Evaluation of Chemical Inhibitors for UDP-glucuronosyltransferase (UGT) Reaction Phenotyping Assays in Human Liver Microsomes

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Introduction

In the development of a new chemical entity (NCE) reaction phenotyping studies are carried out to determine specific enzymes involved in the metabolism of the new drug candidate. These studies increase the understanding of how a drug is cleared and it's potential to be subject to drug-drug interactions (DDI) with co-administrated medications. Polymorphisms and DDIs involving non-CYP metabolism routes (*e.g.,* UDP-glucuronosyltransferases [UGT]) are becoming more widely recognized by regulatory agencies and therefore a need for further investigation exists (Harper *et al.*, 2008, Rowland *et al.*, 2013).

One approach to identify which UGTs are responsible for the metabolism of a drug is the use of chemical inhibitors in an *in vitro* test system utilizing pooled human liver microsomes (HLM), or pooled cryopreserved human hepatocytes (CHH) (Ogilvie *et al.*, 2008). Factors such as cross-reactivity of inhibitors, protein binding, and depletion of the cofactor, etc. must be considered when designing these studies. Additionally, *in vitro* metabolism incubations should be conducted under initial rate conditions to obtain an accurate assessment of inhibition (Parkinson *et al.*, 2011).

In the present study, we examined the selectivity of UGT inhibition with a range of protein concentrations and incubation times in HLM, for a variety of commonly used UGT inhibitors with the goal of identifying selective chemical inhibitors.

Materials & Methods

Buprenorphine, desloratadine, ibuprofen and niflumic acid were purchases from Sigma-Aldrich (St. Louis, MO). Indinavir, erlotinib, fluconazole, ritonavir and troglitazone were purchased from Toronto Research Company (Toronto, Canada). 5-Hydroxytryptophol was purchased from Biosynthesis (Lewisville, TX), and hecogenin was purchased from Steroids (Newport, RI). The sources of all other reagents have been described previously.

Test system

Pooled human liver microsomes (HLM, n=200, mixed gender) were prepared from non-transplantable livers and characterized at XenoTech, LLC (Lenexa, KS) as described previously (Pearce *et al.*, 1996; Parkinson *et al.*, 2004).

***In vitro* chemical inhibition under initial rate conditions in HLM**

Briefly, UDPGA-fortified HLM (n=200) at 0.005, 0.1, 0.5 and 1 mg/mL, were pre-incubated (0, 30, 120 min) with various UGT inhibitors (as shown in **Table 1**), namely ibuprofen, ritonavir, indinavir, desloratadine, 5-hydroxytryptophol, hecogenin, niflumic acid, fluconazole, buprenorphine, erlotinib and troglitazone. Following a pre-incubation step (0, 30, or 120 min) with inhibitor, residual UGT enzyme activities were determined with marker substrate incubations (5 or 10 min) under initial rate conditions with marker substrate concentrations approximately equal to the experimentally determined K_m value. Specifically, probe substrates included estradiol (UGT1A1), chenodeoxycholic acid (UGT1A3), trifluoperazine (UGT1A4), naphthol UGT1A6, propofol (UGT1A9), morphine (UGT2B7), levomedetomidine (UGT2B10), oxazepam (UGT2B15) and testosterone (UGT2B17). Reactions were quenched with the addition of stop reagent containing deuterated internal standards, followed by protein precipitation by way of centrifugation. Metabolite formation was determined by LC-MS/MS analysis as described previously (Parkinson *et al.*, 2011).

