The Strategy for UGT Enzyme Phenotyping

Lifang Jiang[1], Yajuan Bi [2],Tao Zhang[2], Qiuling Zhou[2], Huan Liu[1], Liang Shen[1], Genfu Chen[1]
1. DMPK Department, WuXi AppTec, 31 Yiwei Rd., Waigaoqiao Free Trade Zone, Shanghai, China
2. DMPK Department, WuXi AppTec, Building 6, Juhui Park, # 71 Longshan North Road, Pukou District, Nanjing, Jiangsu, China, 200131

WuXi AppTec

Poster#: P68

Abstract

In enzyme phenotyping studies, CYP enzyme phenotyping typically employs two approaches, namely recombinant enzyme and chemical inhibitor, simultaneously. However, applying a similar strategy to UGT enzyme phenotyping—using specific inhibitors for all isoforms—presents significant challenges. This is because not all UGT isoforms have inhibitors listed in ICH M12[1], and even the inhibitors recommended by ICH M12 are not specific to that isoform. The recent emphasis on the importance of UGT enzyme phenotyping in the ICH M12 guideline underscores the need for a more robust and comprehensive testing platform to accurately assess UGT enzyme activity. In this study, we implemented a strategy for UGT enzyme phenotyping, which was subsequently applied to the characterization of unknown compounds.

Method

The distribution of UGT isoforms varies across different organs (liver, intestinal and kidney), and the probability of UGT conjugation reactions occurring in the liver is the highest. Therefore, the strategy of this study primarily focuses on the UGT isoforms expressed in the liver. After the recombinant enzyme assay identifies the enzymes involved in the metabolism of compounds of interest, inhibitors of these specific isoforms are used in chemical inhibition experiments to confirm their contributions. In this strategy, the screening of specific inhibitors for some isoform under common conditions is crucial. After identifying specific inhibitors, we applied this strategy to validate two known commercial compounds. Upon confirming its feasibility, we extended the approach to a unknown compound for broader application.

Results

In our experimental system, Nilotinib and Regorafenib, two UGT1A1 inhibitors recommended by ICH M12, were found to have off-target effects on UGT1A3 and UGT1A9, respectively, with off-target inhibition rates exceeding 60% (Figure 1). As a result, we have also verified the specificity of some of the inhibitors for UGT1A1, UGT1A3, UGT1A4, UGT1A9, UGT2B7 and UGT2B10 to support further research.

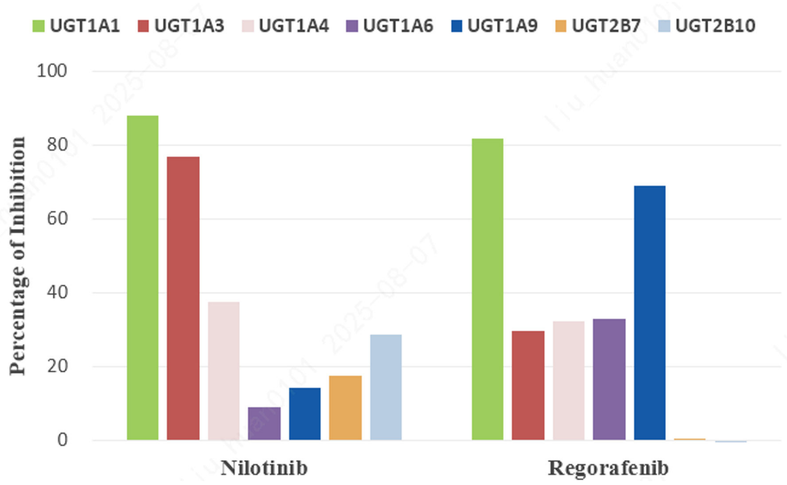

Figure 1. Nilotinib and Regorafenib have off-target effects on UGT1A3 and UGT1A9, respectively.

Validation 1:
After the recombinant enzyme assay identifies UGT1A1, UGT1A3, UGT1A4, UGT1A9 and UGT2B7 involved in the metabolism of β-Estradiol (Figure 2A), inhibitors of these specific isoforms are used in chemical inhibition experiments to confirm their contributions (Figure 2B). Both approaches confirmed that UGT1A1 was the major isoform for the metabolism of β-Estradiol. The result is consistent with that in literature[2].

