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Introduction

Cytokrom P450 (CYP) isofoms are responsible for the metabolism of the majority of drugs in human. An intrinsic clearance (CLint) determined from in vivo intravenous clearance can be used to predict the in vivo hepatic metabolic clearance (CLH) of drugs in humans. However, in vitro to vivo extrapolation (IVIVE) commonly underestimates the in vitro CLint and CLH of drugs metabolized by CYP isofoms.

Recently, it was reported that the addition of albumin to the in vitro incubation system increased the metabolic activities of some CYP isofoms due to inhibition of the effect of polyunsaturated long-chain fatty acids (PUFAs). Therefore, we evaluated the effect of bovine serum albumin (BSA) on the metabolic activities of CYP isofoms in human liver microsomes (HLM).

This study characterized the effect of albumin (BSA) supplementation on human liver CYP activities. An intrinsic clearance (CLint) was calculated using the average value of metabolic activity and t1/2 in each BSA concentration.

Methods

<Measurement of CYP metabolic activities using HLM and BSA>

Table 1 Reaction conditions for each CYP isofom

<table>
<thead>
<tr>
<th>CYP isofom</th>
<th>Metabolic activity</th>
<th>BSA concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td></td>
<td>0% BSA</td>
</tr>
<tr>
<td>CYP2B6</td>
<td></td>
<td>0% BSA</td>
</tr>
<tr>
<td>CYP2C9</td>
<td></td>
<td>0% BSA</td>
</tr>
<tr>
<td>CYP2C19</td>
<td></td>
<td>0% BSA</td>
</tr>
<tr>
<td>CYP3A</td>
<td></td>
<td>0% BSA</td>
</tr>
</tbody>
</table>

Incubation samples contained phosphate buffer (50 mM, pH 7.4) including fatty acid-free BSA (0.1, 2 and 4%), HLM (0.1 mg/ml protein), and mixed gender, pooled pool of 56, Sekisui Xeno Tech, LLC, NADPH generating system (Coming) and each model substrates (the concentrations were set to around 1/5 of K0.5). Reactions were performed at 37°C. The incubation time was 5 min. CYP2B6, 2C9 and 3A (Mizalidim), 3A (CYP3A) and CYP3A (Mizalidim) were labeled with acetylated solution containing internal standard.

<Protein binding of each CYP model substrates in HLM and BSA>

Protein binding was determined by equilibrium dialysis using Rapid Equilibrium Dialysis Device (RED Device, Thermostich Scientific). Protein solution samples (Donor side) contained phosphate buffer (50 mM, pH 7.4) including fatty acid-free BSA (0.1, 2 and 4%), HLM (0.1 mg/ml protein), and each model substrates (the concentrations were set to around 1/5 of K0.5). Reactions were performed at 37°C. The reaction time was 5 min. CYP2B6, 2C9, 2C19, 2D6, CYP3A (Testosterone) and CYP3A (Mizalidim) were labeled with acetylated solution containing internal standard.

<Calculation of metabolic activity>

Metabolic activity of each CYP isofom was calculated according to the following equation.

\[
\text{Metabolic activity} = \frac{\text{Marker metabolic concentration (nm)}}{\text{HLM concentration} \times \text{Inubation time (min/mg/ protein)}}
\]

<Calculation of f_unbound>

The fraction unbound in the microsomal binding of each CYP model substrate was calculated to the following equation.

\[
f_u = \frac{\text{CL}_\text{int}}{\text{CL}_\text{int,app}}
\]

\[
\text{CL}_\text{int,app} = \frac{\text{Activity}\times (\text{protein} \times \text{mg/ protein})}{\text{Molar substrate concentration} \times \text{t}_1/2}
\]

Table 2 CLint and t1/2 each CYP isofom by HLMs in the presence and absence of BSA

<table>
<thead>
<tr>
<th>CYP isofom</th>
<th>0% BSA</th>
<th>1% BSA</th>
<th>2% BSA</th>
<th>4% BSA</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>1.00</td>
<td>0.738</td>
<td>0.527</td>
<td>0.388</td>
<td>0.975</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>1.00</td>
<td>0.590</td>
<td>0.862</td>
<td>1.14</td>
<td>0.857</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>0.769</td>
<td>0.458</td>
<td>0.769</td>
<td>0.458</td>
<td>0.926</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>0.855</td>
<td>0.672</td>
<td>0.980</td>
<td>2.28</td>
<td>0.897</td>
</tr>
<tr>
<td>CYP3A</td>
<td>0.843</td>
<td>0.728</td>
<td>0.673</td>
<td>1.33</td>
<td>0.532</td>
</tr>
</tbody>
</table>

Results

From Table 2, CYP3A (Testosterone) and CYP3A (Mizalidim) did not show significant alteration in CLint and t1/2 by adding BSA (Table 2 and Figure 1). This study indicated that the effect of BSA on CYP activities in HLM is substrate dependent.

Discussion

This study characterized the intrinsic effect of BSA (supplementation on human liver microsomal CYP activities. CLint of CYP2B6 (0.954 μL/min/mg protein) was almost the same as that of CYP2C9 (0.897 μL/min/mg protein) in the presence of 1% BSA. We showed that the effect of BSA on the metabolic activity of each CYP isofom in HLM. These results may help us to evaluate the metabolic intrinsic clearance of CYP isofoms.

References