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Research Article

Pharmacokinetics and Pharmacogenomics of Bupropion in Three Different Formulations with Different Release Kinetics in Healthy Human Volunteers

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Abstract. The purpose of this pharmacokinetics (PK) study was to investigate whether different release kinetics from bupropion hydrochloride (HCl) immediate release (IR), sustained release (SR), and extended release (ER) formulations alter its metabolism and to test the hypothesis that the unsuccessful bioequivalence (BE) study of the higher strength (300 mg) of bupropion HCl ER tablets based on the successful BE study of the lower strength (150 mg) was due to metabolic saturation in the gastrointestinal (GI) lumen. A randomized six-way crossover study was conducted in healthy volunteers. During each period, subjects took a single dose of IR (75/100 mg), SR (100/150 mg), or ER (150/300 mg) formulations of bupropion HCl; plasma samples for PK analysis were collected from 0–96 h for all formulations. In addition, each subject's whole blood was collected for the genotyping of various single-nucleotide polymorphisms (SNPs) of bupropion's major metabolic enzymes. The data indicates that the relative bioavailability of the ER formulations was 72.3–78.8% compared with IR 75 mg. No differences were observed for ratio of the area under the curve (AUC) of metabolite to AUC of parent for the three major metabolites. The pharmacogenomics analysis suggested no statistically significant correlation between polymorphisms and PK parameters of the various formulations. Altogether, these data suggested that the different release kinetics of the formulations did not change metabolites-to-parent ratio. Therefore, the differing BE result between the 150 and 300 mg bupropion HCl ER tablets was unlikely due to the metabolic saturation in the GI lumen caused by different release patterns.

KEY WORDS: bioequivalence; bupropion; carbonyl reductase; CYP2B6; metabolism.

INTRODUCTION

Bupropion hydrochloride (HCl) is a clinically available drug product used for major depressive disorder, smoking cessation, and seasonal affective disorder (1). Bupropion was first marketed as an immediate release (IR) product in the 1980s for three times daily administration. In 1996 and 2003, a sustained release (SR) formulation (dosed twice a day) and extended release (ER) formulation (dosed once a day) became available, respectively, all utilizing the trade name of Wellbutrin (2).

Bupropion HCl is considered to be a biopharmaceutical classification system (BCS) class I drug (high permeability and high solubility) that exhibits linear pharmacokinetics (PK) in clinically available products (up to 450 mg) (3,4). Bupropion HCl is rapidly absorbed; the T_{max} for an IR formulation is on the order of 1 h, whereas the T_{max} for the SR and ER formulations are on the order of 3 and 5 h, respectively (5). Bupropion HCl is extensively metabolized to form three active metabolites; hydroxybupropion and threo/erythrohydrobupropion (5). It is thought that these metabolites might exhibit potency up to 25–

"Andrew Babiskin and Xinyuan Zhang" Disclaimer: The views expressed in this article are those of the authors and not necessarily those of the Food and Drug Administration (FDA).

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50% of bupropion, as shown in an animal model (6,7). In addition, hydroxybupropion and threohydrobupropion have been shown to have higher plasma concentrations than bupropion. A radiolabel study indicated that radioactive bupropion and metabolites are primarily eliminated in urine (87%) and feces (10%); however, only 0.05% of bupropion in urine is unchanged drug (8).

Bupropion is highly metabolized; Sager *et al.* highlight bupropion's metabolism based on current literature (9). CYP2B6 has been shown to be involved in the formation of hydroxybupropion. CYP2B6 polymorphic enzymes have been shown to exhibit as much as 20 to 250% differences in expression and activity (10). Furthermore, our recent study showed that bupropion is also metabolized by two major carbonyl reductases (11β-dehydrogenase isozyme 1 in the liver and AKR7 in the intestine) to form threohydrobupropion and erythrohydrobupropion (11). Carbonyl reductase enzymes have also been found to be highly polymorphic (12–14). In addition, the expression of these enzymes changes through the gastrointestinal (GI) tract and liver (10,15). Additionally, there is evidence that CYP2C19 and CYP3A4 may play a role in bupropion's metabolism (9,16); however, these enzymes are less characterized compared to CYP2B6 and carbonyl reductase.

