A Comprehensive Platform for Evaluating the *In Vitro* CYP Reaction Phenotyping of New Molecular Entities (NME)

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Abstract

Although the non-CYP enzymes are frequently involved in the elimination of many drugs, CYP still is the major enzyme involved in metabolic clearance. Careful assessment of the contribution of individual CYP toward the overall metabolism of a new molecular entity (NME) is therefore crucial to accurately assess the object DDI liability of an NME in the clinic.

Method

A validated method was used to monitor substrate loss or metabolite formation in two recognized test systems. Pooled human liver microsomes with specific chemical inhibitors and/or recombinant individually expressed human enzyme systems are both validated in our lab. Major CYPs (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A(CYP3A4 and CYP3A5)) are used as the standard design. If a discrepancy is found in these two systems for the identification of the major metabolic enzyme(s), additional efforts would include the following:

1) Addition of a time-dependent inhibitor for a CYP to ensure the specificity of the inhibitory effect on that specific CYP (Table 1)

Table 1: Validated inhibitors used in the human liver microsomes system for CYP

CYP enzyme	Reversible inhibitor	Time-dependent inhibitor
1A2	α-Naphthoflavone	Furafylline
2B6	Ticlopidine	PPP
2C8	Montelukast	Gemfibrozil Glucuronide
2C9	Sulfaphenazole	Tienilic Acid
2C19	N-3-Benzyl-nirvanol	Ticlopidine
2D6	Quinidine	Paroxetine
3A	Ketoconazole	Azamulin (CYP3cide as CYP3A4 inhibitor)

2) Additional CYPs (CYP1A1, CYP2A6, CYP2E1, CYP2J2, CYP 3A5, CYP4F2) are all validated in our lab for the comprehensive assessment of the enzyme(s) involved. (Table 2)

Table 2a: Validated kinetic analysis for additional recombinant CYPs

CYP enzyme	Substrate	K_m (μm) range in house	K_m (μM) in publication
1A1	7-Ethoxycoumarin	4.35~5.98	10
2A6	Coumarin	3.37~3.52	0.826 or 6.0
2E1	Chlorzoxazone	301~353	135~590
3A5	Midazolam	2.44~2.81	1.4~5.2
4F2	Pafuramidine	0.704~1.14	0.7

Table 2b: Validated kinetic analysis for recombinant CYP2J2

CYP enzyme	Substrate	CL_{int} (μL/min/pmol) range in house	CL_{int} (μL/min/pmol) range in publication
2J2	Astemizole	2.02~3.93	1.14±0.1

Results

Application 1. Results from the chemical inhibition study showed that CYP3A played a major role and CYP2C8 played a secondary role in the metabolism of compound A (Figure 1A). However, CYP2C8 was identified as the major enzyme by a recombinant CYP metabolism study (Figure 1B). Additional CYPs that might be inhibited by ketoconazole, the chemical inhibitor of CYP3A used in the chemical inhibition study, were subsequently included in this study, which identified CYP3A5 as the major enzyme in the reaction phenotyping study (Figure 1C).

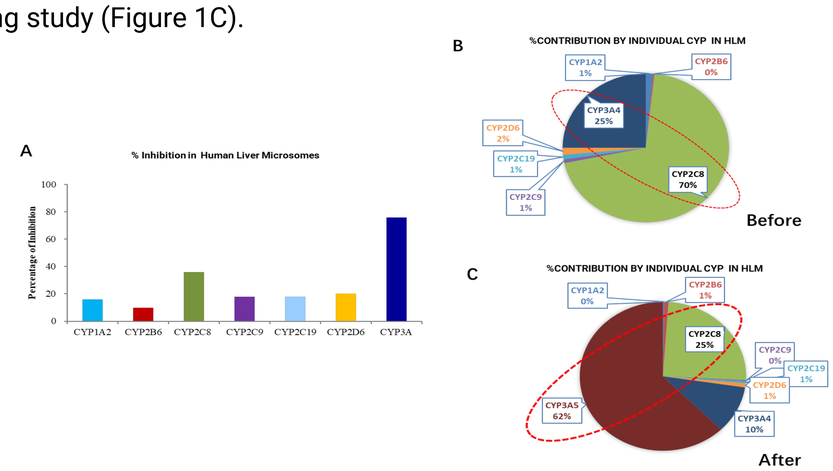

Figure 1. The addition of CYP3A5 in the recombinant CYPs system effectively solved the discrepancy (A and B) and CYP3A5 was identified as the major metabolic enzyme of compound A.

Application 2. Results from the chemical inhibition study showed that CYP1A2 was the major enzyme (Figure 2A). However, CYP2C19 was identified as the metabolic enzyme contributing most to the clearance of compound B by recombinant CYP metabolism study (Figure 2B). CYP1A1 was subsequently included in the recombinant CYP metabolism study due to the off-target potential of the CYP1A2 inhibitor (α-Naphthoflavone), which identified CYP1A1 as playing a role in the metabolism of compound B (Figure 2C).

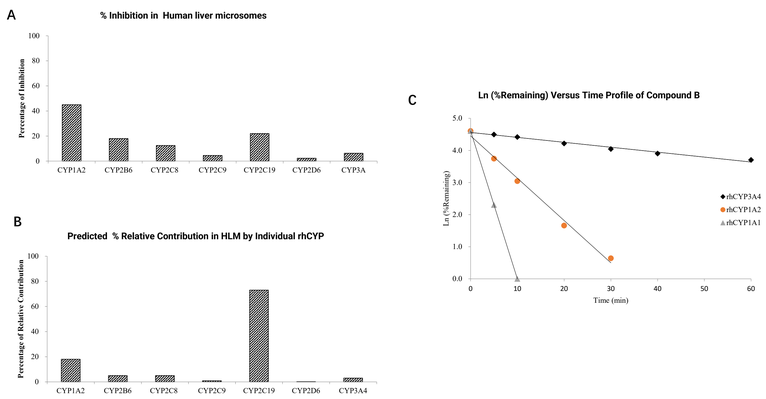

Figure 2. The addition of CYP1A1 in the recombinant CYPs system effectively solved the discrepancy (A and B) and CYP1A1 was identified as playing a role in the metabolism of compound B.

Conclusion

Careful evaluation of the contribution of individual CYP enzymes to the overall metabolism of a new molecular entity (NME) is essential for accurately assessing the victim DDI liability of an NME in studies. Utilizing a validated method with pooled human liver microsomes and recombinant human enzyme systems, we can precisely determine the role of specific CYPs. The expertise and insights gained from this process can be readily applied to the study of other enzymes as well.

References

[1] Caroline A,et al. Drug Metabolism and Disposition. 2010, 38:347–356
[2] FDA guidance for industry: In Vitro Drug Interaction Studies-Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions, January 2020.
[3] Yoshiya Yamamura, et al. Xenobiotica, 2015; 45(4): 353–360.

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