Table 1. Summary of inhibitor concentrations

Inhibitor	HLM
Ibuprofen	200 µM
Ritonavir	10 µM
Indinavir	30 µM
Desloratadine	10 µM
5-Hydroxytryptophol	10 µM
Hecogenin	75 µM

Inhibitor	HLM
Niflumic acid	2 µM
Fluconazole	2500 µM
Buprenorphine	100 µM
Erlotinib	10 µM
Troglitazone	100 µM

Results

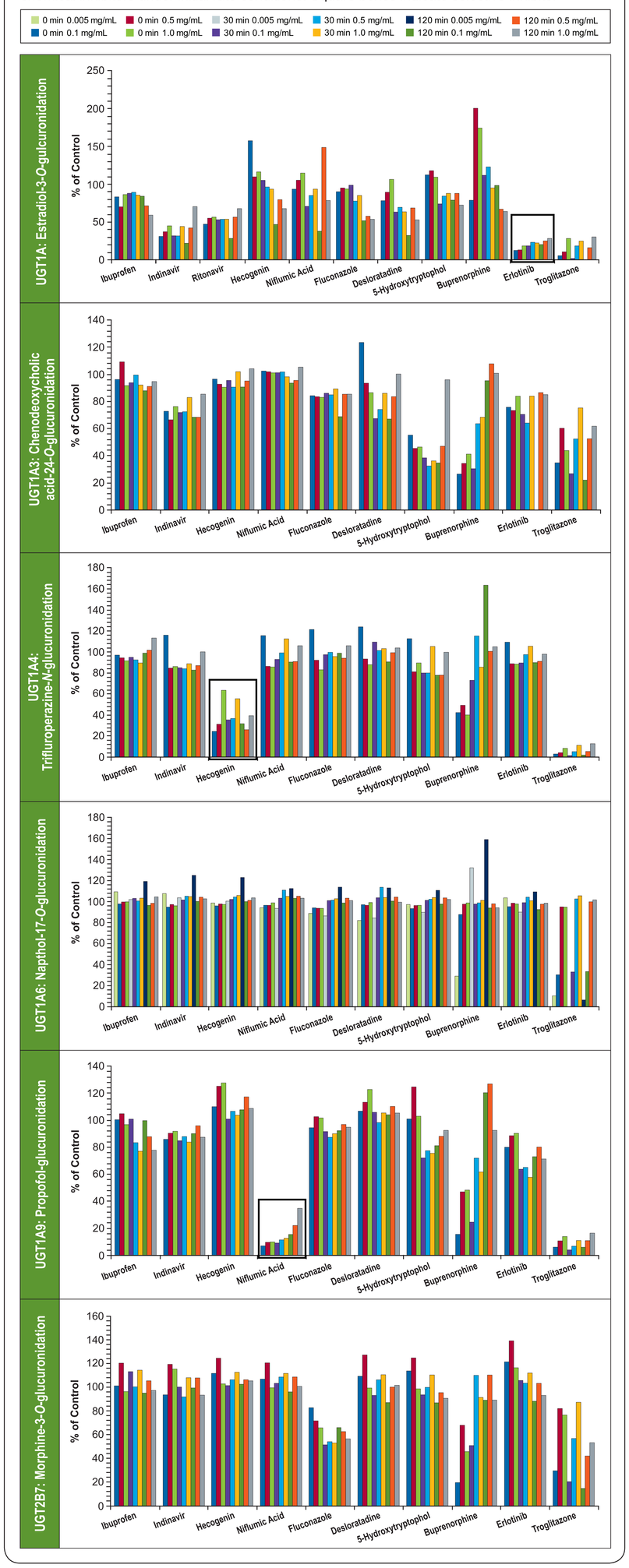
Inhibition of specific UGTs in HLM was accomplished in the presence of a potentially selective inhibitor and was not dependent on pre-incubation.

