Assessment of *in vitro* Methods for UGT1A1 Inhibition in Recombinant Isozyme

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Introduction

Human UGT1A1 is one of the major enzymes responsible for drug metabolism via Phase 2 biotransformation. It is recommended by regulatory agencies to further evaluate inhibitory potential of the investigational drug on UGT1A1 if direct glucuronidation product(s) are observed as one the major elimination routes. Despite that SN-38 has been the recommended substrate of UGT1A1 for clinical drug-drug interaction (DDI) studies and it has been characterized *in vitro* previously[1], its application for *in vitro* DDI evaluation as a substrate for UGT1A1 inhibition remains inconclusive. The objectives of this study are to establish initial rate conditions in recombinant human UGT1A1 (rhUGT1A1), followed by evaluating the inhibitory effect of a universal UGT inhibitor zafirlukast using SN-38 and 4-methylumbelliferone (4-MU).

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Methods

- To establish linear incubation condition, rhUGT1A1 up to 0.2 mg/mL were incubated with SN-38 for up to 20 minutes in the presence of UDPGA.
- To characterize enzyme kinetics parameters, SN-38 at various concentrations up to 50 µM were incubated with rhUGT1A1 under determined linear condition.
- UGT inhibitor zafirlukast at concentrations up to 50 µM and 100 µM were incubated in rhUGT1A1 with SN-38 and with 4-MU, respectively, to evaluate the inhibitory effect.
- The UGT-mediated reactions were monitored by quantitatively measuring the formation of metabolite SN-38 β-D-glucuronide (SN-38G) and 4-MU β-D-glucuronide (4-MUG) by LC-MS/MS. Data were analyzed and plotted by GraphPad Prism®.

Results and Discussion

- Under conditions tested, the rhUGT1A1-mediated glucuronidation of SN-38 appeared to be linear and protein-concentration dependent (**Figure 1**).
- Formation of SN-38G at up to 50 µM followed allosteric sigmoidal model as suggested by Eadie-Hofstee transformation analysis (**Figure 2A**). The model is distinct from the results previously reported by Xiao *et al*., 2017.
- Regression analysis using allosteric sigmoidal model revealed *K'* and V_{max} values of 0.794 µM and 199 pmol/min/mg protein, respectively (**Figure 2B** and **Table 1**). Further analyzing using alternative kinetics models suggests that allosteric sigmoidal is the preliminary model of SN-38 glucuronidation by rhUGT1A1.
- Zafirlukast incubated with substrate SN-38 and 4-MU showing activity inhibition with calculated IC_{50} value of 0.455 µM and 0.507 µM, respectively (**Figure 3A**).
- 4-MU was a suboptimal substrate (high K_m) for rhUGT1A1 despite of its wide use, SN-38 appeared to be an effective alternative substrate for inhibition evaluation using recombinant human UGT1A1 *in vitro*.

Conclusion

- Human recombinant UGT1A1-mediated SN-38 glucuronidation followed allosteric sigmoidal model.
- Universal UGT inhibitor zafirlukast showed comparable IC_{50} values from substrate SN-38 and 4-MU in rhUGT1A1.

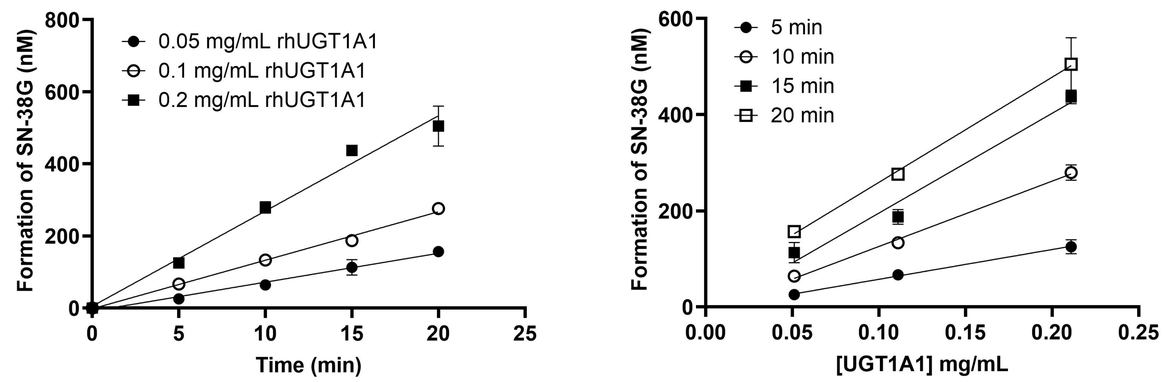

Figure 1. SN-38 metabolism in rhUGT1A1 at various protein concentrations and incubation times.

