Bupropion is an atypical antidepressant that is biotransformed in humans to its major active metabolite hydroxybupropion by cytochrome P450 2B6 (CYP2B6). Co-administration of bupropion with an inhibitor of CYP2B6 can result in a serious drug interaction, leading to bupropion related adverse effects (e.g., seizures). The antiplatelet agent ticlopidine has been identified as a potent \textit{in vitro} inhibitor of bupropion hydroxylation, however it is unknown if it interacts \textit{in vivo} in rodents. In this study we investigated the potential pharmacokinetic (PK) drug interaction between bupropion and ticlopidine in mice. Using a destructive sampling design, male CF-1 mice were administered ticlopidine 1.0 mg/kg daily for 5 d, followed by single-dose bupropion 50 mg/kg. Bupropion and hydroxybupropion levels were measured by HPLC-UV in plasma and brain tissues at 30, 60, 90, 120 and 180 min post-dose, and compared between treatment groups. There was a strong trend in both plasma and brain data towards greater bupropion levels and smaller hydroxybupropion levels in ticlopidine treated mice. Analysis of variance indicated statistical differences ($p<0.05$) at many time points. The variance associated with the area under the curve was calculated using Bailer’s method and significant differences were found between treatment groups. Taken together, the concentration time point statistical analysis followed by PK modeling demonstrate a significant PK drug interaction between bupropion and ticlopidine. To our knowledge, this is the first study to document an \textit{in vivo} drug interaction between these drugs in mice. Our findings support future \textit{in vivo} drug interaction studies in mice between bupropion and CYP2B6 inhibitors.

**Key words** bupropion; drug–drug interaction; ticlopidine; cytochrome P450 2B6; pharmacokinetics

Bupropion is a monocyclic antidepressant and non-nicoti- 

tine aid to smoking cessation that is structurally similar to 
phenylethylamines.\textsuperscript{1} Bupropion exhibits its pharmacological 
effects \textit{via} blockade of the neuronal uptake of two key neuro-
transmitters, dopamine (DA) and norepinephrine (NE), as 
well as antagonizing neuronal nicotinic acetylcholine recep-
tors (nAChRs).\textsuperscript{3} It is metabolized to a series of active 
metabolites (Fig. 1), including hydroxybupropion and the 
diastereomers erythrohydrobupropion and threo-hydrobupro-
propion.\textsuperscript{1} In antidepressant screening tests, hydroxybupropion 
was one-half as potent as bupropion, whereas the erythro-
hydrobupropion and threo-hydrobupropion metabolites were 
one-fifth as potent as bupropion.\textsuperscript{3,4} Hydroxybupropion has 
been identified in humans,\textsuperscript{5,6} guinea pigs,\textsuperscript{7} dogs,\textsuperscript{6} rats,\textsuperscript{6,7} and 
mice.\textsuperscript{6,7} Cytochrome P450 2B6 (CYP2B6) is the major CYP 
enzyme responsible for the metabolic conversion of bupro-
propion to hydroxybupropion in humans,\textsuperscript{3} while the reductive 
formation of erythrohydrobupropion and threo-hydrobupro-
propion is non-CYP mediated.\textsuperscript{4,8} Although not fully elucidated, 
similar CYP2B isoforms metabolize bupropion to hydroxy-

bupropion in animal species.\textsuperscript{7,9}

Bupropion toxicity leads to serious adverse drug effects, 
including seizures, psychosis and even fatalities.\textsuperscript{10—14} Al-
though the exact underlying mechanism of these adverse drug 
events is currently unknown, it appears that seizure in-

cidence is highly correlated with bupropion concentra-
tions.\textsuperscript{10,15} For instance, the incidence of seizures is doubled 
in patients receiving unintentional extra doses of bupropion 
compared to patients receiving therapeutic doses.\textsuperscript{15} Rapid 
nasal insufflation of bupropion, which results in elevated 
brain bupropion concentrations, has produced seizures also.\textsuperscript{16,17} Hence, whenever bupropion is co-administered 
with a potent CYP2B6 inhibitor in the clinic that leads to ele-
vated circulating bupropion concentrations, a potential for 
serious drug–drug interactions exists.

