Results

We evaluated the inhibitory effect of typical inhibitors for each Non-CYP enzyme using an aerobic condition with human liver hepatocytes. We tested the metabolic activities of Non-CYP enzymes in anaerobic conditions using human liver hepatocytes. In this presentation, we have established an evaluation method for metabolic activities of Non-CYP enzymes (AO, X0, AR) and CR.

### Method

**Model substrate, substrate metabolites, and typical inhibitors selected on References.**

**<Experimental method.>**

Reactions were conducted using potassium phosphate buffer (100 mM, pH 7.4) liver cytosol (H600C, male, 50 µM, Monkey P900C, male, 100 µM, Dog D100C, male, 10 µM, Mouse M1000C, male, 1000 µM, Rat R1000C, female, 454 µM, with a standard concentration of 250 µM, NADPH generating system (Cytosolic and CR) and each model substrate. Reactions were terminated by adding acetonitrile solution containing internal standard (Barr).

**<Calculation of velocity, Vm, and Km.>>**

Velocity = Model substrate metabolite concentration (pmol/min/mg protein)

Liver concentration × Incubation time (mg protein/mL)

Km and Vmax values were calculated from the relationship between the velocity and substrate concentration according to the equation shown below (Michaelis-Menten model).

\[
V = \frac{V_{max} \cdot S}{K_m + S}
\]

Vmax: Maximum velocity (pmol/min/mg protein), Km: Michaelis constant (µmol/L), S: model substrate concentration (µmol/L)

**<Calculation of % of control.>>**

Remaining activity (% of control) of each Non-CYP enzyme was calculated from the following equation:

\[
\% \text{ of control} = \left(1 - \frac{\text{peak area ratio in inhibitory group}}{\text{peak area ratio in control group}} \right) \times 100
\]

**<Discussion.>>**

This study contributed to understanding the interspecies differences in metabolic activities of Non-CYP enzymes (AO, AR, CR) in liver cytosol (human, monkey, rat, mouse, and dog). AO, AR, and CR were shown to inter-species differences for CLint (Figure 1 and Table 2). AO and AR showed high CLint in human, indicating that the metabolic activity of the compounds metabolized by these enzymes is underestimated in vitro studies with CYP, compared to animal studies with CYP. These results led us to evaluate the metabolic activities of Non-CYP enzymes in an anaerobic and aerobic conditions using human liver hepatocytes. However, we observed clear differences between conditions (Data not shown).

**<Conclusion.>>**

We have established an evaluation method for metabolic activities of Non-CYP enzymes (AO, AR, CR). These results will help us to identify the metabolic enzymes for the compounds metabolized by Non-CYP enzymes.

### References

8. T. Inoue, et al. (2020) Allopurinol (AO inhibitor) and Furosemide (CR inhibitor) showed specific inhibition for AO and CR. Inhibitory effect of typical inhibitors for each Non-CYP enzyme showed high CLint in human, indicating that the metabolic activity of the compounds metabolized by these enzymes is underestimated in vivo studies with CYP, compared to animal studies with CYP. These results lead us to evaluate the metabolic activities of Non-CYP enzymes in an anaerobic and aerobic conditions using human liver hepatocytes. However, we observed clear differences between conditions (Data not shown).

### Table 1 Interspecies differences (human, monkey, dog, mouse, and rat) at kinetics for each Non-CYP enzyme in liver cytosol.

<table>
<thead>
<tr>
<th>Species</th>
<th>Km (µmol/L)</th>
<th>Vmax (pmol/min/mg protein)</th>
<th>IC50 (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>5.60 µmol/L</td>
<td>700 pmol/min/mg protein</td>
<td>10.8 µmol/L</td>
</tr>
<tr>
<td>Monkey</td>
<td>1.48 µmol/L</td>
<td>1800 pmol/min/mg protein</td>
<td>45.1 µmol/L</td>
</tr>
<tr>
<td>Dog</td>
<td>1.48 µmol/L</td>
<td>2000 pmol/min/mg protein</td>
<td>10.2 µmol/L</td>
</tr>
<tr>
<td>Mouse</td>
<td>1.48 µmol/L</td>
<td>2200 pmol/min/mg protein</td>
<td>10.1 µmol/L</td>
</tr>
<tr>
<td>Rat</td>
<td>1.48 µmol/L</td>
<td>2600 pmol/min/mg protein</td>
<td>10.5 µmol/L</td>
</tr>
</tbody>
</table>

**<Discussion.>>**

We have established an evaluation method for metabolic activities of Non-CYP enzymes (AO, AR, CR). These results will help us to identify the metabolic enzymes for the compounds metabolized by Non-CYP enzymes.