Short Communication

Ritonavir, Efavirenz, and Nelfinavir Inhibit CYP2B6 Activity in Vitro: Potential Drug Interactions with Bupropion

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ABSTRACT:

Since antiretroviral drugs are known to inhibit many cytochrome P450 isoforms, the inhibition of CYP2B6 by non-nucleoside reverse transcriptase inhibitors and viral protease inhibitors was studied in vitro in human liver microsomes using bupropion hydroxylation as the CYP2B6 index reaction. Mean IC_{50} values (µM) for inhibition of bupropion hydroxylation were: nelfinavir (2.5), ritonavir (2.2), and efavirenz (5.5). The reaction was only weakly inhibited by indinavir, saquinavir, amprenavir, delavirdine, and nevirapine. The inhibition of bupropion hydroxylation in vitro by nelfinavir, ritonavir, and efavirenz indicates inhibitory potency versus CYP2B6 and suggests the potential for clinical drug interactions.

Depression frequently accompanies HIV infection (Fernandez and Levy, 1991; Judd and Mijch, 1996; Lyketsos et al., 1996; Ferrando et al., 1997), and studies estimate that depression affects 32 to 56% of HIV-infected persons (Ferrando et al., 1997). Depression associated with HIV infection may be a result of dealing with a progressive physical disability, discrimination, or loss of a social support system (Chesney and Folkman, 1994; Judd and Mijch, 1996; Lyketsos et al., 1996). Alternatively, depression may be due to the virus itself affecting the central nervous system (Lyketsos et al., 1996).

Several studies have examined the efficacy and tolerability of different antidepressants in treating depression in HIV-infected individuals. Bupropion is an atypical antidepressant that is often administered when other agents have been ineffective (Walker et al., 1993). Bupropion treatment of depression in HIV patients has been described in uncontrolled studies (Ferrando and Levy, 1991; Avants et al., 1998).

Viral protease inhibitors inhibit a number of cytochrome P450 (CYP) isoforms in vitro, including CYP3A4 (Eagling et al., 1997; Malaty and Kuper, 1999; von Moltke et al., 1998, 2000). The non-nucleoside reverse transcriptase inhibitors efavirenz and delavirdine inhibit CYP3A4, -2C9, and -2C19 (Voorman et al., 1998). The effects of antiretroviral agents on CYP2B6 activity is not established. CYP2B6 is the primary enzyme mediating the formation of hydroxybupropion from bupropion in human liver microsomes and can be used as a probe for CYP2B6 activity in vitro (Faucette et al., 2000; Hesse et al., 2000). The objective of this study was to investigate the possibility of in vitro inhibition of CYP2B6 by several antiretroviral agents, using bupropion hydroxylation as the index reaction.
addition of 1 N HCl. Trazodone was used as an internal standard. Incubations with 10 μM inhibitor were performed using four different human livers, and incubations with 100 μM inhibitor were performed using one human liver with high CYP2B6 activity.

To generate IC \(_{50}\) estimates for antiretroviral drugs that significantly inhibited bupropion hydroxylation at 10 μM, varying concentrations (0–50 μM) of these agents (nelfinavir, ritonavir, efavirenz) were added to incubation tubes. Microsomes from four different human livers were used. Incubations were performed as previously described (Hesse et al., 2000).

Concentrations of hydroxybupropion were determined by high-performance liquid chromatography using a method adapted from Cooper et al. (1984) and described by Hesse et al. (2000). Briefly, a 300- × 3.9-mm Bondapak C\(_{18}\) column (Waters Associates, Milford, MA) was used with a flow rate of 2 ml min\(^{-1}\), ultraviolet detection at 214 nm, and a mobile phase of 79% 50 mM KH\(_2\)PO\(_4\) (pH 3) and 21% acetonitrile.

**Preincubations.** To evaluate the effect of preincubation on inhibitory potency, 1 or 10 μM inhibitor (nelfinavir, ritonavir, efavirenz, or delavirdine) was preincubated for 15 min with cofactor, buffer, and microsomes from one human liver with high CYP2B6 activity (von Moltke et al., 2000). As a control, cofactor, buffer, and microsomes were added to a tube containing no inhibitor and were preincubated for 15 min. The reaction was started by addition of an aliquot of the mixture to another tube containing bupropion (50 μM final concentration). The reaction was terminated and processed as described above.

**Data Analysis.** IC\(_{50}\) values for chemical inhibitors were determined by nonlinear regression analysis of data using a fixed concentration of substrate as previously described (Hesse et al., 2000).
Antiretroviral drugs inhibit the activity of several human cytochromes (Eagling et al., 1997; von Moltke et al., 1998, 2000, 2001), but interactions with CYP2B6 have not been established. Although bupropion hydroxylation is a probe for CYP2B6 activity in vitro (Faucette et al., 2000; Hesse et al., 2000), bupropion may be coadministered with antiretroviral agents to treat depressive disorders accompanying HIV infection. The present study demonstrated that nelfinavir, ritonavir, and efavirenz have low IC50 values for inhibition of bupropion hydroxylation. Although the clinical significance of these IC50 values is not fully established, the IC50 values are below or within the usual ranges of clinical plasma concentrations, indicating the possibility of clinically important CYP2B6 inhibition by nelfinavir, ritonavir, and efavirenz in vivo.

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Table 2

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Percentage of Control Activity With Preincubation</th>
<th>No Preincubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nelfinavir (1 μM)</td>
<td>87</td>
<td>71</td>
</tr>
<tr>
<td>Nelfinavir (10 μM)</td>
<td>23</td>
<td>37</td>
</tr>
<tr>
<td>Ritonavir (1 μM)</td>
<td>99</td>
<td>86</td>
</tr>
<tr>
<td>Ritonavir (10 μM)</td>
<td>39</td>
<td>29</td>
</tr>
<tr>
<td>Efavirenz (1 μM)</td>
<td>67</td>
<td>75</td>
</tr>
<tr>
<td>Efavirenz (10 μM)</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>Delavirdine (10 μM)</td>
<td>82</td>
<td>91</td>
</tr>
<tr>
<td>Delavirdine (100 μM)</td>
<td>67</td>
<td>80</td>
</tr>
</tbody>
</table>

Results and Discussion

Inhibitory activity of antiretroviral agents at 10 and 100 μM is shown in Table 1. Structures of bupropion, hydroxybupropion, and agents causing substantial CYP2B6 inhibition are shown (Fig. 1, A and B). Mean IC50 values ± S.E. for nelfinavir, ritonavir and efavirenz versus hydroxybupropion formation from bupropion (50 μM) were 2.5 ± 0.4, 2.2 ± 0.1, and 5.5 ± 0.8 μM, respectively (Fig. 2). Amprenavir (100 μM) was a weak inhibitor of bupropion hydroxylation, inhibiting to 49% of control, whereas other antiretroviral agents at 100 μM produced minimal inhibition of bupropion hydroxylation (Table 1). Preincubation of CYP2B6 with nelfinavir, ritonavir, efavirenz, and delavirdine did not substantially alter the inhibitory potency of these antiretroviral agents. Compared with non-preincubated samples, inhibition of CYP2B6 activity due to nelfinavir (1 μM) and ritonavir was slightly diminished by preincubation, whereas inhibition by efavirenz and delavirdine was slightly enhanced by preincubation (Table 2).

References