Numerous clinically relevant drugs have been shown to 
hibit bupropion hydroxylation \textit{in vitro}, including the 
thienopyridine antiplatelet agent ticlopidine.\textsuperscript{18—20} In two sepa-
rate \textit{in vitro} studies conducted in human liver microsomes,
ticlopidine inhibited bupropion hydroxylation with an IC_{50} of 0.15 μM and 0.20 μM, respectively. In humans, repeated treatment of ticlopidine increased the area under the concentration curve (AUC) and maximal plasma concentration (C_{max}) of bupropion by 60% and 40%, respectively. Ticlopidine has also been found to be a selective, mechanism-based inhibitor of CYP2B6. However, to date there have been no published in vivo studies in rodents studying potential drug interactions between bupropion and ticlopidine or other CYP2B6 inhibitors.

Using the prototypical CYP2B6 inhibitor ticlopidine as a model inhibitor, we sought in this study to investigate the potential pharmacokinetic (PK) drug interaction between bupropion and ticlopidine in mice. Our results reported herein are the first to our knowledge that document a significant PK drug interaction between bupropion and ticlopidine in mice. Furthermore, these findings will support future in vivo PK drug interaction studies in mice between bupropion and other CYP2B6 inhibitors.

MATERIALS AND METHODS

Chemicals All solvents and reagents used were of HPLC grade or higher. Bupropion hydrochloride and hydroxybupropion were purchased from Toronto Research Chemicals Inc. (Toronto, Ontario, C.A.). Ticlopidine and 0.9% NaCl solution (sterile filtered) were obtained from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.) and sterile water for injection (USP grade) was obtained from Abbott Laboratories (North Chicago, IL, U.S.A.).

Animals Mice were selected as a suitable animal species for this project since previous studies have shown that the mouse is the most similar species to man in studying the activity of cytochrome P450 2B. Also, bupropion conversion to the hydroxybupropion metabolite in mice appears to be more closely related to humans than in rats. Male CF-1 mice (30—35 g), aged 3 months, were obtained from Charles River Laboratories (Wilmington, MA, U.S.A.) and housed one mouse per cage in a temperature controlled environment. Mice were selected as suitable for this project since previous studies have shown that the mouse is the most similar species to man in studying the activity of cytochrome P450 2B. Also, bupropion conversion to the hydroxybupropion metabolite in mice appears to be more closely related to humans than in rats. Male CF-1 mice (30—35 g), aged 3 months, were obtained from Charles River Laboratories (Wilmington, MA, U.S.A.) and housed one mouse per cage in a temperature controlled environment with a 12 h light/dark cycle. Mice were allowed to acclimate to their new environment at least 2 weeks before study initiation. The experiment protocols were reviewed and approved by the Drake University Institutional Animal Care and Use Committee in accordance with the National Institutes of Health (NIH, U.S.A.) Guide for the Care and Use of Laboratory Animals.

In Vivo Interaction Study Mice were administered ticlopidine (1 mg/kg) or sterile water (1 μl/g) intraperitoneally (i.p.) once daily for 5 d. The ticlopidine dose is pharmacologically relevant and was estimated via in vitro inhibition data. On the 6th day using a destructive sampling technique, mice (cohorts of 4 animals per time point) were administered i.p. bupropion (50 mg/kg; calculated as free-base)±ticlopidine (1 mg/kg), then sacrificed by CO2 asphyxiation at 30, 60, 90, 120 and 180 min post-dose. The selection of the bupropion dose was based on a previous interaction study and the following criteria: (1) The effective bupropion dose and measured plasma concentrations of bupropion and hydroxybupropion should be comparable with human clinical situations; and (2) the lowest concentration of compounds should be above the lower limit of detection of the HPLC assay. Immediately following sacrifice, blood was collected via heart puncture into heparinized syringes and centrifuged at 1800×g for 15 min. The plasma was immediately stored at −80 °C until further analysis. Whole brain tissue was surgically removed following decapitation, blotted dry, weighed and immediately stored at −80 °C until later analysis. Ticlopidine was dissolved in sterile water whereas bupropion was dissolved in 0.9% sodium chloride solution.