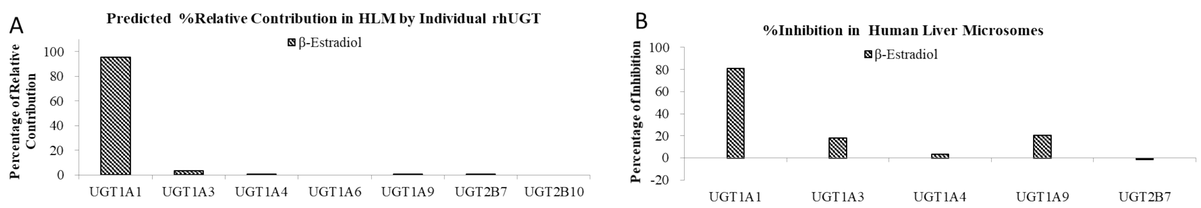

Figure 2. Both chemical inhibition study and recombinant CYP metabolism study confirmed that UGT1A1 was the major isoform for the metabolism of β-Estradiol.

Validation 2:
After the recombinant enzyme assay identifies UGT1A4 and UGT2B10 involved in the metabolism of Amitriptyline (Figure 3A), inhibitors of these specific isoforms are used in chemical inhibition experiments to confirm their contributions (Figure 3B). Both approaches confirmed that UGT2B10 was the major isoform for the metabolism of Amitriptyline. The result is consistent with that in literature[2].

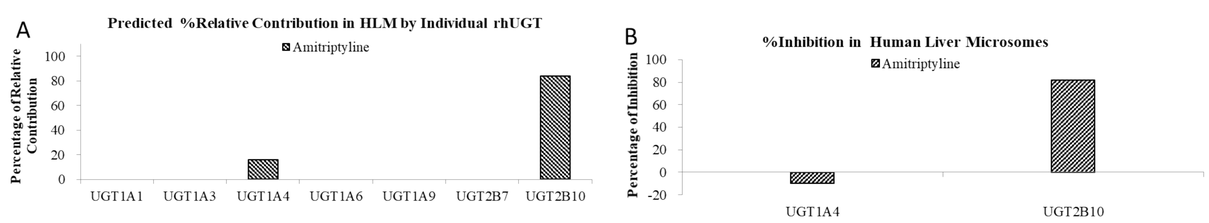

Figure 3. Both chemical inhibition study and recombinant CYP metabolism study confirmed that UGT2B10 was the major isoform for the metabolism of Amitriptyline.

Application 1:
After the recombinant enzyme assay identifies UGT1A3, UGT1A9 and UGT2B7 involved in the formation of M1 from compound A (Figure 4A), inhibitors of these specific isoforms are used in chemical inhibition experiments to confirm their contributions (Figure 4B). Both approaches confirmed that UGT2B7 was the major isoform and UGT1A3 was the minor isoform for the formation of M1 from compound A.

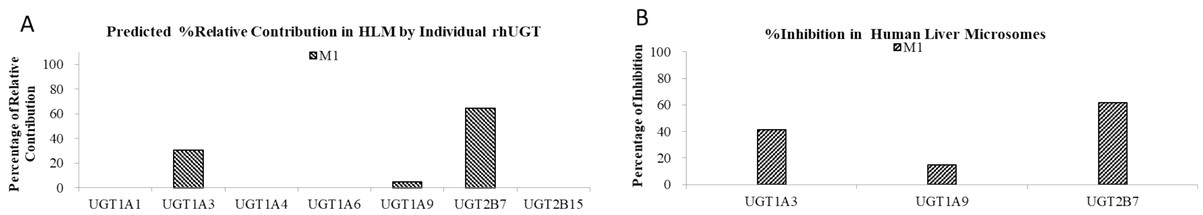

Figure 4. Both chemical inhibition study and recombinant CYP metabolism study confirmed that UGT2B7 was the major isoform and UGT1A3 was the minor isoform for the formation of M1 from Compound A .

Conclusion

The validation using commercial compounds and the application to a unknown compound demonstrated the success of our UGT enzyme phenotyping strategy. Taking the liver as an example, this strategy may also be extended to the identification of UGT phenotypes predominantly expressed in the intestine and kidneys.

References

[1] ICH guidance: M12 Drug Interaction Studies, May 2024.
[2] Miners J.O., Rowland A., et.al. Evidence-based strategies for the characterisation of human drug and chemical glucuronidation *in vitro* and UDP-glucuronosyltransferase reaction phenotyping. Pharmacology Therapeutics. 2021 Feb; 218.

Scan for a copy