Prior to 2013, establishing bioequivalence (BE) for bupropion HCl 300 mg ER tablets referencing Wellbutrin XL was based on acceptable *in vivo* BE study of the 150-mg strength, formulation proportionality, and similar dissolution profiles between the 150- and 300-mg strengths in multiple pH conditions. However, later on, it was reported that a generic of bupropion HCl 300 mg ER tablet was not equivalent to the reference product (17). The purpose of this study was to investigate fundamental mechanisms that may cause the observed BE with the 150-mg strength and the lack of BE with the 300-mg strength, when these two strengths demonstrate formulation proportionality and similar *in vitro* dissolution profiles. The T_{max} disparity between the generic and reference products suggested different *in vivo* release patterns considering that bupropion is a BCS class 1 drug (17). Therefore, it was hypothesized that various SNP or the GI regional metabolic enzymes might interact differently with the 300 mg tablets having different release patterns, which caused BE evaluation in the 150-mg strength to fail to extrapolate up to the 300-mg strength.

To address how different release kinetics of different formulations impact the PK and metabolism of bupropion, a randomized six-way crossover study of a single dose of bupropion HCl [IR (75 or 100 mg), SR (100 or 150 mg), or ER (150 or 300 mg)] was conducted. Plasma PK samples were collected until 96 h for all formulations. Both bupropion and the major metabolites (hydroxybupropion, threohydrobupropion, and erythrohydrobupropion) were monitored by LC-MS/MS. In addition, pharmacogenomics was analyzed for CYP2B6, CYP2C19, and AKR7A3 and examined in relation to the PK of bupropion and its metabolites.

METHODS

Study Population and Recruitment

The study was posted on ClinicalTrials.gov with the registry number of NCT02078180. Both the informed consent and study protocol were approved by the Food and Drug Administration Institutional Review Board/Research Involving Human Subjects Committee (RIHSC, #13-087D) and the University of Michigan Institutional Review Board (HUM00081894). In total, 33 subjects were recruited for this study and 30 completed the study (Table II). All subjects were voluntary, met the inclusion/ exclusion criteria, and provided informed consent prior to participation. All subjects were between the ages of 25 and 55 years old and had a body mass index between 18.5 and 35. Subjects were excluded if they had concomitant medical issues that would alter bupropion metabolism (liver disease, gastric bypass, etc.), nicotine dependence, and alcohol dependence or were either pregnant or nursing. Subjects were not excluded based on gender, race, or ethnicity.

Study Design

This study utilized a six-period, six-treatment crossover design under fasting conditions where each participant was administered a single dose of bupropion HCl IR 75 mg, IR 100 mg, SR 100 mg, SR 150 mg, ER 150, and ER 300 mg tablet for each period. Prior to baseline, each participant fasted for at least 10 h pre-dosing and 4 h post-dosing. No water was given 1 h pre-dosing or post-dosing, with the exception of 240 mL of water that was taken with each pill. A minimum of a 10-day washout period occurred between each period. For sample collection, blood (~3 to 4 mL) was drawn at 0, 0.5, 1, 2, 3, 4, 6, 8 (12 h for ER tablets), 24, 48, 72, and 96 h.

LC-MC/MS Method

Whole blood was collected from all subjects and the actual time of sample collection was recorded and used for the data analysis. Whole blood was centrifuged at 15,000 rpm at 4°C. The supernatant (plasma) was collected and transferred to an EDTA K2 tube. Plasma samples were stored at −80°C.

