INVESTIGATIONS WITH A HUMANISED CYP3A4/3A7 MOUSE MODEL
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Introduction

Humanised transgenic mice were developed in an effort to create a more reliable in vivo system to study and predict human response to xenobiotics. However, this is an emerging field and proof of their utility is required.

The aim of our investigations was to evaluate the use of the hPXR-hCAR-hCYP3A4/3A7 mouse for predicting mechanism-based inhibition. Three well known mechanism-based inhibitors (erythromycin, diltiazem, nefazodone) were administered and the relationship between the clinical situation and the mouse model compared in terms of rank order and percent inhibition.

Methods

Phase 1a: Determination of human CYP3A4 kMet

Rifampicin (10mg/kg, 3 daily doses) was administered intraperitoneally to 12 hPXR/hCAR/hCYP3A4/3A7 mice. After injection of the last dose, two mice were euthanized by rising concentration of CO2 at each of the following time points: 48, 60, 72, 84, 96 and 120 hours post dose. Microsomes (0.25mg/mL) were prepared from fresh liver and CYP3A4 activity measured using triazolam (50µM) and quantification of 1-hydroxytriazolam formation.

Phase 1b: Determination of the fraction fm of triazolam metabolised by human CYP3A4

Rifampicin (10mg/kg, 3 daily doses) was administered intraperitoneally to 4 hPXR/hCAR/hCYP3A4/3A7 and 4 CYP3A KO mice. Control groups (hPXR/hCAR/hCYP3A4/3A7 and C57BL/6j, 4 mice each) received injections of vehicle (corn oil). 48 hours after the final dose, triazolam (0.5mg/kg) was administered orally to all mice. Food was withdrawn ~2 hours prior to triazolam administration. 10µL blood samples were collected at 0.035, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours following the triazolam dose. Triazolam in whole blood was quantified using LC/MS/MS.

Results

Triazolam α-hydroxylation by liver microsomes from hPXR/hCAR/hCYP3A4/3A7 mice isolated at 48, 60, 72, 84, 96 and 120 hours post rifampicin administration (A, B and C – separate experiments).

- Mean experimental t1/2 = 0.052h corresponding to a half-life of 13.9h.
- A study showed that the mean kMet value for hepatic CYP3A4 in the population studied was approximately 0.03h-1 (t1/2 of 23h) –range2 (26h-140h).

Figure 2: Determination of human CYP3A4 kMet in the humanized CYP3A4 model

The fraction of victim drug metabolised (fm) can be calculated from the difference in AUC between rifampicin (Ri) treated humanised mice and KO mice.

Figure 3: Triazolam PK is indicative of CYP3A4-mediated elimination in hPXR/hCAR/hCYP3A4/3A7 mice

<table>
<thead>
<tr>
<th>Mouse line</th>
<th>Inducer</th>
<th>AUC(0-inf) ng.h/mL</th>
<th>fm</th>
</tr>
</thead>
<tbody>
<tr>
<td>hPXR/hCAR/hCYP3A4/3A7</td>
<td>Corn oil</td>
<td>315 ± 207</td>
<td>0.70</td>
</tr>
<tr>
<td>hPXR/hCAR/hCYP3A4/3A7</td>
<td>Rifampicin</td>
<td>102 ± 16.2</td>
<td>0.90</td>
</tr>
<tr>
<td>CYP3A KO</td>
<td>Rifampicin</td>
<td>1040 ± 188</td>
<td></td>
</tr>
<tr>
<td>C57BL/6j</td>
<td>Corn oil</td>
<td>201 ± 18.3</td>
<td>0.81</td>
</tr>
</tbody>
</table>

The fraction of victim drug metabolised (fm) can be calculated from the difference in AUC between rifampicin (Ri) treated humanised mice and KO mice.

Conclusions

- Intrinsic parameters of the model were determined and compared with the clinical situation; indicating that the model with rifampicin was more sensitive than that without rifampicin.
- Without rifampicin the model is not sufficiently sensitive.
- The use of rifampicin makes the model more complex with possible unexpected rifampicin-based DDI (e.g. transporter inhibition), not manageable.
- The model with rifampicin showed the three tested compounds to be MBI, as expected. Nevertheless, only one is accurately predicted from a quantitative perspective. The over-prediction observed with diltiazem is partially explained be the N-desmethyl metabolite concentration.
- Taken together, the DDI predictions are in the 2-fold range compared with clinical outcomes.

Predicted clinical ratios based on model outcomes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>clinical</th>
<th>3A4/3A7</th>
<th>clinical</th>
<th>3A4/3A7</th>
<th>clinical</th>
<th>3A4/3A7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inducer</td>
<td>1:1</td>
<td>1:2</td>
<td>1:2</td>
<td>1:1</td>
<td>1:4</td>
<td>1:4</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.25 mg kg-1</td>
<td>0.5 mg kg-1</td>
<td>1.0 mg kg-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diltiazem</td>
<td>0.08 mg kg-1</td>
<td>0.16 mg kg-1</td>
<td>0.25 mg kg-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.01 mg kg-1</td>
<td>0.02 mg kg-1</td>
<td>0.04 mg kg-1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No clear explanation considering model and clinical concentration comparison for diltiazem. For nefazodone, the low concentration found in mouse might explain the under-prediction.

Considering N-desmethyl diltiazem.

N-desmethyl diltiazem is described as a stronger MBI than the parent compound.

Diltiazem + R = 1.25µM, Kini = 0.017min-1
N-desmethyl diltiazem + R = 1.0µM, Kini = 0.047min-1

Combining the concentrations in the two situations of the perpetrator, and the MBI parameters respectively, through a static method, the theoretical inhibition strength of the humanised model is 45% higher than the clinical situation, thereby partially explaining the over-estimation.

References

1. Y.R. Wang, Drug Metabolism and Disposition, 2010, vol 39, 1094-1104
2. J. Yang, Current Drug Metabolism, 2008, vol 9, 384-393

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