GLUCURONIDATION, SULFATION, AND PHARMACOKINETICS OF CONJUGATED METABOLITES

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NJDMDG, Somerset NJ
Outline

• A renewed interest in xenobiotic conjugation
  • FDA guidelines
  • Poor IVIVE of conjugation and prediction of DDI

• Resveratrol as a model substrate
  • Tissue-specific glucuronidation and sulfation
  • Possibly active metabolites, pharmacogenetics
  • Auto-induction of glucuronidation

• Pharmacokinetics of resveratrol and its conjugates
  • Simple compartmental modeling
  • Incorporation of transporters
Why evaluate metabolite kinetics?
Metabolite disposition

- Active metabolites

- Enzyme regulation
  - Enzyme inhibition
  - Enzyme induction
  - Genetic polymorphisms

- Complex disposition
  - Reversible metabolism and enterohepatic recycling
  - Interplay between DMEs and transporters
UGT cellular location and orientation

Glucuronidation and DDIs

- Much evidence in vitro for both UGT inhibition and induction

- Not many clinical studies

- Complicating factors
  - In vitro protocols not standardized across labs
  - Overlapping substrate specificity, compensatory mechanisms
  - In vivo inhibitor concentrations below their Ki
  - Therefore, AUCi/AUC not as dramatic as with CYPs

UGT2B7 inhibition – fluconazole and AZT

- In HIV-infected males (n=12), fluconazole significantly decreased CL/F of AZT, and increased formation of AZT-G
  - $\text{AUCi/AUC}=1.92$

- IVIVE was possible when using a Ki estimate with either HLM or UGT2B7 (with BSA)
UGT1A1 and irinotecan

UGT1A4 and lamotrigine

UGT2B17 and MK-7246

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Resveratrol as a model substrate

- Substrate for glucuronidation
- Substrate for sulfation
- Possible transporter involvement
- Possibly active metabolites
- Possible role in DME regulation
- Human studies indicate poor F
- Potential chemopreventive
- High profile research on its anti-cancer and anti-obesity potential
- NIH programmatic interest
  - Hope of $$
Brill et al, Pharm Pharmacol 2006; 58: 469 – 479.
## Tissue-specific RES conjugation

![Graphs showing HLM and HIM conjugation](image)

<table>
<thead>
<tr>
<th>Conjugation Product</th>
<th>Protein Source</th>
<th>$V_{max}$</th>
<th>$K_m$</th>
<th>$K_i$</th>
<th>Type of Fit</th>
<th>Goodness of Fit ($r^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R3G</td>
<td>HLM</td>
<td>7.4 ± 0.25</td>
<td>280.4 ± 21.6</td>
<td>1022 ± 71.5</td>
<td>PSI</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>HIM</td>
<td>12.2 ± 0.34</td>
<td>505.4 ± 29.4</td>
<td>600.8 ± 20.5a</td>
<td>PSI</td>
<td>0.95</td>
</tr>
<tr>
<td>R4'G</td>
<td>HLM</td>
<td>$V_{max1} = 0.45 ± 0.01$</td>
<td>$V_{max2} = 1.3 ± 0.03$</td>
<td>$K_{m1} = 65.2 ± 29$</td>
<td>na</td>
<td>BPM</td>
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<tr>
<td></td>
<td>HIM</td>
<td>8.9 ± 0.14</td>
<td>454.5 ± 21.8</td>
<td>564.3 ± 38.1</td>
<td>PSI</td>
<td>0.95</td>
</tr>
</tbody>
</table>

RES is conjugated in the lung – in vitro

<table>
<thead>
<tr>
<th>Conjugation product</th>
<th>Protein source</th>
<th>Vmax (pmol/min/mg)</th>
<th>Km (uM)</th>
<th>Ki (uM)</th>
<th>Goodness of Fit (r²)</th>
<th>Type of Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>R3G</td>
<td>Mouse lung S9</td>
<td>324.40 ± 13.05</td>
<td>7.34 ± 1.60</td>
<td>6632 ± 1198</td>
<td>0.93</td>
<td>Partial substrate inhibition</td>
</tr>
<tr>
<td>R3S</td>
<td>Mouse lung S9</td>
<td>7.05 ± 0.28</td>
<td>2.69 ± 0.45</td>
<td>2021 ± 717.6</td>
<td>0.96</td>
<td>Partial substrate inhibition</td>
</tr>
<tr>
<td>R3S</td>
<td>Human lung S9</td>
<td>16.15 ± 0.48</td>
<td>4.45 ± 0.79</td>
<td>23238 ± 7305</td>
<td>0.95</td>
<td>Partial substrate inhibition</td>
</tr>
</tbody>
</table>

