EVALUATION OF HEPATIC DRUG UPTAKE IS AN IMPORTANT DISCIPLINE IN DRUG DEVELOPMENT

- Evaluation of hepatic drug uptake is an important discipline in drug development.
- DMPK: Definition of hepatic clearance
- Pharmacology: Selection of drug candidates specifically targeting the liver
- Drug-Drug Interactions: Evaluate effects of known transporter inhibitors
- Toxicology: Eliminate drug candidates with hepatotoxicity due to drug accumulation in the liver
- In the evaluation of drug uptake in hepatocytes, organic solvents are commonly used in the preparation of stock solutions.
  - Organic solvents are known to inhibit P450 activities, especially CYP3A4
  - Their effects on drug uptake transporter activities have not been reported.
- We report here an evaluation of the effects of 4 commonly used organic solvents on pravastatin uptake in human hepatocytes:
  - DMSO
  - Acetonitrile
  - Ethanol
  - Methanol

999Elite™ Cryopreserved Human Hepatocytes

999Elite™ Plateable Cryopreserved Human Hepatocytes Lot1086 was used in the generation of the data.

999Elite™ hepatocytes have the following properties:
- >90% confluency
- >90% post-thawed viability
- >9 days in culture retaining the >90% confluency

999Elite™ Cryopreserved Human Hepatocytes are ideal for the following studies:
- Plateable hepatocytes uptake study (this poster)
- Efflux transport
- P450 induction (CYP1A2, CYP2B6, CYP3A4 as well as CYP2C8, CYP2C9 and CYP19)
- In vitro hepatotoxicity assays

Materials and Methods

Cell Culture Reagents: 999Elite™ cryopreserved human hepatocytes (Lot HH1086) was used for the study. The hepatocytes were recovered from cryopreservation using Universal Cryopreservation Recovery Medium (UCRM), and plated in Universal Cryopreservation Plating Medium (UCPM). Uptake studies were performed in Hepatocyte Incubation Medium (HQM) in collagen-coated 96-well Cell-a-Fix™ plates. All cell culture reagents were from In Vitro ADMET Laboratories (IVAL), Columbia, MD.

Procedures:
1. The 96-well collagen coated plates were seeded with 100-µl / well of HH1086 at 56k cells/well in UCPM and allowed to attach for 6 hrs.
2. Medium was changed to 50 µl of prewarmed HQM containing solvents at the designated concentrations and pre-incubated for 30 min.
3. At the end of the preincubation, 50 µl of pravastatin at 2X of the final concentrations containing 1X of the designated solvent concentration was added to each well.
4. The hepatocytes were incubated with pravastatin for 6 min, followed by aspiration of the medium and wash 3X with 200 µl HQM at 4 deg. C.
5. The hepatocytes were extracted with methanol and acetonitrile followed by quantification of pravastatin by LC/MS-MS (SCIEX API 5000).

RESULT

Transporter-Mediated Uptake of Pravastatin in 999Elite™ Human Hepatocytes from Multiple Donors:

<table>
<thead>
<tr>
<th>Lot</th>
<th>Speciation (µM)</th>
<th>Mean</th>
<th>Std Dev</th>
<th>% Inhibition of Pravastatin Uptake Mediated by Uptake Transporters</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH1117</td>
<td>Rifampin (25 µM)</td>
<td>5.27</td>
<td>0.57</td>
<td>69.29</td>
</tr>
<tr>
<td>HH1121</td>
<td>Rifampin (25 µM)</td>
<td>2.76</td>
<td>0.22</td>
<td>47.49</td>
</tr>
<tr>
<td>HH1086</td>
<td>Rifampin (25 µM)</td>
<td>2.33</td>
<td>0.65</td>
<td>62.04</td>
</tr>
<tr>
<td>HH1142</td>
<td>Rifampin (25 µM)</td>
<td>4.02</td>
<td>0.77</td>
<td>67.05</td>
</tr>
<tr>
<td>HH1144</td>
<td>Rifampin (25 µM)</td>
<td>3.20</td>
<td>0.60</td>
<td>36.76</td>
</tr>
<tr>
<td>HH1106</td>
<td>Rifampin (25 µM)</td>
<td>3.98</td>
<td>0.89</td>
<td>62.19</td>
</tr>
<tr>
<td>HH1143</td>
<td>Rifampin (25 µM)</td>
<td>6.69</td>
<td>0.95</td>
<td>67.15</td>
</tr>
<tr>
<td>HH1137</td>
<td>Rifampin (25 µM)</td>
<td>3.85</td>
<td>0.24</td>
<td>67.08</td>
</tr>
<tr>
<td>HH1113</td>
<td>Rifampin (25 µM)</td>
<td>3.81</td>
<td>0.60</td>
<td>49.73</td>
</tr>
<tr>
<td>HH103</td>
<td>Rifampin (25 µM)</td>
<td>3.81</td>
<td>0.37</td>
<td>57.07</td>
</tr>
</tbody>
</table>

**Morphology of Uptake Qualified**

- The hepatocytes were tightly attached onto the collagen-coated plates, thereby allowing the performance of uptake study reported here.
- Efflux transporter function is expressed after 4 days of culture.
- The hepatocytes can be cultured for >30 days, allowing the performance of prolonged experimentation.

**Effects of Organic Solvents on Pravastatin Uptake**

Left: Inteferenceship of Pravastatin and Solvent Concentrations
Right: Dose-dependent Solvent Effects (1.56 µM Pravastatin)

**Summary and Conclusions**

1. The results show that organic solvents can affect drug uptake in human hepatocytes. Inhibitory effects were observed for DMSO, methanol and ethanol but not for acetonitrile.
2. DMSO at 1% and 2% increased pravastatin uptake at the highest concentration evaluated of 100 µM, resulting in linear rather than saturation kinetics. The result suggests that DMSO at these high concentrations may increase diffusion of pravastatin into the hepatocytes.
3. Acetonitrile as a solvent, while showing no evidence of inhibition, showed approx. 2X increases in uptake at the 2% concentrations.

**Conclusions**

Our results suggest that organic solvents may affect uptake transporter activities.

- In general, solvent concentrations should be kept at 0.1% or lower to avoid artifacts due to interaction of the solvents with the activity of the transporter or plasma membrane permeability.
- Use of IVAL 999Elite™ Plateable Cryopreserved Human Hepatocytes allows the generation of drug uptake information for the definition of drug properties in drug development.