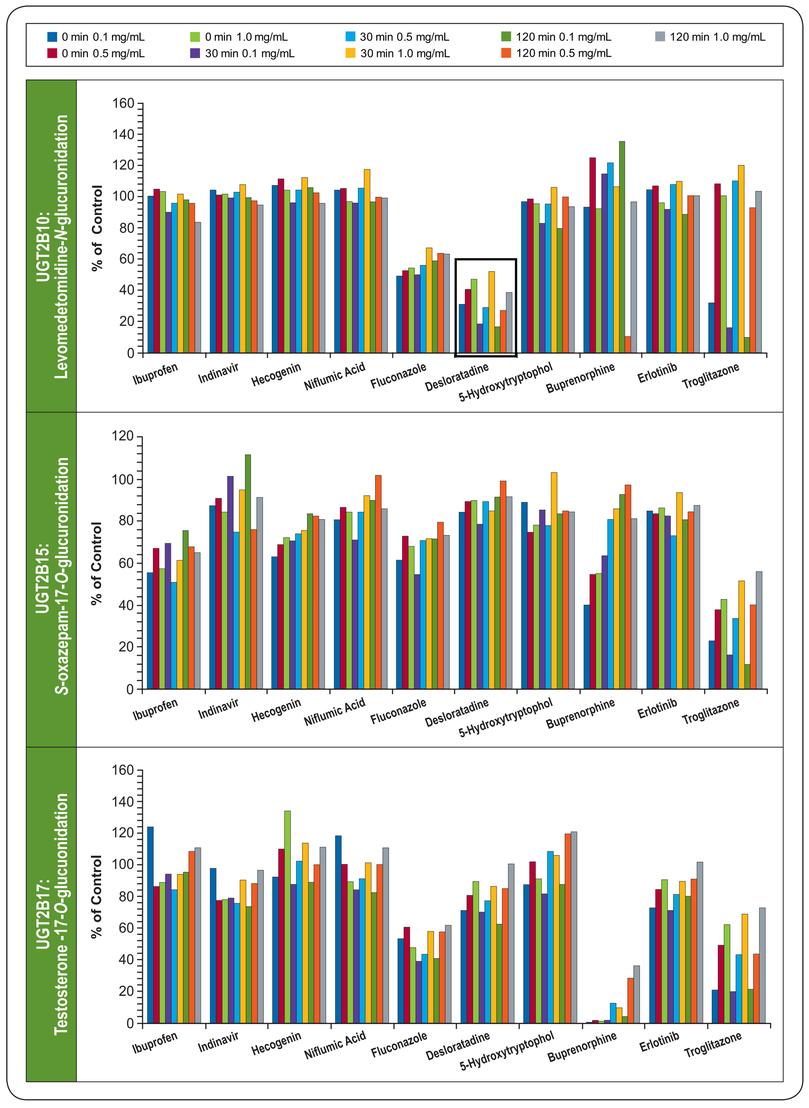
As summarized in **Figure 1**, in HLM at 0.005, 0.1, 0.5 and 1.0 mg/mL over the time course of the incubation:

- Erlotinib (10 µM) selectively inhibited UGT1A1 activity by 70-80%
- Hecogenin (75 µM) selectively inhibited UGT1A4 by 35-75%
- Niflumic acid (2 µM) selectively inhibited UGT1A9 by 60-90%
- Desloratadine (10 µM) selectively inhibited UGT2B10 by 50-80%
- 5-Hydroxytryptophol, a substrate for UGT1A6, inhibited UGT1A3 by 45-60% at 10 µM and its target UGT (1A6) by 33-62% at concentrations of 100 and 1000 µM at the lowest protein concentrations (0.005-0.1 mg/mL)
- Buprenorphine (100 µM) was found to inhibit UGT2B17 (60-90%), but also moderately inhibited other UGTs
- Fluconazole (2.5 mM) did not selectively inhibit UGT2B7 as it also moderately inhibited UGT2B10, 2B15 and 2B17

Results (cont.)

Figure 1. Time course of inhibition of UGTs in HLM at 0.005, 0.1, 0.5 and 1.0 mg/mL by various chemical inhibitors under initial rate conditions. Square boxes indicate selective inhibitor





Conclusions

- These findings suggest that certain chemical inhibitors can be utilized in UGT reaction phenotyping, with implications for the selection of inhibitors with low cross-reactivity.
- Erlotinib was found to be selectively inhibit UGT1A1, hecogenin selectively inhibited UGT1A4 niflumic acid selectively inhibited UGT1A9 and desloratadine selectively inhibited UGT2B10.
- Further investigation is currently being conducted to identify selective inhibitors of UGT1A3, UGT1A6, UGT2B7, UGT2B15 and UGT2B17.

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