The LC/MS/MS analysis was conducted using either an Agilent 1200 HPLC system coupled to an API 3200 mass spectrometer (Applied Biosystems, MDS Sciex Toronto, Canada) or Shimadzu HPLC coupled with an API4500 with an electrospray ionization (ESI) source. Quantitative analysis was accomplished on a Supelco C18 (150 × 4.6 mm I.D., 5 μm) column. The mobile phases were 0.04% formic acid in purified water (A) and 0.04% formic acid in methanol (B). The LC was ran either using isocratic at 35% methanol and the flow rate was set at 0.8 mL/min on API3200, or with API4500, the LC separation started at 30% B, maintained for 8 min, then rose to 90% B and kept for 4 min, switched back to 30% B afterward, and kept for another 4 min with a flow rate of 1 mL/min.

The LC/MS/MS method was operated at positive ESI ionization mode. The multiple reaction monitoring (MRM) transitions and collision energies determined for bupropion, hydroxybupropion, threohydrobupropion, erythrohydrobupropion, and internal standard are listed in Table I. The analytical data were processed by Analyst software (versions 1.2 and 1.6; Applied Biosystems, Foster City, CA, USA).

The quantification of bupropion, hydroxybupropion, threohydrobupropion, and erythrohydrobupropion at different times and concentration were performed by MRM of the $[M - H]^+$ ion, using venlafaxine as an internal standard (IS) to establish peak area ratios for calibration curve fitting.

Table I. MRM Parameters for Bupropion, Hydroxybupropion, Threo/Erythrohydrobupropion, and IS Determination

Compound name	MRM transition	Collision energy (CE)	Declustering potential (DP)	Entrance potential (EP)	Collision cell exit potential (CXP)
Bupropion	240.1 → 184.0	10 (25)	50 (51)	10	3 (10)
Hydroxybupropion	256.0 → 238.0	12 (25)	50 (51)	10	3 (10)
Threo/erythrohydrobupropion	242.0 → 168.1	5 (25)	50 (51)	10	3 (10)
Venlafaxine (IS)	278.0 → 260.0	10 (25)	50 (51)	10	3 (10)

Some MS parameters used on the API4500 were different to ones used on the API3200. These parameters were indicated in the parentheses

Method Validation

The LC-MS/MS method was validated according to the FDA Bioanalytical Method Validation. The following test passed according to the guidance for bupropion and the three major metabolites: selectively, specificity, precision, matrix effect, recovery, carryover, standard curves, QC samples, short-term stability, and accuracy.

Pharmacogenomic Analysis

Selection of SNPs and Genotypes. The *CYP2B6* polymorphisms, rs8192709 (variant allele present in *CYP2B6*2*), rs3745274 (variant allele present in *CYP2B6*6* and *CYP2B6*9*), rs45482602 (variant allele present in *CYP2B6*3*), rs2279343 (variant allele present in CYP2B6*4 and CYP2B6*6), and rs3422 3104 (variant allele present in *CYP2B6*22*), were chosen based on previous reports suggesting that selected variants alter CYP2B6 activity (16,18–22).

Selected polymorphisms for *CYP2C19* genotyping, rs4986893 (variant allele present in *CYP2C19*3*), rs4244285 (variant allele present in *CYP2C19*2*), and rs12248560 (variant allele present in *CYP2C19*17*), were chosen based on their associations with drug metabolism (23–25).

Due to the small sample size of this investigation and paucity of literature pertaining to AKR7A3 and bupropion metabolism, *AKR7A3* polymorphisms, rs2231198, rs12405546, rs7525791, and rs2013249, were selected based a reported minor allele frequency (MAF) of at least 0.2 (www.1000genomes.org).

