148 Inhibitory effect of the CYP typical inhibitors under long term incubation in human liver microsomes

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Introduction

Cytochrom P450 (CYP) isoforms are responsible for the metabolism of the majority of drugs in human. In case of the metabolic enzyme identification of CYP isoforms for the low intrinsic clearance (CL_{int}) candidate compounds, it is necessary to perform metabolic reaction with long term incubation. In addition, when performing the metabolic enzyme identification with long term incubation, the specificity and sustainability of a typical inhibitor are important. In this research, we evaluated the inhibitory effect of typical inhibitor for each CYP isoform in human liver microsomes (HLM) under long term incubation (incubation time of CYP typical inhibitors was 60 minutes, final concentration of HLM was 1 mg protein/mL) to clarify an optimal concentration of the CYP typical inhibitors under long term incubation.
In this presentation, we have confirmed specificity and sustainability of each typical inhibitor for CYP isoform under long term incubation in HLM.

Method

< Reaction condition and typical inhibitor for each CYP isoform>

CYP isoform	Metabolic activity	* Model substrate concentration (μM)	Typical inhibitor
CYP1A2	Phenacetin *O*-deethylation	30	α-Naphthoflavone Furafylline
CYP2B6	Bupropion hydroxylation	100	Thio-TEPA
CYP2C8	Amodiaquine *N*-deethylation	2.5	Montelukast
CYP2C9	Diclofenac 4'-hydroxylation	4	Sulfaphenazole
CYP2C19	*S*-(+)- Mephenytoin 4'-hydroxylation	30	Benzylnirvanol
CYP2D6	(±)- Bufuralol 1'-hydroxylation	10	Quinidine
CYP3A4	Testosterone 6β-hydroxylation	60	Ketoconazole
CYP3A4	Midazolam 1'-hydroxylation	1	Ketoconazole

*The model substrate concentrations were set to around K_m values based on internal data of Sekisui Medical Co., Ltd.
Incubation samples contained phosphate buffer (100 mM, pH 7.4), HLM (final concentration: 1 mg protein/mL, H0610, mixed gender, pool of 50, Sekisui Xeno Tech, LLC), NADPH generating system (Corning) and each typical inhibitor were incubated at 37° C for 60 min. The reaction was started by addition of the each model substrate solution, and the incubation samples were incubated at 37° C for 1 min. After incubation, the incubation samples were terminated by adding acetonitrile solution containing internal standard. The metabolite concentrations were determined by LC-MS/MS (HPLC system : LC-10ADvp, Shimadzu, MS/MS system :API4000 and 4000QTRAP, SCIEX).

<Calculation of % of control>
Remaining activity (% of control) of each CYP isoform was calculated to the following equation.

$$\% \text{ of control} = \frac{\text{Peak area ratio in typical inhibitor group}}{\text{Peak area ratio in control group}} \times 100$$

Table 3 and Figure 3 Inhibitory effect of Thio-TEPA for each CYP isoform in HLM

Thio-TEPA	% of control			
	CYP1A2	CYP2B6	CYP2C8	CYP2C9
0 μmol/L	100.0	100.0	100.0	100.0
10 μmol/L	100.2	7.2	105.4	103.2
30 μmol/L	102.7	5.4	95.1	101.0
100 μmol/L	97.0	3.5	77.3	91.6

Thio-TEPA	% of control			
	CYP2C19	CYP2D6	CYP3A4 (T)	CYP3A4 (M)
0 μmol/L	100.0	100.0	100.0	100.0
10 μmol/L	101.1	95.1	81.6	81.3
30 μmol/L	99.2	97.8	58.9	66.6
100 μmol/L	85.3	96.5	42.2	42.4

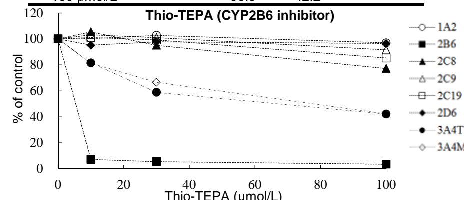

Table 6 and Figure 6 Inhibitory effect of Benzylnirvanol for each CYP isoform in HLM

Benzylnirvanol	% of control			
	CYP1A2	CYP2B6	CYP2C8	CYP2C9
0 μmol/L	100.0	100.0	100.0	100.0
1 μmol/L	92.3	82.7	101.3	91.5
5 μmol/L	86.2	74.1	84.5	93.5
25 μmol/L	93.5	65.9	67.2	100.4

Benzylnirvanol	% of control			
	CYP2C19	CYP2D6	CYP3A4 (T)	CYP3A4 (M)
0 μmol/L	100.0	100.0	100.0	100.0
1 μmol/L	78.6	94.1	93.1	94.5
5 μmol/L	13.8	93.0	77.6	77.6
25 μmol/L	2.0	114.9	49.2	37.1

