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-administered 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 purchased 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 Biosearch Technologies (Novato, CA), 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, propfol (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).

Results

Table 1. Summary of inhibitor concentrations

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>HLM</th>
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<tbody>
<tr>
<td>Ibuprofen</td>
<td>200 µM</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>10 µM</td>
</tr>
<tr>
<td>Indinavir</td>
<td>30 µM</td>
</tr>
<tr>
<td>Desloratadine</td>
<td>10 µM</td>
</tr>
<tr>
<td>5-Hydroxytryptophol</td>
<td>10 µM</td>
</tr>
<tr>
<td>Hecogenin</td>
<td>75 µM</td>
</tr>
<tr>
<td>Niflumic acid</td>
<td>2 µM</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>2500 µM</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>100 µM</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>10 µM</td>
</tr>
<tr>
<td>Troglitazone</td>
<td>100 µM</td>
</tr>
</tbody>
</table>

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 UGT1A6 by 35-75%
- Niflumic acid (2 µM) selectively inhibited UGT1A9 by 60-90%
- Desloratadine (10 µM) selectively inhibited UGT2B10 by 50-80%
- 5-Hydroxytrypophol, 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 (60-80%), but also moderately inhibited other UGTs
- Fluconazole (2.5 mM) did not selectively inhibit UGT2B7 as it also moderately inhibited UGT2B10, 2B15 and 2B17

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, UGT1M6, UGT2B7, UGT2B15 and UGT2B17.

References


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