Genotyping Methods

A separate whole blood (10 mL) was collected from each subject and was used for genomic DNA extraction by

Table II. Subject Demographics and Baseline Lab Data

Variable		Value	Normal range
Demographics			
Sex, *n* (%)	Female	17 (51.5%)	
	Male	16 (48.5%)	
Ethnicity, *n* (%)	Hispanic	2 (6.1%)	
	Non-Hispanic	31 (93.9%)	
Race, *n* (%)	African American	4 (12.2%)	
	Asian	3 (9.0%)	
	Caucasian	24 (72.7%)	
	Other	2 (6.1%)	
Patient characteristics, median (range)			
Height (cm)		67.5 (62.0, 76.4)	
Weight (lb)		176 (124.2, 236)	
BMI		25.9 (20.6, 36.0)	<18.5– > 35
AST (IU/L)		23 (17, 34)	8–30 IU/L
ALT (IU/L)		23 (13, 40)	≤35 IU/L
WBC (10^3/μL)		6.7 (4.0, 13.4)	4–10 K/μL
Hemoglobin (g/dL)		14.2 (11.9, 17.8)	12–16 g/dL
HCT (%)		41.9 (35.5, 50.6)	36–48%
Platelet count (10^3/μL)		256 (153, 340)	150–400 k/μL
BUN (mg/dL)		14 (8, 26)	8–20 dL
HDL (mg/dL)		53 (39, 99)	>40 mg/dL
LDL (mg/dL)		106	<130 mg/dL

The median (percent of population) is presented for each demographic and the median (range) patient characteristics
BMI body mass index, *AST* aminotransferase blood test, *ALT* alanine aminotransferase, *WBC* white blood cell, *HCT* hematocrit, *BUN* blood urea nitrogen, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein

