Introduction

Recently, lead compound screenings has become widely used in drug development. The hit rate of the development of increasing range of lead compound structures with metabolic stability for Cytochrome P450 (CYP) is increasing. However, because the unpredictability of metabolic reactions and species differences by Non-CYP enzymes, there has been a case that the test compound’s blood concentration was found to be significantly lower than expected in the clinical phase, resulting in the discontinuation of the development. Therefore, it is necessary to establish an in vitro evaluation system for the clinical activity of Non-CYP enzymes.

We evaluated interspecies differences in metabolic activities for Non-CYP enzymes (aldehyde oxidase (AO), aldo-keto reductase (AKR) and carbonyl reductase (CR)). And, we tested the effect of typical inhibitors on Non-CYP enzyme (AO, xanthine oxidase (XO), AKR and CR). And, we tested the metabolic activities of Non-CYP enzymes in anisic acid conditions using human liver hepatocytes.

In this presentation, we have established an evaluation method for metabolic activities of Non-CYP enzymes (AO-XD ARX-CR).

Method

Table 1 Model substrate, model substrate metabolite, and typical inhibitor for each Non-CYP enzyme-mediated metabolism.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Model substrate</th>
<th>Model substrate metabolite</th>
<th>Typical inhibitor</th>
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<tbody>
<tr>
<td>AO</td>
<td>Naltrexone</td>
<td>Naltrexol</td>
<td>Carbazeran</td>
</tr>
<tr>
<td>AKR</td>
<td>Allopurinol</td>
<td>Allopurinol</td>
<td>Naltrexone</td>
</tr>
<tr>
<td>CR</td>
<td>Quercetin</td>
<td>8-OH-Quercetin</td>
<td>Naltrexone</td>
</tr>
</tbody>
</table>

Results

Figure 1 Kinetics for each Non-CYP enzyme in human liver cytosol. Assay was performed in duplicate. Each model substrate (0.1 to 10 µmol/L carbazeran for AO activity, 3 to 300 µmol/L naltrexone for ARX activity and 5 to 500 µmol/L doxorubicin for CR activity) was incubated in 0.1 mg/ml protein of human liver cytosol at 37°C for 5 min (AO and ARX) or 10 min (CR), respectively. The IC₅₀ values for carbazeran, naltrexone and doxorubicin were 5.60, 1.48 and 349 µmol/L, respectively.

Figure 2 Inhibitory effect of typical inhibitor for each oxidase in human liver cytosol. Assay was performed in duplicate. Each model substrate (0.5 µmol/L carbazeran for AO activity and 100 µmol/L 6-mercaptopurine for XO activity) was incubated in 0.5 mg protein/mL of human liver cytosol at 37°C for 5 min (AO), or 60 min (XO), respectively. The IC₅₀ values were calculated using the following equation ‘% of control = (IC₅₀ / [model substrate concentration] ) × 100’.

Figure 3 Inhibitory effect of typical inhibitor for each reductase in human liver cytosol. Assay was performed in duplicate. Each model substrate (300 µmol/L doxorubicin for CR activity and 10 µmol/L naltrexone for ARX activity) was incubated in 0.1 mg/ml protein of human liver cytosol at 37°C for 10 min. The IC₅₀ values were calculated using the following equation ‘% of control = (IC₅₀ / [model substrate concentration] ) × 100’.

Discussion

This study characterized the interspecies differences in metabolic activities of Non-CYP enzymes (AO, ARX and CR) in liver cytosol (human, monkey, rat, mouse and dog). AO, ARX and CR were observed to show inter species differences for IC₅₀ (Figure 1 and Table 2). AO and AKR showed high CL in human. We compared the metabolic activities of Non-CYP enzymes in anaerobic and aerobic conditions using human liver hepatocytes, but no clear difference was observed under both conditions (Data not shown).

The model substrate metabolites were measured by LC-MS/MS. The model substrate metabolites concentration was calculated by LC-MUNS (HPLC system: LC-10Amp, Shimadzu, MSMS system: AP4000 QTRAP, SCIEX). The velocity Vmax and IC₅₀ values for carbazeran, naltrexone and doxorubicin were 7130, 1587 and 107 L/min/mg protein, respectively. The model substrate metabolites concentration was calculated by the following equation ‘CL = Vmax × [model substrate concentration] / (IC₅₀ + [model substrate concentration])’.

The metabolic activities of Non-CYP enzymes in anisic acid conditions using human liver hepatocytes were determined using in vitro studies with CYP mediated metabolites. These results may help us to predict the compound pharmacokinetics in human.

References


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8P-20 Evaluation of interspecies metabolic activities for Non-CYP enzymes and into a suitable incubation condition for human

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