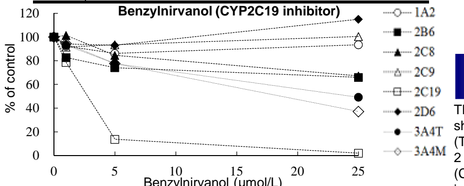

Table 9 IC_{50} of typical inhibitor for each CYP isoform in HLM

Typical inhibitor	IC_{50} (μmol/L) Incubation time 15 min	IC_{50} (μmol/L) Incubation time 60 min
α-Naphthoflavone (CYP1A2)	0.191	0.273
Furafylline (CYP1A2)	0.605	0.386
Thio-TEPA (CYP2B6)	1.94	1.41
Montelukast (CYP2C8)	1.16	1.69
Sulfaphenazole (CYP2C9)	0.530	0.652
Benzylnirvanol (CYP2C19)	0.441	1.88
Quinidine (CYP2D6)	0.557	1.78
Ketoconazole (CYP3A4 (T))	0.156	0.401
Ketoconazole (CYP3A4 (M))	0.185	0.467

IC_{50} values were calculated using the following equation
"% of control = (IC_{50} / (IC_{50} + typical inhibitor (μmol/L))) × 100".

Results

Table 1 and Figure 1 Inhibitory effect of α-Naphthofravone for each CYP isoform in HLM

α-Naphthoflavone	% of control			
	CYP1A2	CYP2B6	CYP2C8	CYP2C9
0 μmol/L	100.0	100.0	100.0	100.0
1 μmol/L	17.3	96.2	101.3	92.0
5 μmol/L	14.0	92.2	86.7	86.2
25 μmol/L	14.3	86.7	44.9	32.9

α-Naphthoflavone	% of control			
	CYP2C19	CYP2D6	CYP3A4 (T)	CYP3A4 (M)
0 μmol/L	100.0	100.0	100.0	100.0
1 μmol/L	89.0	100.8	92.3	98.7
5 μmol/L	68.5	106.6	79.0	119.1
25 μmol/L	27.4	98.0	16.8	36.3

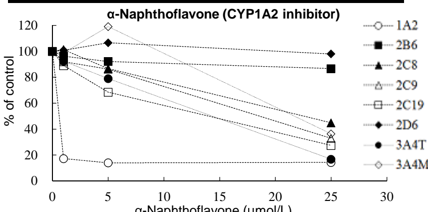

Table 2 and Figure 2 Inhibitory effect of Furafylline for each CYP isoform in HLM

Furafylline	% of control			
	CYP1A2	CYP2B6	CYP2C8	CYP2C9
0 μmol/L	100.0	100.0	100.0	100.0
1 μmol/L	23.3	100.5	101.9	99.8
5 μmol/L	16.8	98.1	96.3	99.3
25 μmol/L	15.9	95.5	98.4	94.4

Furafylline	% of control			
	CYP2C19	CYP2D6	CYP3A4 (T)	CYP3A4 (M)
0 μmol/L	100.0	100.0	100.0	100.0
1 μmol/L	97.8	96.9	104.8	99.1
5 μmol/L	91.8	99.1	98.3	98.3
25 μmol/L	67.7	93.0	104.8	98.0

Furafylline (CYP1A2 inhibitor)
% of control
Furafylline (μmol/L)

Table 4 and Figure 4 Inhibitory effect of Montelukast for each CYP isoform in HLM

Montelukast	% of control			
	CYP1A2	CYP2B6	CYP2C8	CYP2C9
0 μmol/L	100.0	100.0	100.0	100.0
0.2 μmol/L	113.1	103.3	95.5	114.3
1 μmol/L	107.6	99.7	66.9	101.4
5 μmol/L	103.9	97.2	17.1	89.4

Montelukast	% of control			
	CYP2C19	CYP2D6	CYP3A4 (T)	CYP3A4 (M)
0 μmol/L	100.0	100.0	100.0	100.0
0.2 μmol/L	99.2	95.1	97.3	110.2
1 μmol/L	99.4	97.8	93.2	111.2
5 μmol/L	89.5	96.5	92.3	103.9

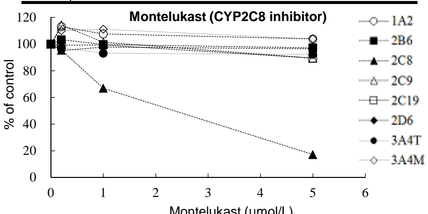

Table 5 and Figure 5 Inhibitory effect of Sulfaphenazole for each CYP isoform in HLM

Sulfaphenazole	% of control			
	CYP1A2	CYP2B6	CYP2C8	CYP2C9
0 μmol/L	100.0	100.0	100.0	100.0
1 μmol/L	94.5	91.9	92.3	39.4
5 μmol/L	91.3	92.0	95.3	11.2
25 μmol/L	93.7	88.8	94.7	3.7

