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Evidence-Based Strategies for the Characterization of New Chemical Entity (NCE) *In Vitro* CYP and Non-CYP Enzyme Reaction Phenotyping at the Development Stage

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PURPOSE

Successful prediction of the major routes of human clearance of the New Chemical Entity (NCE) is critical for the pharmaceutical industry. Reaction phenotyping has become a standard practice in the process of drug discovery and development. Various *in vitro* and *in vivo* methodologies are available for reaction phenotyping at different stages of drug discovery and development.

Decisions regarding which methods to be used and which drug metabolic enzyme (DME)s should be involved depend on a number of factors, including turnover rate[1] (low clearance vs. high clearance), clearance pathways, metabolic pathways, metabolite structures, availability of metabolite standards and stages of the projects (discovery vs. pre-clinical development vs. clinical development) et al.

The aim of the present work was to establish evidence-based strategies to accurately search for metabolic enzymes of drugs and optimize and broaden the identification system at the development stage. Both parent depletion and metabolite formation approaches were applied in this presentation.

METHOD(S)

Phenotyping assay is designed and conducted based on the information from metabolite identification studies and metabolic stability assays. Information about the major metabolic pathways and the knowledge of enzymatic reactions guides the design of enzyme reaction phenotyping assays.

1. If an NCE undergoes direct conjugation, involvement of enzymes such as UDP-glucuronosyltransferase (UGT), sulfotransferase (SULT), Glutathione-S-transferase (GST) or N-acetyltransferase (NAT) is evaluated based on the major metabolites identified.

2. If an NCE undergoes NADPH-independent metabolism, involvement of enzymes such as aldehyde oxidase (AO), xanthine oxidase (XO), monoamine oxidase (MAO)[5], carboxylesterase (CES), alcohol dehydrogenase (ADH), or aldehyde dehydrogenase (ALDH) is evaluated based on the NCE structure as well as whether species specific metabolism exists.

3. If an NCE undergoes NADPH-dependent metabolism, involvement of enzymes such as cytochrome P450 (CYP)s or flavin-containing monooxygenase (FMO)s is evaluated based on the knowledge of enzymatic reactions and pre-test results.

4. If data suggest that contribution from CYPs is the major metabolic pathway, two methods (recombinant CYPs and chemical inhibitor approaches) are used to confirm whether the major cytochrome P450 enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A) are responsible for the drug metabolism. Additional CYPs (CYP2A6, CYP2J2, CYP4F2, CYP2E1, CYP1A1, CYP17A, and CYP19A) should also be tested if the outcomes for the major DMEs from the two methods mentioned above are inconsistent with each other.

RESULT(S)

Case 1： Metabolism by direct conjugation via UGT.

The major metabolic pathway of compound A showed that the relative abundance of direct conjugation metabolites was much higher than that of CYP-mediated metabolites by UV absorption (Figure 1a), therefore a UGT reaction phenotyping assay was applied. The formation of metabolites (M4 or M5) was monitored by LC-MS/MS in the recombinant UGTs system by a semi-quantitative method. The results showed that UGT1A9 could catalyze the formation of M4 and UGT1A1 as well as UGT2B7 could catalyze the formation of M5 (Figure 1b and 1c).

Case 2：The major metabolic pathway of a test compound was in a NADPH-independent manner.

The pre-test of reaction phenotyping study showed that compound B could be metabolized by human liver microsomes in a NADPH-independent manner (Figure 2a). Based on the structure information of the parent compound and its major metabolite, CES was considered as the potential enzyme (Figure 2b). Following incubations of the test compound in the recombinant CES1 and CES2 systems, a conclusion was drawn that CES2 could catalyze the metabolism of compound B.

Case 3: The major metabolic pathway for a test compound was in a NADPH-dependent manner and the additional CYPs should be included.

The chemical inhibition study showed that CYP3A was the major metabolic enzyme (Figure 3a), however, CYP2C9 was identified as the major metabolic enzyme by recombinant CYP metabolism study (Figure 3b). Additional CYP enzymes (CYP2A6, CYP2J2, CYP3A5, CYP3A7, CYP4F2)that might be inhibited by Ketoconazole [2-4], which is usually used as the chemical inhibitor for CYP3A, were included in this study (Figure 3c). CYP2J2 was identified as the major metabolic enzyme in reaction phenotyping study (Figure 3d).

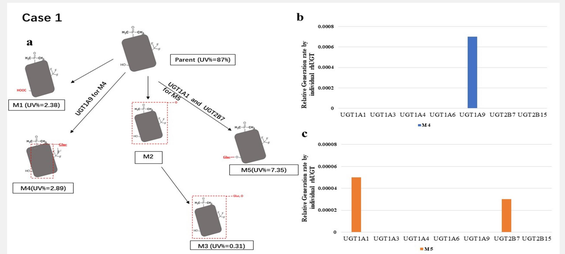

Figure 1. *UGT1A9, UGT1A1, and UGT2B7 were identified as the main enzymes responsible for the metabolism of compound A via metabolite formation method.*

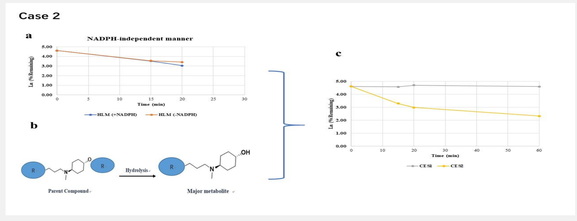

Figure 2. *CES2 was identified as the main enzyme responsible for the metabolism of compound B via parent depletion method.*

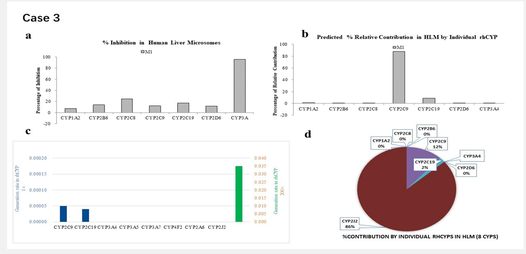

Figure 3. *CYP2J2 was identified as the main enzyme responsible for the metabolism of compound C via metabolite formation method.*

CONCLUSION(S)

We present evidence-based strategies for the evaluation of the reaction phenotyping for both CYPs and Non-CYPs of the candidate compound *in vitro*. We anticipate that the strategies will inform practice, encourage development of standard experimental procedures where feasible, and guide ongoing research in the field.

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