Development of an *In Vitro* Platform for Screening UGT Inhibitors Using Human Liver Microsomes

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Abstract

UGT-mediated conjugation with glucuronic acid ('glucuronidation'), derived from the cofactor UDP-glucuronic acid (UDPGA), is a major clearance pathway in phase II metabolism. In particular, after cytochromeP450 (CYP) mediated biotransformation, glucuronidation is the most important metabolic pathway for small molecule drugs[1]. With the release of the new ICH M12 guidelines, the screening of UGT inhibitors is becoming increasingly important in drug development. Therefore, the objective of this study is to meet the need for the implementation of accurate and cost-effective high-throughput in vitro methods.

Method

The evaluation of the UGT inhibition potential of the compound of interest is performed using a human liver microsome (HLM) with relatively selective substrates. The inhibition potential of the compound of interest on UGT1A1 (β-Estradiol or bilirubin as the substrate), UGT1A3 (Telmisartan as the substrate), UGT1A4 (Trifluoperazine as the substrate), UGT1A6 (Serotonin as the substrate), UGT1A9 (Propofol as the substrate), UGT2B7 (Zidovudine as the substrate), and UGT2B10 (Cotinine as the substrate) was evaluated in pooled human liver microsomes.

Results

These newly established methods utilize different concentrations (0.01~0.1mg/mL) of human liver microsomal protein selected based on the results from the initial condition determination. Unlike cytochrome P450, UGT activity remains stable over extended incubation periods. In some instances, we have incorporated incubation periods of up to 40 minutes to enhance metabolite production. This approach improves detection sensitivity while maintaining initial-rate conditions (Figure 1). The substrate concentration selected for each assay was determined after characterizing the enzyme kinetics of each reaction (Figure 2). Finally, the optimized conditions and IC_{50} range of the positive controls used in the evaluation of UGT inhibition potential were summarized in Table 1.

Table 1. Assay conditions for evaluating UGT inhibition in HLM

UGT Enzyme	Substrate	HLM Conc. (mg/mL)	Inhibitor	IC_{50} (μM)
UGT1A1	Bilirubin	0.01	Nilotinib	0.0809-0.122/0.0571-0.0627
	β-Estradiol	0.1		
UGT1A3	Telmisartan	0.1	Zafirlukast	0.651-0.993
UGT1A4	Trifluoperazine	0.05	Hecogenin	0.180-0.194
UGT1A6	Serotonin	0.05	Zafirlukast	1.04-1.33
UGT1A9	Propofol	0.025	Magnolol	0.0111-0.0261
UGT2B7	Zidovudine	0.1	Zafirlukast	21.2-23.7
UGT2B10	Cotinine	0.1	Desloratadine	3.17-4.16

