Test System-Dependent Clearance of CYP2D6 and CYP3A4/5 Substrates: A Comparison of Human Liver Microsomes and Cryopreserved Human Hepatocytes

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Introduction

The in vitro to in vivo extrapolation (IVIVE) of drug clearance involves the determination of intrinsic clearance in vivo (CLint) based on in vitro measurements of \( V_{\text{max}} \) or 

\[
\text{CLint} = \frac{V_{\text{max}}}{\text{Km}} \times \frac{66,000}{\text{PBSF}}.
\]

Microsomes were incubated with substrate (0.5 µM, 10 µM, 20 µM, 50 µM, and 100 µM) for 10 min at 37°C. Complexes of CYP3A4/5 and CYP2D6 activity were determined in human liver microsomes (pooled gender; n = 16) and compared with those determined in human liver microsomes from cryopreserved human hepatocytes. The in vivo intrinsic clearance of midazolam (CLH,int) was calculated as follows:

\[
\text{CLH,int} = \frac{\text{Vmax}}{\text{Km}} \times \frac{198,000}{\text{PBSF}}.
\]

Materials and Methods

Chemicals and test system:

Dextromethorphan and midazolam were purchased from Sigma-Aldrich (St. Louis, MO). Phorbol ester-free buffer (PEF) was prepared in XenoTech LLC (Lenexa, KS). Microsomes were isolated from human hepatocytes as described previously. The saponified microsomes were isolated from the supernatant fraction by ultracentrifugation. The resulting pellets were washed twice with a sucrose buffer (150 mM KCl and 10 mM EDTA at pH 7.4) and then resuspended in the same buffer. The microsomal pellets were resuspended in 250 mM sucrose. Protein concentration was determined with a Pierce BCA assay (Pierce Chemical, Rockford, IL). CYP3A4 and CYP2D6 activity in microsomes isolated from the pooled hepatocytes was compared with that in pooled human liver microsomes (both at 0.1 mg/mL) with midazolam and dextromethorphan at two substrate concentrations: 1 µM and 5.0 µM. The results are shown in Table 1.

Results

Table 1. Summary of scaled values of hepatic intrinsic clearance (CLH,int) from cryopreserved human hepatocytes and human liver microsomes

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Human liver microsomes</th>
<th>Cryopreserved human hepatocytes</th>
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<tbody>
<tr>
<td>Midazolam</td>
<td>56.0 ± 1.1</td>
<td>147.0 ± 2.1</td>
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<tr>
<td>Dextromethorphan</td>
<td>81.0 ± 1.0</td>
<td>102.0 ± 1.3</td>
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Table 2. Comparison of metabolic rates between microsomes isolated from cryopreserved human hepatocytes and a standard preparation of human liver microsomes

<table>
<thead>
<tr>
<th>Protein Source</th>
<th>Rate (pmol/min)</th>
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<tr>
<td>HLM (n = 200)</td>
<td>263 1132 45 259</td>
</tr>
<tr>
<td>HLM isolated from CHH (n = 50)</td>
<td>206 893 46 220</td>
</tr>
</tbody>
</table>

Figure 1. The in vitro clearance of dextromethorphan and midazolam from pooled cryopreserved human hepatocytes (n = 50; 1x10⁶ cells/mL) at 1 µM and a substrate concentration equivalent to their in vivo plasma concentrations. The estimates of intrinsic clearance determined in human liver microsomes and cryopreserved human hepatocytes are compared. The in vivo intrinsic clearance of midazolam was 45.0 L/h, while the half-life of midazolam in microsomes was estimated to be 50 min (compared with 60 min in hepatocytes).

Figure 2. The in vitro clearance of dextromethorphan and midazolam in pooled human liver microsomes at 0.33 µM, a concentration equivalent to the microsomal content of cryopreserved human hepatocytes at 1x10⁶ cells/mL.

Figure 3. Kinetics for dextromethorphan (CYP2D6) and 1'-hydroxymidazolam (CYP3A4/5) formation in pooled human liver microsomes.

Conclusions

• The scaled CLH,int for the low intrinsic clearance CYP2D6 substrate midazolam agrees well between human liver microsomes and cryopreserved human hepatocytes.

• The scaled CLH,int for the high intrinsic clearance CYP3A4 substrate midazolam is significantly different between human liver microsomes and cryopreserved human hepatocytes, confirming previous reports that clearance of high turnover substrates for CYP3A4 in human liver microsomes is substantially greater (by an order of magnitude or more) than that in human hepatocytes.

• Microsomes isolated from cryopreserved human hepatocytes have roughly the same CYP3A4/5 and CYP2D6 activity as those in pooled human liver microsomes.

• These results suggest that some factor, such as membrane permeability, cofactor availability or an unknown factor substantially limits the rate of metabolism of midazolam by CYP3A4/5 in human hepatocytes.

References