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An in Vitro Mechanistic Study to Elucidate the Desipramine / Bupropion Clinical Drug-Drug Interaction

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Running Title: An in Vitro Study to Elucidate the CYP2D6/Bupropion DDI

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Nonstandard abbreviations: AUC, area under the drug concentration-time curve;  AUCi/AUC, area under the drug concentration-time curve of the substrate, in the presence and absence of inhibitor; Cmax, maximum plasma concentration; CYP, cytochrome P450; Em, emission wavelength; Ex, excitation wavelength; fmCYP2D6, the fraction of a substrate drug cleared by the inhibited pathway via a particular CYP enzyme; fu, fraction unbound in plasma; HPLC, high-performance liquid chromatography; I, inhibitor; IC50, concentration required for 50% inhibition; Ki, concentration at which 50% of the enzyme is inhibited; KI, inactivator concentration at which half-maximal inactivation rate is achieved; k_inact, maximal inactivation rate at saturating concentration of the inactivator; Km, substrate concentration corresponding to 50% maximum reaction velocity; LC/MS, liquid chromatography/mass spectrometry; qWBA, quantitative whole body autoradiography
Abstract

There are documented clinical drug-drug interactions between bupropion and the CYP2D6-metabolised drug, desipramine, resulting in marked (5-fold) increases in desipramine exposure. This finding was unexpected as CYP2D6 does not play a significant role in bupropion clearance, and bupropion and its major active metabolite, hydroxybupropion, are not strong CYP2D6 inhibitors in vitro. The aims of this study were to investigate whether bupropion’s reductive metabolites, threohydrobupropion and erythrohydrobupropion, contribute to the drug interaction with desipramine. In human liver microsomes using the CYP2D6 probe substrate bufuralol, erythrohydrobupropion and threohydrobupropion were more potent inhibitors of CYP2D6 activity ($K_i = 1.7 \mu M$ and $5.4 \mu M$, respectively) than hydroxybupropion ($K_i = 13\mu M$) or bupropion ($K_i = 21\mu M$). Further, neither bupropion nor its metabolites were metabolism-dependent CYP2D6 inhibitors. Using the in vitro kinetic constants and estimated liver concentrations of bupropion and its metabolites, modeling was able to predict within two-fold the increase in desipramine exposure observed when co-administered with bupropion. This work indicates that the reductive metabolites of bupropion are potent competitive CYP2D6 inhibitors in vivo and provides a mechanistic explanation for the clinical drug-drug interaction between bupropion and desipramine.
Introduction

Bupropion is a norepinephrine/dopamine reuptake inhibitor currently indicated for the treatment of major depressive disorder (Wellbutrin®) and smoking cessation (Zyban®). Clinical interactions involving bupropion and co-administered CYP2D6 substrates desipramine (Jefferson et al., 2005; Shad and Preskorn 1997), dextromethorphan (Guzey et al., 2002; Kotlyar et al., 2005) and venlafaxine (Kennedy et al., 2002) are well-documented. However, CYP2D6 plays an insignificant role in bupropion clearance. In humans, bupropion is extensively metabolized to three active metabolites: hydroxybupropion, which is formed primarily via CYP2B6, and the amino-alcohol isomers threohydrobupropion and erythrohydrobupropion, which are formed via reduction of the carbonyl group (Hesse et al., 2000; Faucette et al., 2000; Shroeder, 1983; Golden et al., 1988). Presumably, the mechanism for the clinical interaction with desipramine, which is primarily eliminated via CYP2D6 (Brøsen et al., 1986; Gram, 1974; Distlerath and Guengrich, 1984), is the result of CYP2D6 inhibition by bupropion and/or its metabolite(s). However, published data demonstrate that bupropion and hydroxybupropion are weak CYP2D6 inhibitors in vitro (IC₅₀ = 58 and 74 µM, respectively; Hesse et al., 2000). Since unbound human plasma concentrations of the active metabolites are 2.3- to 12-fold higher than bupropion levels, it is possible that the CYP2D6 inhibition observed in the clinic is due to more potent CYP2D6 inhibition by threohydrobupropion or erythrohydrobupropion compared to bupropion and hydroxybupropion. In addition, another hypothesis is that bupropion and/or its metabolites are metabolism-dependent CYP2D6 inhibitors. Therefore, the objective of this study was to investigate reversible and metabolism-dependent CYP2D6 inhibition by bupropion, hydroxybupropion, threohydrobupropion and erythrohydrobupropion in vitro in human liver microsomes using the CYP2D6 probe substrate bufuralol. The calculated in vitro kinetic constants and estimated in vivo liver concentrations of bupropion and its metabolites were then
used retrospectively to predict the change in desipramine exposure following co-administration with bupropion.
Materials and Methods