Sulfaphenazole	% of control			
	CYP2C19	CYP2D6	CYP3A4 (T)	CYP3A4 (M)
0 μmol/L	100.0	100.0	100.0	100.0
1 μmol/L	92.6	100.0	95.3	95.5
5 μmol/L	90.6	98.9	89.6	95.5
25 μmol/L	92.1	107.5	84.9	91.5

Sulfaphenazole (CYP2C9 inhibitor)
% of control
Sulfaphenazole (μmol/L)

Table 7 and Figure 7 Inhibitory effect of Quinidine for each CYP isoform in HLM

Quinidine	% of control			
	CYP1A2	CYP2B6	CYP2C8	CYP2C9
0 μmol/L	100.0	100.0	100.0	100.0
1 μmol/L	96.7	97.6	96.2	101.3
5 μmol/L	97.9	97.3	96.5	97.6
25 μmol/L	83.7	80.7	78.0	93.0

Quinidine	% of control			
	CYP2C19	CYP2D6	CYP3A4 (T)	CYP3A4 (M)
0 μmol/L	100.0	100.0	100.0	100.0
1 μmol/L	99.5	57.2	90.3	99.0
5 μmol/L	99.5	30.0	85.4	90.1
25 μmol/L	77.3	20.3	67.9	57.2

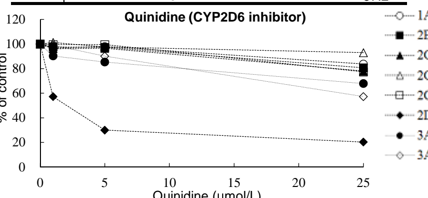

Table 8 and Figure 8 Inhibitory effect of Ketoconazole for each CYP isoform in HLM

Ketoconazole	% of control			
	CYP1A2	CYP2B6	CYP2C8	CYP2C9
0 μmol/L	100.0	100.0	100.0	100.0
0.2 μmol/L	89.8	95.9	96.5	91.3
1 μmol/L	79.0	90.8	107.6	97.5
5 μmol/L	68.9	60.8	77.7	78.3

Ketoconazole	% of control			
	CYP2C19	CYP2D6	CYP3A4 (T)	CYP3A4 (M)
0 μmol/L	100.0	100.0	100.0	100.0
0.2 μmol/L	94.0	96.8	80.1	87.3
1 μmol/L	92.8	89.8	15.7	17.3
5 μmol/L	67.6	90.6	2.6	2.3

Ketoconazole(CYP3A4 inhibitor)
% of control
Ketoconazole (μmol/L)

Discussion

This study characterized the specificity of each CYP typical inhibitor under long term incubation in HLM. At 1 μmol/L α-naphthoflavone (CYP1A2 inhibitor) showed specific inhibition to CYP1A2 . However, at 25 μmol/L, it inhibited other CYP isoform activities (CYP2C8, CYP2C9, CYP2C19 and CYP3A4) (Table 1 and Figure 1). By contrast, furafylline (CYP1A2 inhibitor) showed specific inhibition to CYP1A2 at the maximum concentration (25 μmol/L) (Table 2 and Figure 2). At 10 μmol/L, thio-TEPA (CYP2B6 inhibitor) showed specific inhibition to CYP2B6 (Table 3 and Figure 3). At 5 μmol/L, montelukast (CYP2C8 inhibitor) showed specific inhibition to CYP2C8 (Table 4 and Figure 4). At 5 to 25 μmol/L, sulfaphenazole (CYP2C9 inhibitor) showed specific inhibition to CYP2C9 (Table 5 and Figure 5). At 5 μmol/L, benzylnirvanol (CYP2C19 inhibitor) showed specific inhibition to CYP2C19, but at 25 μmol/L, it inhibited other CYP isoform activities (CYP2B6, CYP2C8 and CYP3A4) (Table 6 and Figure 6). At 5 μmol/L, quinidine (CYP2D6 inhibitor) showed specific inhibition to CYP2D6 (Table 7 and Figure 7). At 1 μmol/L, ketoconazole (CYP3A4 inhibitor) showed specific inhibition to CYP3A4 (Table 8 and Figure 8).
We also compared the IC_{50} values of the CYP typical inhibitors under the different incubation times (15 min and 60 min) (Table 9). As for the typical inhibitors of which the IC_{50} values after 60 min incubation were higher (α-naphthoflavone, montelucast, sulfaphenazole,benzylnirvanol,quinidine and ketoconazole), the inhibitory effects might be weakened by the metabolism of the typical inhibitors themselves. As for the typical inhibitors of which the IC_{50} values after 60 min incubation were lower (furafylline and thio-TEPA), time-dependent inhibition was indicated.
In this presentation, we have confirmed the specificity and sustainability of CYP typical inhibitors under the tough condition (incubation time of inhibitor was 60 min, final concentration of HLM was 1 mg protein/mL). Therefore, these results will help us to identify metabolic enzymes of the low intrinsic clearance (CL_{int}) compounds metabolized by CYP isoforms.

References

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