \( V_{\text{max}} \) and \( \text{CL}_{\text{int},u} \), there was a lack of intestinal data, although \( \text{UGT1A9} \) activity values were available for hepatic, intestinal, and renal tissues as both \( V_{\text{max}} \) and \( \text{CL}_{\text{int},u} \). These results therefore suggest that the relative contribution of drug metabolism by liver may have been assigned incorrectly for UGT substrates. This is when PBPK models assume the same metabolic clearance by UGT per unit of enzyme in various tissues. A renal \( k_{\text{cat}} \) of more than 200-fold greater than the liver (e.g., UGT2B7) can compensate greatly for a 300-fold lower abundance relative to liver (e.g., UGT1A1).

Contribution of any enzyme to overall kinetics also depends on other factors such as the blood flow to the organ and the topological arrangements related to the physiology and anatomy (Nishimura et al., 2007; Pang et al., 2019). In addition, enzyme-specific cofactors [e.g., UDP-glucuronic acid (UDPGA) is a glucuronic donor in glucuronidation reactions] are critical. Lack of UDPGA, which needs to be at least 5 mM for optimum glucuronidation activity, and simplification of kinetic analyses leads to loss of activity in all UGT isoforms. Although specific probe substrates have in fact been identified for UGT enzymes (Miners et al., 2021), the analysis in the current study was limited by the lack of enzyme activity data measured using these specific probe substrates—a common research gap for non-CYP450 enzymes (Argikar et al., 2016). Hence, the data in this study were limited by the nonspecificity of some of the probes used for measuring activity (i.e., ezetimibe and nalofoxone), as data were selected based on availability of comparable probes across the studied tissues. Nevertheless, most of the isoforms could be assessed as data were selected based on availability of comparable probes across the studied tissues.

The abundance measurements suffered much less from these concerns. We were able to use proteomic measurements generated in a single laboratory in this study, so the general lack of interlaboratory consistency in quantifying intestinal and renal UGT enzymes did not apply in the present study (Couto et al., 2020; Al-Majdoub et al., 2021). Moreover, hepatic data taken from the meta-analysis conducted by Achour et al. (2014) uses literature measuring abundance with LC-MS proteomic technology, much like the intestinal and renal studies, maintaining consistency in the standards used for measuring abundance.

![Fig. 1. Comparison of abundance values for UGT enzymes in the gut and kidney as a ratio to the liver. The liver has been used as a reference point, crossing the y-axis horizontally at 1. Plotting the y-axis in logarithmic scale (log10) demonstrates the fold difference in abundance for intestinal and renal tissues with respect to the liver (specific fold values labeled on the graph).](image-url)
Going forward, it is imperative to develop and use standardized experimental conditions for future UGT enzyme kinetic research to generate reliable cross-study comparisons.

In conclusion, this preliminary analysis provides a starting point for building tissue-specific IVIVE data. The methods are generalizable to other enzymes involved in drug metabolism. These are important for continuous improvements to PBPK simulations in the development of new drugs. As this case study for UGTs illustrates, accurate estimates of functional enzyme kinetics in various tissues are still limited. It would be desirable to conduct functional assays on the same samples as proteomic measurements to confirm the preliminary findings presented here. Nevertheless, our results suggest that $k_{cat}$ may vary from tissue to tissue, perhaps even within similar tissues depending upon disease state.

Authorship Contributions

Participated in research design: Ahmed, Rostami-Hodjegan, Al-Majdoub.

Performed data analysis: Ahmed.

Wrote or contributed to the writing of the manuscript: Ahmed, Rostami-Hodjegan, Barber, Al-Majdoub.

References


Fig. 2. Comparison of activity values ($k_{cat}$) (A) and intrinsic clearance ($CL_{int,k}$) (B) for UGT enzymes in the gut and kidney as a ratio to the liver. The liver has been used as a reference point, crossing the y-axis horizontally at 1. Plotting the y-axis in logarithmic scale (log10) demonstrates the fold difference in abundance for intestinal and renal tissues with respect to the liver (fold values labeled specifically on the graph). Error bars are included where there is sufficient data (i.e., $\geq 2$ data sets).
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Gill KL, Gertz M, Houston JB, and Galetin A (2013) Application of a physiologically based phar-