RES conjugation in the lung – in vivo

<table>
<thead>
<tr>
<th></th>
<th>RES 15 mg/kg i.a.</th>
<th>RES 15 mg/kg i.v.</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td></td>
</tr>
<tr>
<td>AUC0-inf</td>
<td>591.08 ± 167.29</td>
<td>294.98 ± 137.87*</td>
<td>min*uM</td>
</tr>
<tr>
<td>CI</td>
<td>118.77 ± 33.36</td>
<td>280.04 ± 158.25</td>
<td>mL/min/kg</td>
</tr>
<tr>
<td>Vss</td>
<td>37.59 ± 23.70</td>
<td>34.90 ± 20.10</td>
<td>L/kg</td>
</tr>
<tr>
<td>t1/2</td>
<td>190.58 ± 69.65</td>
<td>101.30 ± 43.41*</td>
<td>min</td>
</tr>
</tbody>
</table>

**R3G**

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<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>AUC0-inf</td>
<td>921.23 ± 457.07</td>
<td>2268.35 ± 517.00*</td>
<td>min*uM</td>
</tr>
</tbody>
</table>

**R3S**

<p>| | | | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>AUC0-inf</td>
<td>174.94 ± 45.75</td>
<td>157.21 ± 77.77</td>
<td>min*uM</td>
</tr>
</tbody>
</table>

Implications of tissue-specific conjugation

• IVIVE of clearance needs to incorporate extrahepatic tissue metabolism

• Local tissue levels of active metabolites
  • Colorectal cancer chemoprevention
  • Lung cancer chemoprevention
RES induces UGT1A1 transcription

Human variability in RES glucuronidation

High variability in trans-resveratrol glucuronidation a human liver bank

Variability not explained by UGT1A1 TA repeat polymorphism

Reference: Iwuchukwu et al, DMD 2009; 37: 1726 - 1732
Active metabolites of RES

Anti-estrogenic activity of R3S

Colorectal cell death by RES metabolites

Cell antiproliferative activity of R3S

S-phase arrest

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Mouse PK study design

- C57BL/6J black male mouse
- Right carotid artery was cannulated and 20 uL blood serially sampled
- Sampling time points 2.5, 5, 10, 15, 45, 90, 180, 300, 420, 600 min & 24 hrs
- Oral, intravenous (I.V.) and intra arterial (I.A.) dosing was performed
- Blood samples were centrifuged at 14000 rpm for 2 minutes and plasma samples analyzed with LC-MS
RES 15 mg/Kg i.a.

R3S 5 mg/Kg i.a.

\[ V_{C, R3S} \]

R3G 3.5 mg/Kg i.a.

In vivo formed versus preformed metabolites

- Different barriers to tissue access
- Zonal expression of metabolizing enzymes in the organ (not every organ is well-stirred)
- Complex metabolism (e.g. sequential metabolism)
- Different exposure to drug transporters

Model 4

RES 15 mg/Kg i.a.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>In-vivo formed metabolite</th>
<th>Preformed Metabolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl,R3G (ml/min/Kg)</td>
<td>67.86</td>
<td>13.78 ± 5.75</td>
</tr>
<tr>
<td>Cl,R3S (ml/min/Kg)</td>
<td>313.08</td>
<td>76.29 ± 37.07</td>
</tr>
<tr>
<td>fm, R3G (%)</td>
<td>52.00</td>
<td>17.08</td>
</tr>
<tr>
<td>fm,R3S (%)</td>
<td>48.00</td>
<td>16.87</td>
</tr>
</tbody>
</table>

Simulating UGT inhibition

Simulated R3G profiles

Baseline
UGT inhibition

R3G concentration (uM)

Time (min)
Simulating SULT inhibition

Simulated R3S profiles

Baseline

SULT inhibition
Simulating UGT induction (plus MRP2 induction)
Simulating UGT induction (plus MRP2 induction)

**Simulated R3S profiles**

- Baseline
- UGT induction
- UGT MRP2 induction

**Graph:**
- X-axis: Time (min)
- Y-axis: R3S concentration (uM)
- Data points and curves showing concentration changes over time.
Summary

• Xenobiotic conjugation can be complicated by issues such as reversible metabolism, enterohepatic recycling, enzyme orientation in the cell, and interplay with transporters
• Standardized in vitro techniques, and tools like specific substrates/inhibitors/antibodies are needed
• Regulation of UGTs and genetic polymorphisms can alter the extent of clinical DDIs
• Interplay of conjugation and transport is important
• Compartmental modeling can provide a step toward predicting alterations in systemic drug and conjugated metabolite exposure
Future directions

• Single versus multiple dosing
  • Time-dependent UGT induction

• Combination dosing
  • Interaction between combinations of UGT substrates and inducers
  • Comparison between synthetic phytochemicals and dietary components
  • Prediction of food- and herb- drug interactions

• Transporter-UGT interactions
  • Clearance of conjugated metabolites
  • Common regulatory pathways
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