**Materials.** Bupropion hydrochloride, threohydrobupropion, erythrohydrobupropion, (S)-hydroxybupropion, paroxetine and [13C,²H₃]-1-hydroxybufuralol were synthesized at GlaxoSmithKline, Inc (Research Triangle Park, NC). Quinidine, glucose-6-phosphate, glucose-6-phosphate dehydrogenase and β-nicotinamide adenine dinucleotide phosphate (NADP⁺) were purchased from Sigma-Aldrich (St Louis, MO). Bufuralol hydrochloride and hydroxybufuralol maleate were purchased from Ultrafine Chemicals (Manchester, UK). Pooled human liver microsomes (prepared from at least 15 donors) were obtained from Xenotech LLC (Lenexa, KS). Microsomal protein and CYP content were provided by the manufacturer.

**CYP inhibition assays.** Inhibition of bufuralol 1'-hydroxylase (CYP2D6) activity was investigated in human liver microsomes in the presence and absence of bupropion and its metabolites. Bupropion, hydroxybupropion, threohydrobupropion, erythrohydrobupropion, and a positive control inhibitor (quinidine) were pre-incubated for 5 min with microsomes (0.1 mg/mL) and bufuralol (5 µM, approximately = Kₘ) in 50 mM potassium phosphate buffer, pH 7.4 at 37°C. Final concentrations of inhibitor were 0.003-30 µM (bupropion), 0.02-200 µM (hydroxybupropion) and 0.1-100 µM (threohydrobupropion, erythrohydrobupropion). Reactions were initiated by the addition of an NADPH-regenerating system and terminated after 10 min for threohydrobupropion and erythrohydrobupropion and 30 min for bupropion and hydroxybupropion by the addition of an equal volume of acetonitrile. Incubations absent of cofactor were performed as controls. All incubations were performed in duplicate. A solution containing internal standard ([13C,²H₃]-1-hydroxybufuralol) was added to the threohydrobupropion and erythrohydrobupropion samples and centrifuged prior to LC/MS/MS.
analysis. $K_i$ determinations for threohydrobupropion and erythrohydrobupropion were conducted at concentrations corresponding to approximately $0.33 \times IC_{50}$, $IC_{50}$, $3 \times IC_{50}$, and $10 \times IC_{50}$, and at final bufuralol concentrations of 1.25, 2.5, 5, 10, and 25 $\mu$M. $K_i$ determinations for bupropion and hydroxybupropion were conducted using final bufuralol concentrations of 1, 3, 10, and 30 $\mu$M, bupropion concentrations of 0.003, 0.03, 0.3, 3, and 30 $\mu$M, and hydroxybupropion concentrations of 0.02, 0.2, 2, 20 and 50 $\mu$M. $K_i$ values were also determined for the CYP2D6 positive control inhibitor quinidine. To evaluate metabolism-dependent CYP2D6 inhibition, bupropion, threohydrobupropion, erythrohydrobupropion, hydroxybupropion or positive control paroxetine were pre-incubated for 20 min with cofactor, buffer and microsomes prior to addition of bufuralol (5 $\mu$M). The reactions were then processed as described above. Metabolism-dependent inhibition was investigated by measuring the shift in $IC_{50}$ rather than determining the metabolism-dependent inhibitor parameters, $k_{inact}$ and $K_i$.