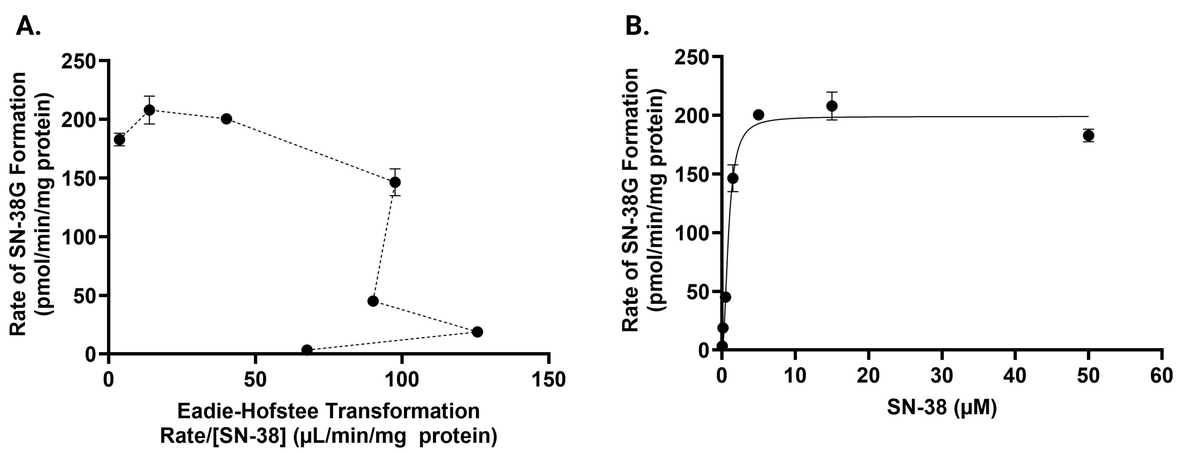

Figure 2. (A) Eadie-Hofstee transformation and (B) Enzyme Kinetics of SN-38 metabolism in rhUGT1A1.

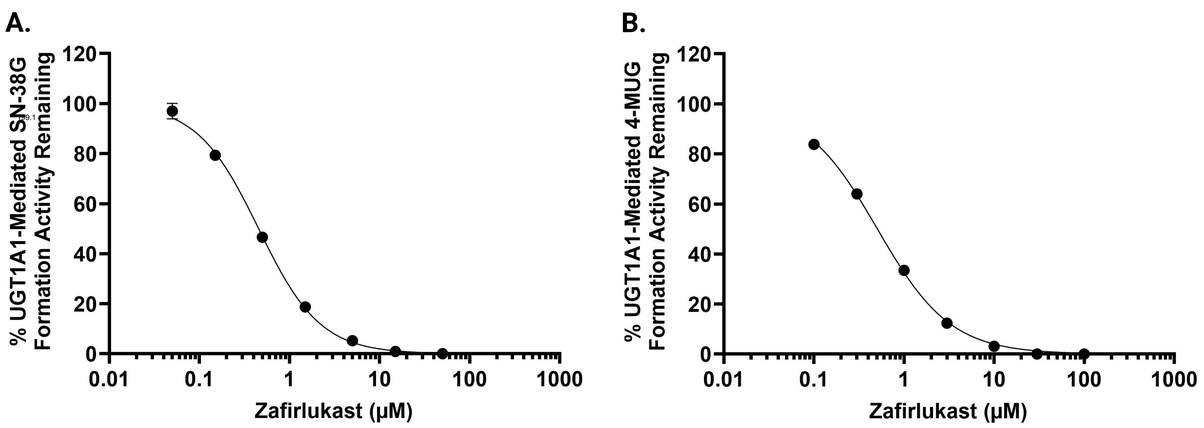

Figure 3. Inhibition of rhUGT1A1-mediated metabolism of (A) SN-38 and (B) 4-MU by zafirlukast.

Table 1. Enzyme kinetics parameters and IC_{50} values for UGT1A1 inhibition

	Enzyme Kinetics		UGT Inhibition		
Substrate	**Kinetics Model**	**Parameters**	**Substrate Conc. (µM)**	**Inhibitor**	**IC_{50} (µM)**
SN-38	Allosteric Sigmoidal	V_{max} = 199 pmol/min/mg protein K' = 0.794 µM K_{half} = 0.891 µM (h = 2.00)	1	zafirlukast	0.455
4-MU	Michaelis-Menton[a]	V_{max} = 300 pmol/min/mg protein[a] K_m = 113 µM[a]	100	zafirlukast	0.507

[a] WuXi AppTec internal data; Data not shown

Reference

[1] Xiao, L., *et al*. *Basic Clin. Pharmacol. Toxicol.* **2017**; 122:424-248.