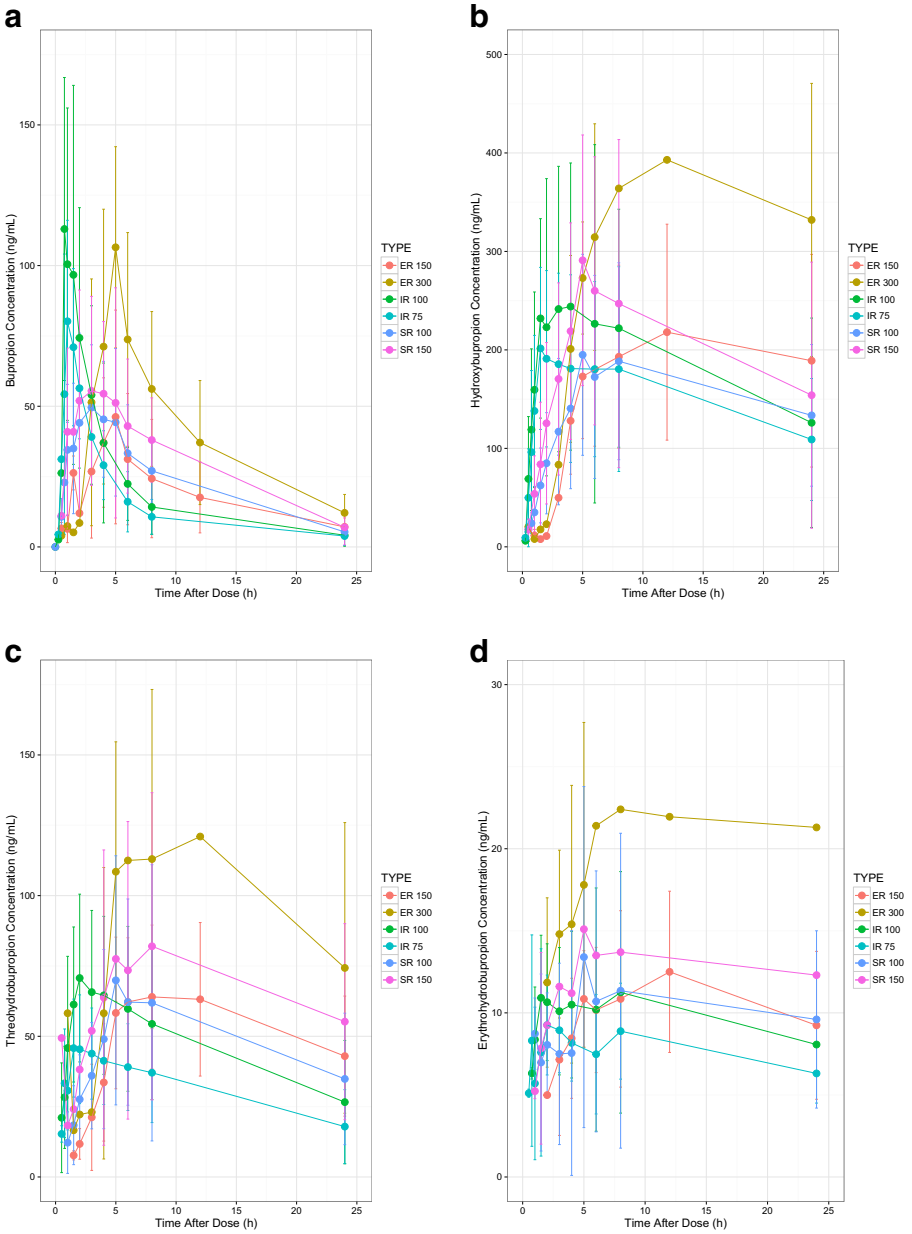

Fig. 1. Plasma concentration *versus* time profiles for bupropion and metabolites. The mean ± standard deviation of the plasma concentration for bupropion and three major metabolites are represented for each formulation. **a** Bupropion. **b** Hydroxybupropion. **c** Threohydrobupropion. **d** Erythrohydrobupropion

the salt precipitation method as described previously (26). Genotyping was completed by polymerase chain reaction (PCR), followed by genotyping using Pyrosequencing™ technology (Qiagen, Hilden, Germany) for *CYP2B6* and *AKR7A3* (27). PCR primers were designed using Pyrosequencing SNP Primer Design version 1.01 software (www.pyrosequencing.com). Assay conditions are available upon request. Genotyping for *CYP2C19* was performed using the LightCycler® 480 Instrument II (Roche Life Science, Indianapolis, IN) and TaqMan Genotyping Kits (Thermo Fisher Scientific Inc., Waltham, MA) by protocols designed by the manufacturer.

Pharmacokinetic Parameter Estimates

The parameters estimates were calculated using non-compartment analysis in Phoenix WINNONLIN (Pharsight, version 6.4.). The PK parameter estimates ($t_{1/2}$, T_{max}, C_{max}, $AUC_{0-\infty}$, CL/F, and V_z/F) were estimated from all individual concentration *versus* actual time profiles for each subject and all treatments of bupropion. The PK parameters $t_{1/2}$, T_{max}, C_{max}, and $AUC_{0-\infty}$ were estimated for the major metabolites for each subject and all treatments. A minimum of three points excluding the C_{max} in the terminal phase was needed in order to calculate lambda$_z$ and associated PK parameters

Table III. Relative Bioavailability of Bupropion

	AUC_{0-inf} (h × ng/mL)	Dose-normalized AUC_{0-inf}	Relative bioavailability (%)
IR 75	469 (38%)	6.25 ± 2.4	100%
IR 100	688 (62%)	6.87 ± 4.3	110%
SR 100	706 (43%)	7.06 ± 3.0	113%
SR 150	1000 (51%)	6.68 ± 3.4	107%
ER 150	740 (60%)	4.93 ± 2.9	78.8%
ER 300	1360 (47%)	4.52 ± 2.1	72.3%

The AUC_{0-inf} was calculated for each subject and formulation. The table shows the average (CV%) and the dose-normalized AUC_{0-inf} (mean ± SD). The IR 75 formulation was used to calculate the relative bioavailability for each formulation

(CL/F and V_z/F). The mean ± standard deviation is shown for each formulation.