Figure 1. Linearity of UGT-mediated substrate glucuronidation in HLM

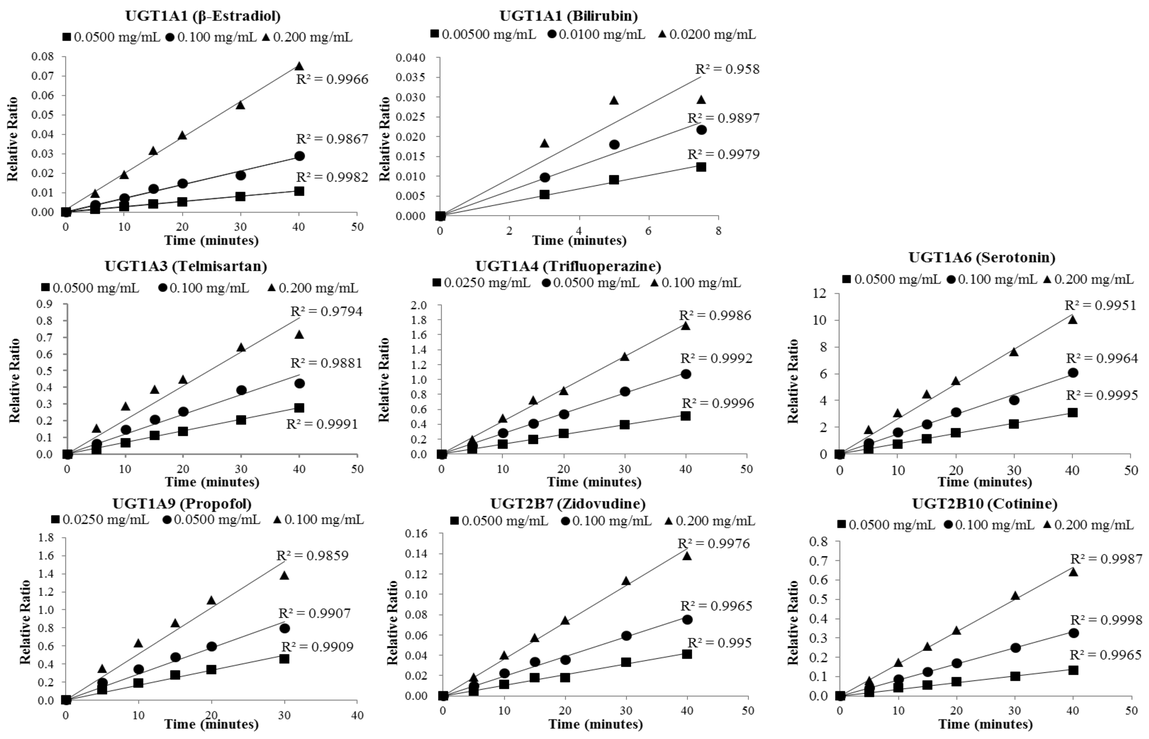
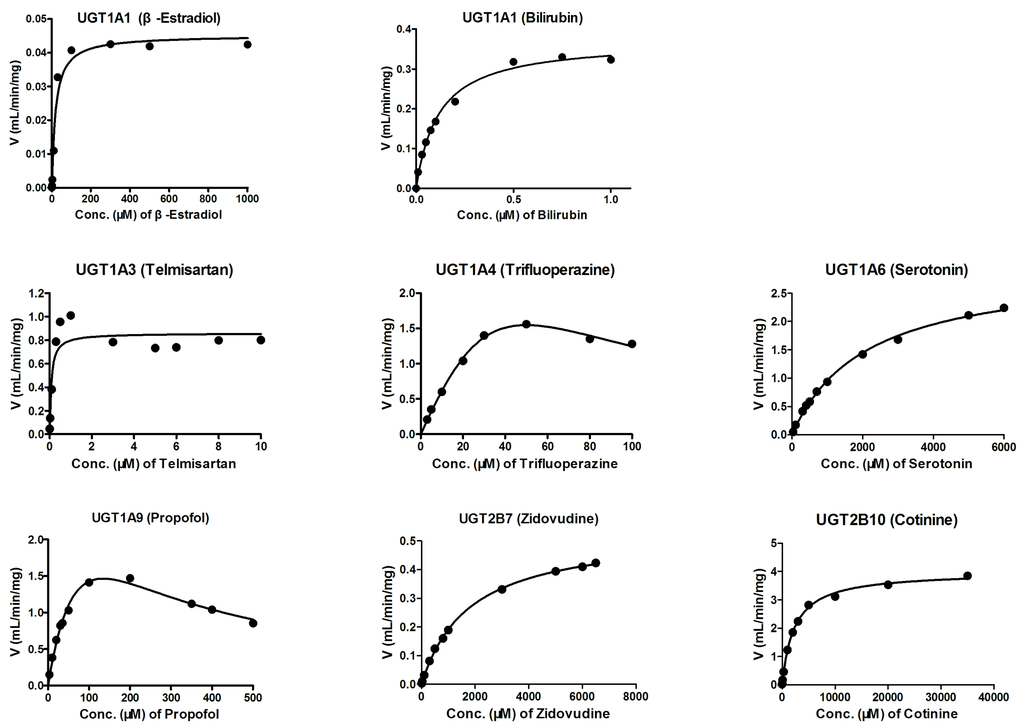


Figure 2. Enzyme kinetics of UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7 and UGT2B10 with ICH M12 recommended probe substrate in HLM



Conclusions

UGT inhibition assays using human liver microsomes as the test system have been developed. As the assays were designed to reduce costs compared with using human recombinant UGTs, they will provide an excellent tool to evaluate the *in vitro* DDI potential of new chemical entities in the early discovery stage.

References

[1] Miners JO, et al.. Analytical Chemistry. 2021, 93, 10850-10861. Pharmacol Ther. 2023 Aug; 248:108459

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