**Data analysis.** 1'-Hydroxybufuralol formation was quantified in the erythrohydrobupropion and threohydrobupropion incubations using an LC/MS/MS system comprised of a PE Sciex API 4000 or API300 with Ionics EP10+ mass spectrometer, CTC HTS PAL autosampler and Shimadzu pump. For the bupropion and hydroxybupropion incubations, 1'-hydroxybufuralol formation was quantified with HPLC and fluorescence detection (Ex 252nm/ Em 302nm) using calibration standards of the authentic reference standard 1'-hydroxybufuralol. For all inhibitors, rates of 1'-hydroxybufuralol production at each concentration of inhibitor were expressed as a percentage of the mean uninhibited control rate. Graphical analysis of the data and $IC_{50}$ calculations were performed using GraFit version 5.0.8 (Erithacus Software Ltd, Staines, UK). For $K_i$ determinations, the data were fitted to the Michaelis-Menten equations for competitive, mixed (competitive and noncompetitive) and noncompetitive inhibition using nonlinear
regression, and graphical analysis was performed according to Eadie-Hofstee plots. Metabolism-dependent inhibition of CYP2D6 activity was inferred from a decrease (> 2-fold) in IC$_{50}$ obtained following a 20 min NADPH pre-incubation relative to that obtained with the control pre-incubation.

**Prediction of clinical AUC change.** The AUC ratios of orally administered CYP2D6 substrates in the presence and absence of inhibitor(s) have been successfully predicted using the following equation, which assumes that all inhibitors act via the same inhibition mechanism (Rostami-Hodjegan and Tucker, 2004; Hinton et al., 2007; Ito et al., 2005):

$$\frac{AUC_i}{AUC} = \frac{1}{\left(\frac{fm_{CYP2D6}}{1+\sum_{j=1}^{n}[I_j]/Ki_j} \right) + \left(1 - fm_{CYP2D6}\right)}$$  \hspace{1cm} (1)

where $AUC_i/AUC$ is the area under the plasma concentration-time curve of substrate in the presence and absence of inhibitor(s) (after repeat oral dosing), $fm_{CYP2D6}$ is the fraction of a substrate drug cleared by the inhibited pathway via a particular CYP enzyme, $[I_j]$ is the $j^{th}$ inhibitor concentration, $1-fm_{CYP2D6}$ is the clearance via other CYP enzymes and/or renal clearance, and $K_i$ is the $j^{th}$ *in vitro* inhibition constant. Substituting the clinical unbound plasma $C_{max}$ of bupropion and hydroxybupropion for $[I]$, the calculated $K_i$ parameters for these entities (Table 1), and desipramine $fm_{CYP2D6}$ (0.9; Brøsen et al., 1993; Ito et al., 2005) into equation 1 (reflecting the previously published data from Hesse et al. 2000), the apparent $AUC_i/AUC$ ratio was predicted. The calculations were repeated using estimated liver concentrations of these two entities shown in Table 1. Liver concentrations were estimated by multiplying the clinical unbound plasma $C_{max}$ by the liver : plasma ratio of 5.5 to 9.4, determined from rat quantitative
whole body autoradiography (qWBA) studies (internal data; not published). Adding the clinical unbound plasma concentrations for threohydrobupropion and erythrohydrobupropion and the calculated $K_i$ parameters for these entities (Table 1), the $AUC_i/AUC$ ratio was predicted using equation 1. The predicted AUC change was re-calculated using the estimated liver concentrations of all four entities shown in Table 1.

**Clinical study design.** An open-label study investigating the effects of multiple doses of bupropion on the pharmacokinetics of a single dose of desipramine was performed in 15 male subjects genotyped as CYP2D6 extensive metabolizers. Subjects received a single 50 mg oral dose of desipramine on Day 1, bupropion (150 mg/day orally) on Days 8-10 and 150 mg twice daily on Days 11 – 21. On day 22, subjects received a single oral dose of bupropion 150 mg and 50 mg dose of desipramine. The effect of bupropion dosing on desipramine exposure was assessed based on pharmacokinetic parameters calculated from serum concentrations of desipramine before and after treatment with bupropion.
Results and Discussion

Clinical interactions resulting in increased exposure of CYP2D6-metabolised drugs following co-administration with bupropion have been observed despite published in vitro data demonstrating that bupropion and a major active metabolite, hydroxybupropion, are relatively weak CYP2D6 inhibitors (IC$_{50}$ = 58 and 74 µM, respectively; Hesse et al., 2000). The purpose of this study was to evaluate whether bupropion’s other active metabolites, threohydrobupropion and erythrohydrobupropion, are potent CYP2D6 inhibitors in vitro and if bupropion and its metabolites are metabolism-dependent CYP2D6 inhibitors in vitro.

