Contribution of UGT1A1, CYP2A6, CYP2C9 and CYP3A4 in Metabolism of Belinostat

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Abstract
Belinostat, a potent inhibitor of class I and II histone deacetylase enzymes, has been approved for the treatment of patients with relapsed or refractory peripheral T-cell lymphoma. Previous work demonstrated that UGT1A1-mediated glucuronidation was a significant metabolic pathway of belinostat. A separate report suggests that belinostat was metabolized by recombinant human CYP enzymes. In the present study, contribution of UGT1A1, CYP2A6, CYP2C9, and CYP3A4 in metabolism of 14C-belinostat was assessed using in vitro test systems. 14C-Belinostat was extensively metabolized by human liver microsomes (HLM) in the presence of both uridine 5'-diphosphoglucuronic acid (UDP) and dihydronicotinamide-adenine dinucleotide phosphate (NADP). Identified metabolites include belinostat glucuronide (major), belinostat amide (minor) and belinostat acid (minor). For example, belinostat glucuronide, belinostat amide, and belinostat acid accounted for 54.6 to 55.1, 2.68 to 2.71 and 1.45 to 1.78% of the total radioactivity, respectively, after 14C-belinostat (10 and 100 μM) was incubated with human hepatic microsomes (1 mg/mL) for 60 minutes. K_{m} and V_{max} of glucuronidation were determined to be 72.8 μM and 2160 pmol/min/mg, respectively. The projected hepatic clearances obtained from time-dependent total metabolism of belinostat (9.67 to 10.8 mL/min/kg) and from K_{m}/V_{max} (12.9 mL/min/kg) of belinostat glucuronidation were equivalent to human in vivo clearance (estimated as 12.6 to 14.7 mL/min/kg). Rates of belinostat glucuronidation by HLM prepared from six individual UGT1A1 genotyped donors were significant different between allelic variants of *1*1 and *28*28 and highly correlated (r² = 0.9613), as shown in Figure 5. Belinostat glucuronidation and estradiol glucuronidation by HLM and glucuronidation of belinostat were highly correlated (r² = 0.9613), as shown in Figure 6. UGT1A1 inhibitor troglitazone markedly decreased belinostat glucuronidation, but CYP2A6, CYP2C9 and CYP3A4 inhibitors did not change production of belinostat amide and belinostat acid (Figure 7).

Results

- 14C-Belinostat was metabolized in HLM in a linear fashion in the presence of UDPGA and NADPH (Figure 3).
- Identified metabolites included belinostat glucuronide (major), belinostat amide (minor) and belinostat acid (minor), as shown in Figure 4.
- Glucuronidation of 14C-belinostat was determined in six UGT1A1 genotyped donors (*1*1, *1*28, and *28*28), as shown in Figure 5.
- Belinostat glucuronidation and estradiol glucuronidation by HLM from six individual UGT1A1 genotyped donors were highly correlated (r² = 0.9613), as shown in Figure 6.
- UGT1A1 inhibitor troglitazone markedly decreased belinostat glucuronidation, but CYP2A6, CYP2C9 and CYP3A4 inhibitors did not change production of belinostat amide and belinostat acid (Figure 7).
- Major contribution of UGT1A1 and minor contribution of CYP enzymes was confirmed by using recombinant human enzymes (Table 1).
- K_{m} and V_{max} of glucuronidation were determined to be 72.8 μM and 2160 pmol/min/mg, respectively, as shown in Figure 8.

Methods

General Incubation Procedure
Incubation mixtures containing 14C-belinostat with microsomal protein or recombinant UGT1A1, CYP3A4, CYP2C9 and CYP2A6 in assay buffer were warmed at 37°C for 5 minutes. The reactions were initiated with the addition of warmed (37°C) UDPGA (2 mM) and/or NADPH (1 mM) and terminated by the addition of stop solution followed by vortex mixing. Supernatants obtained from centrifugation were transferred to a separate plate and stored at approximately 4°C prior to analysis.

Summary

- UGT1A1 mediated glucuronidation was the predominant metabolic pathway for belinostat.
- CYP2A6, CYP2C9 and CYP3A4 did not play a significant role in metabolism of belinostat.

Table 1. Metabolism of 14C-Belinostat (100 μM) by Recombinant Human Enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Belinostat Glucuronide</th>
<th>Belinostat Amide</th>
<th>Belinostat Acid</th>
<th>Remaining Radioactivity (% of the total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector Control</td>
<td>95.8</td>
<td>0</td>
<td>1.82</td>
<td>1.12</td>
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<tr>
<td>UGT1A1</td>
<td>32.1</td>
<td>63.2</td>
<td>1.60</td>
<td>0</td>
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<tr>
<td>CYP2A6</td>
<td>95.5</td>
<td>0</td>
<td>1.92</td>
<td>1.01</td>
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<tr>
<td>CYP2C9</td>
<td>95.6</td>
<td>0</td>
<td>1.56</td>
<td>1.01</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>94.3</td>
<td>0</td>
<td>1.72</td>
<td>1.04</td>
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</tbody>
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