Reactive Metabolite Detection Study -Cysteine Trapping-

Presented by Miki Fujishima
From Drug Development Solutions Center
➢ Drug-induced liver injury (DILI) is caused by various mechanisms and it is difficult to predict it accurately in one type of assay

➢ Conducting various experiments and making a comprehensive judgment leads to accurate DILI risk evaluation

Table 3. Selectivity and Specificity for the in Vitro Panel

<table>
<thead>
<tr>
<th>Severe and Marked concern</th>
<th>Low concern</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 or more signals</td>
<td>13</td>
</tr>
<tr>
<td>1 or less signals</td>
<td>14</td>
</tr>
</tbody>
</table>

\text{PPV (13/14) = 93\%}
\text{NPV (8/22) = 36\%}

\text{Sensitivity (13/27) = 48\%}
\text{Specificity (8/9) = 89\%}
\text{Correct = 58\%}

Table 5. Selectivity and Specificity for the Integrated in Vitro Hazard Matrix

<table>
<thead>
<tr>
<th>Marked and Severe concern</th>
<th>Low concern</th>
</tr>
</thead>
<tbody>
<tr>
<td>integrated in vitro</td>
<td>27</td>
</tr>
<tr>
<td>hazard detected</td>
<td>2</td>
</tr>
<tr>
<td>integrated in vitro</td>
<td>0</td>
</tr>
<tr>
<td>hazard not detected</td>
<td>7</td>
</tr>
</tbody>
</table>

\text{PPV (27/29) = 93\%}
\text{NPV (7/7) = 100\%}

\text{Sensitivity (27/27) = 100\%}
\text{Specificity (7/9) = 78\%}
\text{Correct = 94\%}
➢ Drug-induced liver injury (DILI) is caused by various mechanisms and it is difficult to predict it accurately in one type of assay

➢ Conducting various experiments and making a comprehensive judgment leads to accurate DILI risk evaluation

Reactive Metabolite detection study is very important factor for DILI prediction
Background

To evaluate reactive metabolites...

- Most accurate method is Covalent Binding study, but this study needs radio-labeled test article

- Therefore it is difficult to conduct it in early stage development

- Generally, in early stage, trapping study is conducted to evaluate reactive metabolites instead of Covalent Binding study
  Most major trapping study is Glutathione (GSH) trapping

This time, we has started to offer Cysteine (Cys) trapping study as an alternative study of GSH trapping

✓higher quantitativity ✓ higher throughput ✓ lower cost
Metabolic pathway of Nefazodone

Reactive Metabolites not detectable

GSH adduct
Detoxification, stabilization and detectable

DMD 2008;36(6):1016-29
## Advantage of Cys trapping

### Trapping Studies

<table>
<thead>
<tr>
<th></th>
<th>GSH trapping</th>
<th>Cys trapping</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trapping reagent</strong></td>
<td>³H- or ³⁵S-GSH</td>
<td>³⁵S-Cys</td>
</tr>
<tr>
<td><strong>Detection</strong></td>
<td>HPLC-RAD</td>
<td>HPLC-RAD Liquid Scintillation Counter</td>
</tr>
<tr>
<td><strong>Quantitativity</strong></td>
<td><strong>$^*$</strong></td>
<td>***</td>
</tr>
<tr>
<td><strong>Throughput</strong></td>
<td><strong>$^*$</strong></td>
<td><strong>$^*$</strong></td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td>High</td>
<td>Low</td>
</tr>
</tbody>
</table>

*Our Method!*
Cys Trapping

Reagents
- Enzyme: 50-donor human liver microsomes (Sekisui XenoTech)
- Cofactors: NADPH and UDPGA
- Trapping reagent: Radio-labeled cysteine ($^{35}$S)

Assay procedure
- Mix reagents and incubate at 37°C for 60 min
- Stop the reaction and separate the cys-adduct and non-adduct by solid phase extract plate
- Measure the radioactivity of adduct fraction by liquid scintillation counter
Study Design
- 1 positive control (e.g. Nefazodone) and 1 solvent control
- 1 concentration (100 uM)
- 30 compounds can be evaluated per plate

End point
Formation rate of reactive metabolite (pmol/h/mg protein)
Correlation with GSH trapping

Comparison with previously reported data of Cys trapping

<table>
<thead>
<tr>
<th>Compounds (pmol/h/mg protein)</th>
<th>SMD data</th>
<th>Previously reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>18.3</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Clozapine</td>
<td>944</td>
<td>429</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>329</td>
<td>257</td>
</tr>
<tr>
<td>Nefazodone</td>
<td>2631</td>
<td>2333</td>
</tr>
<tr>
<td>Rimonabant</td>
<td>0</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Troglitazone</td>
<td>358</td>
<td>265</td>
</tr>
</tbody>
</table>

Special Offer
By 20th March, 2022
We are offering special price for this study!
https://www.xenotech.com/about/events/65-off-new-cysteine-trapping-service-for-a-limited-time/

To get more detail information about this offer, please use Contact Us form on Sekisui XenoTech’s website https://www.xenotech.com/
Thank you for watching!