Sample Extraction Plasma samples (0.2 ml) were spiked with timolol (internal standard) 1000 ng/ml then extracted with 0.5 M sodium carbonate buffer (pH 10.0) and 1.5% 3-methyl butanol in n-heptane according to the methods of Loboz et al. The organic layer was separated, back-extracted into 0.1 M HCl, and the bottom aqueous layer was evaporated to dryness under a gentle stream of nitrogen at 45 °C. The residue was reconstituted in mobile phase (0.110 ml) and an aliquot (0.05 ml) was injected onto the HPLC system. Samples for standard curve were prepared in parallel using known concentration of bupropion (3.0—1000 ng/ml), hydroxybupropion (6.0—5000 ng/ml) and internal standard (1000 ng/ml). Mobile phase was selected as the diluent for preparing stock solutions.

To analyze drugs in brain tissue, whole brain tissues were homogenized in 0.01 M HCl using an electronic Omni TH (Omni International; Marietta, GA, U.S.A.) homogenizer and Omni plastic tissue probe. Homogenate was centrifuged at 4200×g for 20 min. The supernatant (1.0 ml) was removed and extracted similar to plasma.

HPLC Analysis According to an earlier method reported by Loboz et al., the concentrations of bupropion and hydroxybupropion were quantified by reversed phase HPLC. The HPLC system consisted of a Shimadzu Scientific (Columbia, MD, U.S.A.) LC-10ADVP Solvent Pump, SIL 10ADVP Auto Sampler and SPD-M20A Photodiode Array Detector powered by EZStart v7.4 software. The mobile phase consisted of 25 mM potassium dihydrogen phosphate buffer (pH 6.4), acetonitrile and triethylamine in a ratio of 75:25:0.1. The analytical column was a Phenomenex Synergi Hydro C18 (Torrance, CA, U.S.A.) protected by an Aqua C18 Security Guard (Phenomenex) cartridge. All analytes were detected by ultraviolet detection at 214 nm. The column oven temperature was maintained at 30 °C, and the flow was delivered at 1.0 ml/min. The limit of detection (LOD) of bupropion and hydroxybupropion in plasma and brain was 6.0 ng/ml and 12.0 ng/ml, respectively. The intra- and inter-day % coefficient of variation at the LOD was ±16% for both plasma and brain samples.

PK Analysis The destructive sampling data obtained from the PK studies were analyzed by the naïve averaging pooled method. Plasma and brain concentrations of bupropion and hydroxybupropion from 2—4 mice at each time point were averaged. Non compartmental analysis was used to estimate various pharmacokinetic parameters using WinNonlin Professional v5.3 (Pharsight Corp., Mountain View, CA, U.S.A.). The estimated PK parameters were: AUC_{inf}= area under the plasma concentration time curve from time 0 to infinity; CL=clearance; V=volume of distribution, t_{1/2}=terminal elimination half-life, C_{max}=maximum plasma concentration, t_{max}=time of C_{max}.

At each time point, the mean sample concentration was estimated by the following method:
\[
\bar{y}_q = \frac{1}{n_q} \sum_{i=1}^{n_q} y_{q,i}
\]

Where \( \bar{y}_q \) is the average concentration of bupropion (or hydroxybupropion) at the \( q \)th time point, \( n_q \) is the number of sample points at \( q \)th point and \( y_{q,i} \) is the concentration of the \( r \)th animal at the \( q \)th time point. The trapezoidal rule was then applied to the average concentrations to estimate the AUC from 0 to 180 min by the following method:

\[
AUC = \sum_{q=0}^{Q} w_q \bar{y}_q
\]

where

\[
w_q = \begin{cases} \frac{(t_q - 0)}{2}, & q = 0 \\ \frac{(t_q - t_{q-1})}{2}, & q = 1, \ldots, Q - 1 \\ \frac{(T - t_{Q-1})}{2}, & q = Q \end{cases}
\]

The variance of the estimated AUC at each time interval was estimated using Bailer’s method as described below:

\[
\text{var}(AUC) = \frac{\sum w_q^2 s_q^2}{n_q}
\]

where \( s_q \) is the variance of the concentration at the \( q \)th time point. Total variance was calculated as the summation of variance at each time interval.

