Overview

Purpose

• Create three separate custom pools of cryopreserved human hepatocytes (low, moderate, and high activity from 75 donors).
• Compare activity across six aldehyde oxidase (AO) substrates that range in clearance (CL).
• Conduct in vitro to in vivo correlation (IVIVC) analysis to assess scaling approaches.

Method

• Five individual donors were selected from the low (<30 mL/min/kg), moderate (30-60 mL/min/kg), and high (>60 mL/min/kg) AO activity ranges and pooled to create custom lots of cryopreserved human hepatocytes, which were compared to a standard commercial 10-donor pool (Lot EAV).
• Analysis was performed by high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC/MS/MS).

Results

• Under-prediction was still noted when comparing in vitro intrinsic clearance to in vivo intrinsic clearance.
• When hepatic clearance was scaled using the well-stirred model and compared to in vivo total clearance, the predictions were within 2-fold for each of the tested AO substrates, with the exception of XK-469.
• Custom pooling of human hepatocytes for maximal AO activity may help minimize under-prediction of total clearance, but factors involved in why in vivo intrinsic clearance is still drastically under-predicted and require further research.

Introduction

Aldehyde oxidase is a cytosolic drug-metabolizing enzyme that has emerged in recent years due to the reported negative impact on numerous clinical programs (1).
• Multiple literature reports have pointed towards an under-prediction of clearance for substrates of AO (2,3), with multiple potential causes (e.g., extra-hepatic AO contribution, polymorphisms in genes involved in AO activity).
• Custom pools of human hepatocytes prepared with pre-selected donors based on their individual activity may be an approach to maximize activity and, thus, minimize under-prediction of metabolic clearance (4).
• Previously published work in human hepatocytes has identified a high level of variability of AO activity across 75 donors (Figure 1), which translates to measuring low-to-high activity (5).

Figure 1. Characterization of AO Activity in Cryopreserved Human Hepatocytes from 75 Individual Donors (Originally published by Hutzel et al. (3)).

Methods

Hepatocyte Incubations

Five individual donors were selected from the low (<30 mL/min/kg), moderate (30-60 mL/min/kg), and high (>60 mL/min/kg) AO activity ranges and pooled to create custom lots of cryopreserved human hepatocytes, which were compared to a standard commercial 10-donor pool (Lot EAV).

Table 1. IVIVC Analysis for Tested AO Substrates

<table>
<thead>
<tr>
<th>AO Substrate</th>
<th>In Vitro Intrinsic</th>
<th>In Vivo Intrinsic</th>
<th>Custom/EAV Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>XK-469</td>
<td>39.9 ± 19 mL/min/kg</td>
<td>98.5 ± 7.1 mL/min/kg</td>
<td>2.5</td>
</tr>
<tr>
<td>Zaleplon</td>
<td>15.7</td>
<td>7.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Zaniporide</td>
<td>15.4</td>
<td>16.0</td>
<td>1.0</td>
</tr>
<tr>
<td>O-Benzylguanine</td>
<td>17.2</td>
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Hepatocyte Incubation Conditions

- Pooled Cryopreserved Human Hepatocytes
  1) Three custom prepared pools consisting of 5 donors each with pre-determined AO activity
  2) Three custom prepared pools consisting of 5 donors each with pre-determined AO activity

Calculations

To predict CLint from in vitro data, scaling factors of 120 x 10⁵ hepatocytes/g liver and 25.7 g liver/kg were used (Equation 1), where t = step of time (min) vs. LN Remaining. Inc = incubation volume, and BW = body weight.

Equation 1: In Vivo Intrinsic Clearance:

\[
\text{CL}_{\text{int}} = \frac{\text{Inc} \times \text{Vol} (\text{ml})}{120 \times 10^5 \times \text{Hepatocytes} \times \text{g Liver}}
\]

Figure 2. Substrate Depletion Plots for AO Substrates Tested in Separate Lots of Cryopreserved Human Hepatocytes

Table 2. Summary of Intrinsic Clearance (CLint) and Custom-Pooled Cryopreserved Human Hepatocytes (Low, Moderate, and High AO)

<table>
<thead>
<tr>
<th>AO Substrate</th>
<th>Standard (Lot EAV)</th>
<th>Custom (Honeywell)</th>
<th>custom/EAV Ratio</th>
</tr>
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<tr>
<td>Zaleplon</td>
<td>15.7 ± 10.9 mL/min/kg</td>
<td>15.4 ± 15.6 mL/min/kg</td>
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</tr>
<tr>
<td>O-Benzylguanine</td>
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Conclusions

• Predicting human clearance for AO substrates remains challenging.
• In general, standard commercial lots of pooled human cryopreserved hepatocytes have not been characterized for AO activity by vendors, but reported data (3) has found that while activity is measurable, it is not reflective of the true in vivo activity.
• Custom pooling of cryopreserved hepatocytes from donors with high activity demonstrated ≥2 to 3-fold higher activity than commonly used lot of pooled cryopreserved hepatocytes.

Figure 3. Substrate Depletion Plots of AO Substrates for IVIVC Analysis Comparison

- Activities for all substrates tested trended in a similar fashion, with intrinsic clearance values rank-ordering as: high AO pool > moderate AO custom pool > commercial pool EAV > low AO custom pool.
- The activity in the high AO custom pool was roughly 2- to 3-fold higher than the commercial pool EAV.

Custom-Pooled Cryopreserved Hepatocytes from donors with high activity demonstrated ≥2 to 3-fold higher activity than commonly used lot of pooled cryopreserved hepatocytes.