In vitro ADME & PK

BSEP, MRP2, MRP3 and MRP4 Inhibition

Background Information

- BSEP (bile salt export pump; ABCB11) is an ATP binding cassette (ABC) efflux transporter located on the canalicular membrane of hepatocytes, and is the major transporter for the secretion of bile acids from hepatocytes into bile in humans.


- MRP2 (multidrug resistance associated protein 2; ABCC2), MRP3 (ABCC3) and MRP4 (ABCC4) are ATP binding cassette (ABC) efflux transporters located on the canalicular membrane (MRP2) or sinusoidal membrane (MRP3, MRP4) of hepatocytes.

- MRP3 and MRP4 efflux transporters are upregulated under cholestatic conditions suggesting they provide a protective role against bile acid-mediated hepatotoxicity by alleviating increases in intracellular bile acid concentrations, which may occur as a result of impaired biliary excretion due to inhibition of BSEP. Understanding whether a compound is able to inhibit MRP transporters may therefore provide useful additional information towards helping evaluate the risk of DILI.

- Cyprotex offer BSEP, MRP2, MRP3 and MRP4 inhibition assays which investigate inhibition of the uptake of prototypical probe substrates (taurocholic acid for BSEP and estradiol 17β-D-glucuronide for MRPs) into inside-out membrane vesicles overexpressing the human ABC-transporter of interest.

Protocol

- Test System
  Sf9 insect cell-derived or mammalian (HEK293) cell-derived inside-out membrane vesicles overexpressing a single transporter (BSEP, MRP2, MRP3 or MRP4) incubated in the presence of ATP and AMP (absence of ATP).

- Probe Substrate
  [3H]-Taurocholic acid
  [3H]-Estradiol 17β-glucuronide

- Test Article Concentrations
  6 concentrations plus 0 µM (triplicate wells) (final test article concentrations dependent on customer requirements)

- Time Points
  Dependent on transporter

- Analysis Method
  Radiochemical detection using scintillation counting

- Data Delivery
  IC₅₀
  Written report available on request

To find out more contact enquiries@cyprotex.com
Figure 1
BSEP-mediated taurocholic acid (A) and MRP-mediated estradiol 17β-D-glucuronide (B-D) transport in the presence of a range of concentrations of inhibitor expressed as a percentage of vehicle control (mean ± standard deviation; n=3-9 wells, triplicate incubations performed on 3 separate occasions).

Table 1
Inhibition of human BSEP- and MRP-mediated transport of the prototypical substrates, taurocholic acid and estradiol 17β-glucuronide, respectively.

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Substrate</th>
<th>Inhibitor</th>
<th>IC₅₀ ± Standard Deviation (µM)</th>
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</thead>
<tbody>
<tr>
<td>BSEP</td>
<td>Taurocholic acid</td>
<td>Ketoconazole</td>
<td>8.78 ± 1.25</td>
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<tr>
<td>MRP2</td>
<td>Estradiol 17β-glucuronide</td>
<td>MK-571</td>
<td>22.6 ± 6.38</td>
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<td>Terfenadine</td>
<td>33.5 ± 6.77</td>
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<tr>
<td>MRP3</td>
<td>Estradiol 17β-glucuronide</td>
<td>MK-571</td>
<td>56.8 ± 7.23</td>
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<tr>
<td></td>
<td></td>
<td>Fidaxomicin</td>
<td>1.06 ± 0.117</td>
</tr>
<tr>
<td>MRP4</td>
<td>Estradiol 17β-glucuronide</td>
<td>MK-571</td>
<td>0.555 ± 0.238</td>
</tr>
</tbody>
</table>

The incubation conditions for each of the species have been fully characterised for the chosen substrates based on time linearity and uptake kinetics (Vₘₚ and Kₘ).

The chosen substrate concentration is much lower than the determined Kₘ, and as such IC₅₀ equates to Kᵢ (assuming competitive inhibition).

References