Population Pharmacokinetic Analysis

The plasma concentrations *versus* actual time data were used for the data analysis. The population PK (PPK) analysis was performed using Nonlinear Mixed Effect Modeling Software, (NONMEM; version 7.3, Icon Development). The PPK structural model was fitted using both a one-compartment and two-compartment models. The model selection was based on the objective function value (OFV), shrinkage, goodness-of-fit plots, and standard error of the random effect parameters. The inter-individual variability (IIV) was described as an exponential error model and the residual variability (RV) was analyzed using the log error model. The IIV for the peripheral volume of distribution (V_3) and blood flow (Q) were fixed.

For the covariate analysis, the SNPs for the three metabolism enzymes were added as covariates to the CL/F and V/F terms linearly. Covariates were grouped in the model based on suggested SNP variant (i.e., homozygous dominant, heterozygous, and homozygous recessive). In addition, CYP2B6 was also further explored using the suggested phenotype for each individual. Covariates were selected if they had a minimum p value <0.05 (ΔOFV ≤3.84) in the forward selection and p value of <0.001 (ΔOFV ≤10.83) in the backward method. Covariates that were selected were validated by performing bootstrap and visual predictive check (VPC).

Data Analysis

Data were graphically analyzed using R (version 3.2.4).

RESULTS

Subject Randomization

A total of 33 subjects were enrolled in the study and 30 completed all 6 periods for data analysis. Each subject received a single dose of bupropion HCl tablets (IR 75 or 100 mg, SR 100 or 150 mg, or ER 150 or 300 mg) during each period.

The demographic and baseline lab data collected for the study are summarized in Table II. The study population was almost half females and half males (16 males and 17 females) and most subjects were Caucasian (72.7%).

Table IV. Bupropion PK Parameters

(A)

		n	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng × h/mL)	CL/F (L/h)	V_z/F (L)
Bupropion	IR 75	30	93 ± 36	1.3 ± 1.1	10 ± 6	469 ± 177	182 ± 68	2420 ± 1330
	IR 100	32	134 ± 70	1.2 ± 0.7	10 ± 8	688 ± 427	181.2 ± 81	2110 ± 800
	SR 100	30	61 ± 29	2.9 ± 1.5	12 ± 11	706 ± 304	169 ± 68	2250 ± 1600
	SR 150	32	78 ± 40	3.2 ± 1.9	12 ± 9	1002 ± 516	188 ± 132	3460 ± 1380
	ER 150	30	61 ± 34	4.7 ± 1.3	15 ± 16	740 ± 449	289 ± 181	4110 ± 2280
	ER 300	30	111 ± 44	4.8 ± 1.8	14 ± 9	1356 ± 637	282 ± 136	4570 ± 2170

(B)

	Parameter	Units	Base model estimate (SE)	Shrinkage
Fixed effects	SR-F		0.96 (0.06)	19.5%
	ER-F		0.68 (0.04)	43.9%
	K_a	1/h	0.47 (0.04)	n.a.
	CL	L/h	154 (68.2)	n.a.
	V_2	L	1070 (306)	20.7%
	V_3	L	2530 (7.3)	
	Q	L/h	103 (7.2)	
IIV	K_a	%	96.0% (36.8%)	
	CL	%	33.6% (19.1%)	
	V_2	%	47.4% (14.8%)	
Residual variability	Proportional	%	54.7% (15.6%)	

PK parameters were estimated by non-compartment analysis (A). The mean ± standard deviation (SD) is shown for each parameter. The population pharmacokinetic model for bupropion was fitted to a two-compartment model (B). The structure model is shown for the fixed and random effects

n number of subjects, C_{max} time to maximum concentrations, T_{max} time to C_{max}, $t_{1/2}$ half life, $AUC_{0-\infty}$ area under the time *versus* concentration plot from 0 to infinity, *CL/F* apparent clearance, V_z/F apparent volume of distribution, *SE* standard error, *F* relative bioavailability (referenced to IR formulation), *IIV* inter-individual variability, K_a absorption rate constant, V_2 central volume of distribution, V_3 peripheral volume of distribution, *CL* clearance, Q blood flow