**Statistical Analysis** Mean plasma and brain concentrations of bupropion and hydroxybupropion were computed at each time point and subjected to pair wise statistical analysis by one-way analysis of variance (ANOVA) followed by Bonferroni \( t \)-test using SigmaStat v3.10 (Systat software, Chicago, IL, U.S.A.). Statistical comparisons among plasma and brain AUC values were determined using Student’s \( t \)-test. For all statistical tests, a value of \( p<0.05 \) was considered to be statistically significant.

**RESULTS**

To investigate the potential drug interaction between bupropion and ticlopidine, male CF-1 mice were repeatedly treated with ticlopidine i.p. 1.0 mg/kg (or sterile water 1 \( \mu \)l/g) for 5 d, followed by administration of single dose bupropion i.p. 50 mg/kg on the morning of the sixth day. Plasma and brain concentrations of bupropion and hydroxybupropion were measured by HPLC. The erythrohydrobupropion and threo-hydrobupropion metabolites were not measurable in most samples. Therefore, PK analyses were only conducted for bupropion and hydroxybupropion. Mean plasma and brain concentration time profiles (ng/ml \pm S.E.) for bupropion and hydroxybupropion were compiled from the data and depicted in Fig. 2. In general, bupropion levels should be greater and hydroxybupropion levels should be lesser in the ticlopidine treated mice if a drug interaction exists, indicating that ticlopidine is inhibiting the metabolism of bupro-
pion. Indeed, visual inspection of the profiles shows such notable differences between treatment groups at numerous time points for plasma bupropion (Fig. 2A), plasma hydroxybupropion (Fig. 2B), brain bupropion (Fig. 2C) and brain hydroxybupropion (Fig. 2D) concentrations. Statistical analysis of plasma bupropion concentrations (Fig. 2A) in the ticlopidine treated mice using ANOVA followed by the Bonferroni post hoc test t-test demonstrated significant differences at all time points (### p<0.001). Although more variability was present in the plasma hydroxybupropion profile (Fig. 2B), statistical differences were observed at 30 min and 180 min post-dose (## p<0.01) in the ticlopidine treated group. In the brain, statistical differences in bupropion concentrations at 60 min, 90 min and 120 min (## p<0.05) were observed in the ticlopidine treated mice; whereas, statistically significant differences were demonstrated at 30 min, 60 min, 120 min and 180 min for hydroxybupropion brain levels.

Using non compartmental analysis, basic PK parameters (AUC inf, CL, V, t1/2, Cmax, Tmax) were computed for plasma and brain bupropion and hydroxybupropion concentration profiles. Due to the destructive sampling design, only single PK parameters for plasma and brain were computed, and are included in Table 1. Mice treated with bupropion and ticlopidine exhibited an increase in AUC, t1/2 and Cmax of plasma bupropion by 2.3, 2.0 and 1.8 fold, respectively, and an increase in brain bupropion AUC by 1.5 fold (Table 1). These same mice showed a decrease in AUC, t1/2 and Cmax of plasma hydroxybupropion levels by 1.2, 6.3 and 1.2 fold, respectively, with a decrease in brain hydroxybupropion AUC by 1.2 fold (Table 1). The brain to plasma AUC ratios for both bupropion and hydroxybupropion was greater in mice treated only with bupropion. Bailer’s method was utilized to calculate the variance associated with the AUC values obtained from both treatment groups. In the ticlopidine treated mice, we found a greater AUC in bupropion exposure and a smaller AUC of hydroxybupropion exposure in both plasma and brain profiles (p<0.05; Student’s t-test).