In human liver microsomes, erythrohydrobupropion and threohydrobupropion were more potent inhibitors of CYP2D6-mediated bufuralol 1’hydroxylation (Ki = 1.7 µM and 5.4 µM, respectively, Figure 1) than hydroxybupropion (Ki = 13µM) or bupropion (Ki = 21µM) (Table 1). The mechanism for CYP2D6 inhibition best fit a competitive model. It has been suggested that the clinical drug-drug interaction with bupropion and CYP2D6 substrates may be due to mechanism-based CYP2D6 inhibition by bupropion (Kotlyar et al., 2005). Our investigations demonstrated that neither bupropion nor its metabolites were metabolism-dependent CYP2D6 inhibitors (data not shown). In addition, neither bupropion nor its metabolites were reversible or metabolism-dependent inhibitors of the major CYP enzymes investigated in pooled human liver microsomes.

The in vitro kinetic constants and unbound human plasma concentrations (C$_{max}$) of bupropion and its metabolites were then used retrospectively to predict the increase in desipramine exposure in vivo. Accurate prediction of the magnitude of clinical drug interactions is dependent on the in vivo inhibitor concentration available to the enzyme in the liver. Since the
actual concentration at the enzyme site cannot be measured directly, the use of surrogate inhibitor *in vivo* concentrations, particularly unbound hepatic inlet concentrations, has been successful in predicting clinical drug interactions (Obach et al., 2006). Hepatic inlet concentration estimations, however, require knowledge of inhibitor dose and absorption rate, and fraction of inhibitor passing through the intestine unchanged. In the case of bupropion, all three metabolites are formed in the liver, which confounds the use of hepatic inlet concentrations for the metabolites. Thus, the most meaningful *in vivo* concentration to use to predict the increase in desipramine exposure (AUCi/AUC) is unbound clinical plasma concentrations (C<sub>max</sub>) of bupropion and its metabolites, along with estimated liver : plasma ratios from preclinical rat qWBA studies. Bupropion qWBA data were not available, and thus qWBA data from rats dosed with <sup>14</sup>C-hydroxybupropion were used as estimates. Therefore, liver : plasma ratios for bupropion and its metabolites could be different if each compound had been individually dosed in separate qWBA studies. The purpose of this study, however, was to provide via *in vitro* experiments a mechanistic explanation for the clinical bupropion - CYP2D6 interaction, rather than to provide an absolute quantitative prediction of a clinical interaction.

The predicted AUC change was compared with the actual AUC ratio observed in CYP2D6-extensive metabolizers administered bupropion 300 mg for 10 days and a single 50 mg dose of desipramine on day 11. In this study a 5.2-fold and 1.9-fold increase in the AUC<sub>0→∞</sub> and C<sub>max</sub> of desipramine, respectively, were observed when desipramine was co-dosed with bupropion (product label; Figure 2). Using the *in vitro* kinetic constants and the unbound plasma concentrations (C<sub>max</sub>) of bupropion and hydroxybupropion only (Table 1; equation 1), the change in desipramine exposure (AUC<sub>i</sub>/AUC) *in vivo* was under-predicted (AUC ratio = 1.07). Since tissue correction factors have been previously shown to improve DDI predictions (Hinton et al., 2007), the liver concentrations of bupropion and hydroxybupropion were estimated using a liver
plasma ratio of 5.5 to 9.4 (from rat whole body autoradiography studies). Incorporating these estimated liver concentrations into equation 1, the predicted AUC ratio increased marginally to 1.4 to 1.6. When erythrohydrobupropion and threohydrobupropion were included in the calculation using only unbound plasma concentrations (C_{max}) for all four entities, the predicted AUC ratio remained marginal at 1.4. In contrast, adjusting the estimated liver concentrations of bupropion, hydroxybupropion, erythrohydrobupropion and threohydrobupropion yielded a predicted AUC change increase of 2.9- to 3.8-fold, which is within 2-fold of the in vivo AUC/AUC of desipramine following co-administration with bupropion (AUC ratio = 5.2; Figure 3). This predicted AUC ratio is considerably greater than if only bupropion or any of its metabolites were used for the prediction (Figure 3B). Thus, incorporation of bupropion metabolites’ in vitro kinetic constants and estimated liver concentrations in addition to those of bupropion improves the in vivo drug interaction prediction.