Table V. PK Parameters for Metabolites

		n	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng × h/mL)	Dose-normalized AUC
Hydroxybupropion	IR 75	30	232 ± 105	4.8 ± 4.1	23.3 ± 9.2	9,020 ± 5,080	120 ± 68
	IR 100	32	296 ± 154	3.4 ± 1.4	22.7 ± 6.6	10,800 ± 7,000	108 ± 70
	SR 100	30	223 ± 99	7.3 ± 3.5	24.0 ± 8.4	10,100 ± 6,010	101 ± 60
	SR 150	32	311 ± 168	6.4 ± 1.3	23.4 ± 8.1	14,300 ± 9,700	95.2 ± 64.9
	ER 150	30	242 ± 110	12.2 ± 6.0	24.9 ± 6.7	12,100 ± 8,000	80.5 ± 53.1
	ER 300	32	402 ± 145	10.0 ± 4.7	23.5 ± 8.0	19,700 ± 9,700	65.7 ± 32.4
Threohydrobupropion	IR 75	30	53.9 ± 17.9	3.1 ± 1.7	35.3 ± 18.5	2,050 ± 990	27.3 ± 13.2
	IR 100	32	85.5 ± 30.9	3.1 ± 1.8	39.5 ± 15.6	3,260 ± 1,950	32.6 ± 19.5
	SR 100	30	67.9 ± 32.6	6.8 ± 3.6	41.4 ± 11.3	3,300 ± 1,600	33.0 ± 16.0
	SR 150	32	104 ± 64	6.5 ± 1.5	44.7 ± 44.1	4,880 ± 2,170	32.5 ± 14.8
	ER 150	30	70.9 ± 28.5	8.4 ± 4.3	41.7 ± 14.4	3,860 ± 1,720	25.7 ± 11.5
	ER 300	30	144 ± 71	9.2 ± 4.7	42.2 ± 14.3	7,350 ± 4,740	24.5 ± 15.8
Erythrohydrobupropion	IR 75	30	8.5 ± 5.9	4.2 ± 2.2[a]	26.9 ± 18.3[b]	439 ± 303[b]	5.8 ± 4.0
	IR 100	32	13.6 ± 8.0	5.0 ± 4.0[c]	46.0 ± 63.8[d]	1,020 ± 1,240[d]	10.2 ± 12.4
	SR 100	30	11.0 ± 6.4	8.0 ± 5.3[e]	38.4 ± 17.3[f]	774 ± 369[f]	7.7 ± 3.7
	SR 150	32	15.2 ± 6.5	9.6 ± 9.0	60.0 ± 95.5[g]	1,510 ± 2,060[g]	10.0 ± 13.7
	ER 150	22	13.8 ± 4.8	12.4 ± 9.5	31.0 ± 14.7[h]	765 ± 432[h]	5.1 ± 2.9
	ER 300	32	24.8 ± 10.6	10.3 ± 6.1	43.3 ± 37.2[i]	1,860 ± 1,480[i]	6.2 ± 4.9

The PK parameters were estimated by non-compartment analysis. The mean ± standard deviation (SD) is shown for each parameter. Number of subjects for parameter estimates may have been adjusted based on the data available

n number of subjects, C_{max} time to maximum concentrations, T_{max} time to C_{max}, $t_{1/2}$ half life, $AUC_{0-\infty}$ area under the time *versus* concentration plot from 0 to infinity

[a] $n = 24$

[b] $n = 10$

[c] $n = 30$

[d] $n = 20$

[e] $n = 25$

[f] $n = 13$

[g] $n = 18$

[h] $n = 14$

[i] $n = 27$

All adverse events (AEs) were summarized in Supplemental Table I. All AEs were mild to moderate; of the 11 AEs reported from 7 subjects, the most common adverse events reported were headaches, fatigue, or nausea ($n = 8$). In addition, these AEs may be due to study conditions rather than investigational products (i.e., no caffeine, 10-h fasting).