**DISCUSSION**

Drug–drug interactions are a serious hindrance in achieving effective pharmacotherapy in the clinic, and account for a significant amount of untoward adverse effects and patient emergency room visits. Seizures resulting from the use of the popular antidepressant bupropion have been correlated with circulating bupropion plasma concentrations. Bupropion is exclusively metabolized to its major active metabolite hydroxybupropion by CYP2B6 in humans (Fig. 1). Numerous clinically relevant drugs have been shown to inhibit bupropion hydroxylation in vitro and in humans, however no studies to our understanding have explored interactions in rodents. Using the well characterized CYP2B6 inhibitor ticlopidine, we investigated the potential PK drug interaction between bupropion and ticlopidine in male CF-1 mice.

Repeated administration of ticlopidine to mice significantly altered the plasma and brain concentration profiles of bupropion and hydroxybupropion (Figs. 2A—D). Statistical analysis of the AUC values provided further evidence of a drug interaction between ticlopidine and bupropion (Table 1). Bupropion AUC values in both plasma and brain were greater in ticlopidine treated mice, whereas hydroxybupropion levels were significantly less in these mice. Collectively, the individual time point comparisons and statistical analysis of AUC are strongly suggestive of a PK drug interaction occurring between bupropion and ticlopidine in mice.

The variability observed at several time points in the hydroxybupropion plasma profile are perplexing. In contrast to the persistent statistical differences found in the bupropion plasma profiles, the hydroxybupropion levels show no differences at 60, 90 and 120 min post-dose. Although not proven by our studies, this may be evidence of a competing biotransformation pathway, such as non-CYP mediated ketone reduction and/or glucuronidation of hydroxybupropion. It cannot be determined from our current study if ticlopidine alters these potential competing pathways. Also, due to the limit of detection for the erythro- and threo-metabolites on our HPLC assay, the plasma and brain time course profiles and PK analyses of these metabolites is not known in this study.

The mouse model as an important in vivo animal model to study bupropion drug interactions based on several factors. In comparison to other animal species, mice more closely simulate human disposition of bupropion, suggesting that a similar CYP2b isofrom metabolizes bupropion to hydroxybupropion. This model when compared to a human model is a more practical (and easier studied) in vivo model. Also, mice present a unique opportunity to measure the brain distribution of bupropion and hydroxybupropion, which is clearly not possible in human subjects. In fact we demonstrated that the plasma to brain ratio of bupropion and hy-
droxybupropion AUC was greater in mice treated with bupropion only (Table 1). Also the brain volume of distribution of bupropion was greater in mice treated with ticlopidine (results not shown), suggestive of a greater brain distribution of bupropion when ticlopidine is co-administered.

Numerous psychoactive agents have been recently identified as in vitro CYP2B6 inhibitors. These include the selective serotonin re-uptake inhibitors (SSRIs) sertraline, paroxetine, norfluoxetine (the major metabolite of fluoxetine) and fluvoxamine, as well as the atypical antidepressant nefazodone. SSRIs in particular are widely prescribed for the treatment of depression and other mood and anxiety disorders. Interestingly, bupropion has emerged as an efficacious and tolerable treatment of depression and other mood and anxiety disorders.

Bupropion is also an efficacious adjunct treatment of depression and other mood and anxiety disorders. Interestingly, bupropion has emerged as an effective augmentation agent in patients exhibiting partial-response or treatment resistant depression to SSRI monotherapy. Bupropion was shown to be efficacious and well tolerated alone and with SSRI augmentation. A randomized double-blind study compared the efficacy and tolerability of bupropion as monotherapy with fluoxetine and escitalopram in adult outpatients with major depressive disorder. Bupropion was shown to be efficacious and well tolerated alone and with SSRI augmentation.

To conclude, this is the first study to our knowledge that demonstrates a significant drug interaction between the antidepressant bupropion and antiplatelet agent ticlopidine in mice. These studies will serve as a template for conducting future in vivo drug interaction studies in our laboratory between bupropion and psychoactive CYP2B6 inhibitors identified in the Walsky et al. and Hesse et al. studies.

REFERENCES