In conclusion, the bupropion metabolites erythrohydrobupropion and threohydrobupropion are more potent CYP2D6 inhibitors in vitro than bupropion or hydroxybupropion. These data offer a mechanistic explanation for the observed clinical drug interaction between bupropion and drugs metabolized by CYP2D6, which cannot be explained by the in vitro inhibitory potency of bupropion alone.
Acknowledgements

We gratefully acknowledge Joseph Polli for helpful discussions and review of this manuscript.
References


Legends for Figures.

Figure 1. Representative Eadie-Hofstee plots for CYP2D6 inhibition by threohydrobupropion (A) and erythrohydrobupropion (B) in human liver microsomes. Bufuralol concentrations were 1.25, 2.5, 5, 10, and 25 µM.

Figure 2. Average serum concentrations of desipramine in extensive CYP2D6 metabolizers given a single oral dose of 50 mg desipramine with (□) and without (■) 300 mg bupropion co-dosing for 11 days.

Figure 3. A. Desipramine AUCi/AUC predicted in vitro using inhibition constants and plasma and estimated liver concentrations of bupropion and hydroxybupropion only, using bupropion, hydroxybupropion, threohydrobupropion and erythrohydrobupropion parameters, and actual AUCi/AUC observed in clinic; B. The predicted desipramine AUC change when only bupropion’s (or its individual metabolites) Kᵢ, plasma and estimated liver concentrations were used in the prediction. See Table 1 for bupropion and metabolites Kᵢ values and plasma and liver concentrations.

Bup, bupropion; OH-bup, hydroxybupropion; threo, threohydrobupropion; erythro, erythrohydrobupropion.
### Tables

Table 1. Estimated human clinical concentrations and $K_i$ values for CYP2D6 inhibition by bupropion and metabolites

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Total Clinical Plasma $C_{\text{max}}$ (µM)</th>
<th>$f_u$</th>
<th>Unbound Clinical Plasma $C_{\text{max}}$ (µM)</th>
<th>Estimated Human Liver Concentration$^a$ (µM)</th>
<th>$K_i$ (µM)</th>
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</thead>
<tbody>
<tr>
<td>Bupropion</td>
<td>0.67</td>
<td>0.16$^c$</td>
<td>0.11</td>
<td>0.605 - 1.03</td>
<td>21</td>
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<tr>
<td>Hydroxybupropion</td>
<td>4.01</td>
<td>0.23$^b$</td>
<td>0.92</td>
<td>5.06 – 8.65</td>
<td>13.3</td>
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<tr>
<td>Threo hydrobupropion</td>
<td>2.34</td>
<td>0.58$^b$</td>
<td>1.35</td>
<td>7.43 – 12.7</td>
<td>5.4</td>
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<tr>
<td>Erythrohydrobupropion</td>
<td>0.43</td>
<td>0.58$^b$</td>
<td>0.25</td>
<td>1.38 – 2.35</td>
<td>1.7</td>
</tr>
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</table>

$^a$ Based on a liver:plasma ratio of 5.5 – 9.4 [determined from quantitative whole body autoradiography (qWB) studies in rats dosed with $^{14}$C-hydroxybupropion]

$^b$ Findley et al., 1981

$^c$ Internal data; not published
Figure 1

A

$K_i = 5.4 \, \mu M$

Threohydrobupropion concentrations

- $0 \, \mu M$
- $1.2 \, \mu M$
- $4 \, \mu M$
- $12 \, \mu M$
- $40 \, \mu M$

B

$K_i = 1.7 \, \mu M$

Erythrohydrobupropion concentrations

- $0 \, \mu M$
- $0.33 \, \mu M$
- $1 \, \mu M$
- $3.3 \, \mu M$
- $10 \, \mu M$
Fig 2.

Desipramine Serum Concentration (ng/mL)

- Desipramine
- Desipramine + bupropion

AUC\(_{0→∞}\) = 2324 ng.h/mL

AUC\(_{0→∞}\) = 446 ng.h/mL
Figure 3

A

- Predicted using [plasma]
- Predicted using [liver]
- Actual Clinical Observation

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<td>0.0</td>
<td>7.0</td>
</tr>
<tr>
<td>bup + OH-bup + erythro + threo</td>
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B

- Predicted using [plasma]
- Predicted using [liver]
- Actual Clinical Observation

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