Use of complex hepatic models for a fit-for-purpose metabolism assessment of ingredients for use in Next Generation Risk Assessment

CHARLES RIVER-SOLVO Poster Blitz
10th May 2024
INTRODUCTION/BACKGROUND

• Next Generation Risk Assessments use a battery of in vitro tools and assays
• Metabolism framework/testing strategy developed
• Metabolism not always well assessed in in vitro cell assays (cell lines have poor metabolic capacity, exposure time not appropriate)
• We present two in vitro assays using of HepaRG cells
Design the HepaRG toroid:
- Optimisation of media
- Cell density with imaging to check sausage-like organoids formation
- mRNA expression of major CYPs
- Check agarose permeability for CYPS probes
- CYPs activity measured using probes (phenacetin, bupropion, testosterone, verapamil)
- LC-MS measurements of media and tissue lysates
HEPARG TOROID IN CO-CULTURE SYSTEM

Co-culture HepaRG Toroid and AR-Calux cells:

- Culture HepaRG toroid 10 days
- Add AR-Calux cells and wait 24h
- Treat with testosterone for 24h
- Effect of testosterone treatment is less pronounced in the AR-Calux cells when HepaRG toroid is present (detoxification)

Development of a human liver microphysiological co-culture system for higher throughput chemical safety assessment

Blanche C Ip 1,2, Samantha J Madnick 1,2, Sophia Zheng 1, Tessa C A van Tongeren 3, Susan J Hall 1, Hui Li 1, Suzanne Martin 4, Sandrine Spriggs 4, Paul Carmichael 4, Wei Chen 5, David Ames 5, Lori A Breitweiser 5, Heather E Pence 5, Andrew J Bowling 5, Kamin J Johnson 5, Richard Cubberley 4, Jeffrey R Morgan 1,2, Kim Boekelheide 1,2

Affiliations + expand

PMID: 38335931 DOI: 10.1093/toxsci/kfae018

HEPARG AS A TOOL TO RISK ASSESS REACTIVE METABOLITES

Cell Stress Panel is a high throughput imaging assay developed to assess up to 36 biomarkers.

Reactive metabolites formed in situ can influence levels of:

- GSH, ROS MMP, LDH and ATP
- DNA (damage)
- PLD and Steatosis

All these biomarkers have shown a good prediction for DILI but can be useful for more generic toxicities in cells.
HEPARG AS A TOOL TO RISK ASSESS REACTIVE METABOLITES

<table>
<thead>
<tr>
<th>Chemical ID</th>
<th>CAS number</th>
<th>MW (g/mol)</th>
<th>Reactive metabolite of interest (a)</th>
<th>Cytotoxicity top dose in assay (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac [sodium salt]</td>
<td>15307-79-6</td>
<td>318.13</td>
<td>Quinoneimine (after CYP formation of 5-hydroxy diclofenac)</td>
<td>500</td>
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<tr>
<td>Acetaminophen</td>
<td>103-90-2</td>
<td>151.16</td>
<td>NAPQI (quinoneimine)</td>
<td>2000</td>
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<tr>
<td>Sunitinib [maleate]</td>
<td>341031-54-7</td>
<td>532.56</td>
<td>Quinoneimine (formed after CYP induced oxidative defluorination)</td>
<td>200</td>
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<tr>
<td>Fialuridine</td>
<td>69123-98-4</td>
<td>372.09</td>
<td>Metabolites generated via CYPs</td>
<td>500</td>
</tr>
<tr>
<td>Troglitazone</td>
<td>97322-87-7</td>
<td>441.54</td>
<td>Quinone and α-quinone methide</td>
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<tr>
<td>Ketoconazole</td>
<td>65277-42-1</td>
<td>531.43</td>
<td>Reactive metabolite via CYP3A4</td>
<td>500</td>
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<tr>
<td>Cyclophosphamide</td>
<td>6055-19-2</td>
<td>279.1</td>
<td>Phosphoramidemustard</td>
<td>2000</td>
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<tr>
<td>Eugenol</td>
<td>97-53-0</td>
<td>164.2</td>
<td>Quinone type</td>
<td>500</td>
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<tr>
<td>Methyl eugenol</td>
<td>93-15-2</td>
<td>178.23</td>
<td>Non-reactive metabolite</td>
<td>500</td>
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<tr>
<td>Hydroquinone</td>
<td>123-31-9</td>
<td>110.11</td>
<td>Quinone</td>
<td>200</td>
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<tr>
<td>Retrorsine</td>
<td>480-54-6</td>
<td>351.39</td>
<td>Dehydroretoresin via CYP3A4</td>
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<tr>
<td>4-Hexylresorcinol</td>
<td>136-77-6</td>
<td>194.27</td>
<td>Quinone type</td>
<td>500</td>
</tr>
</tbody>
</table>

Example:

- Plate design allow for the analysis of 12 compounds
- Chemicals selected have a high potential for reactive metabolite formation (often quinone types)
- Tested in plated HepG2 (up to 72h), plated HepaRG (up to 72h) and HepaRG spheroids (up to 14 days)
- Derive PoDs using internal BIFROST model to assess the impact of metabolism on bioactivity.
CONCLUSION/NEXT STEPS

• HepaRG cells are a useful in vitro tool

• Demonstrated good Metabolic activity when cultured in 3D (here in toroid)

• Demonstrated stable metabolite formation in amounts sufficient to have an effect on target cells

• Further work to demonstrate effect of reactive metabolites on the HepaRG cells themselves currently ongoing
Thank You

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