Table VI. Allele Frequencies of *CYP2B6*, *CYP2C19*, and *AKR7A3*

Gene	SNP	AA	Variant	Reference sequence identification	Observed frequencies
CYP2B6					
	T-82C		*22	rs34223104 genotype, TT/TC/CC	33/0/0
	C64T	R22C	*2	rs8192709 genotype, CC/CT/TT	30/3/0
	G516T	Q172H	*6,*7,*9	rs3745274 genotype, GG/GT/TT	0/26/7
	C777A	S259R	*5	rs45482602 genotype, CC/CA/AA	33/0/0
	A785G	K262R	*4, *5, *6	rs2279343 genotype, AA/AG/GG	7/25/1
CYP2C19					
	G22948A	W212Ter	*3	rs4986893 genotype, GG/GA/AA	33/0/0
	G681A	P227P	*2	rs4244285 genotype, GG/GA/AA	27/4/0[a]
	C-806T		*17	rs12248560 genotype, CC/CT/TT	21/9/2[a]
AKR7A3					
	G489A	V138M		rs2231198 genotype, GG/GA/AA	10/23/0
	A215 − 546G			rs12405546 genotype, AA/AG/GG	21/9/3
	G215 − 697A			rs7525791 genotype, GG/AG/AA	4/20/9
	C214 + 926G			rs2013249 genotype, CC/CG/GG	5/28/0

Each subject was genotyped for selected SNPs in (a) *CYP2B6*, (b) *CYP2C19*, and (c) *AKR7A3*. The amino acid (AA) substitution, SNP, and rs number designations are displayed

Ter termination

[a] One subject's genotype was undetermined

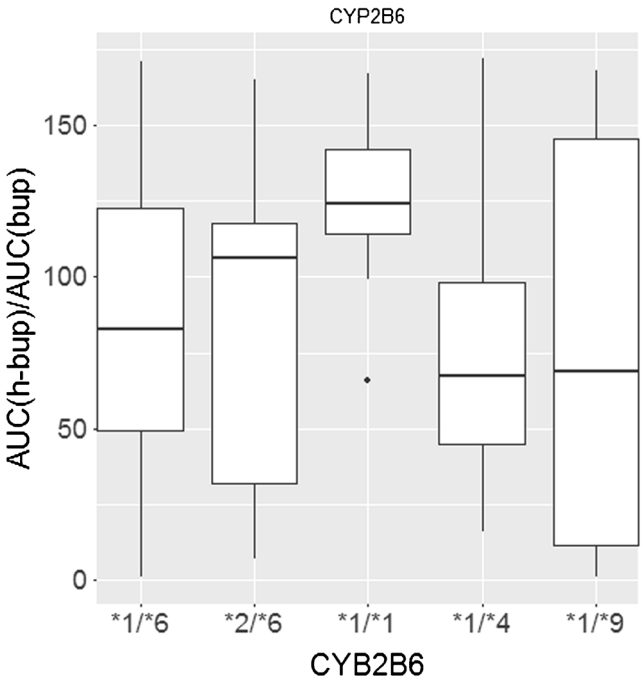

Fig. 2. Ratio of AUC(m)/AUC(p) for hydroxybupropion. The AUC(hydroxybupropion)/AUC(bupropion) is shown for CYP2B6 variants. The *solid black line* represents the median. The height of each box corresponds to the interval between the first and the third quartiles, and the *bar* represents 1.5 times the interquartile range

Bupropion and Major Metabolite Concentrations

The mean plasma concentration *versus* time profiles for bupropion and the major metabolites by formulation at different dose levels are represented in Fig. 1. Hydroxybupropion and threohydrobupropion showed higher plasma concentrations compared to bupropion, with hydroxybupropion mean concentration nearly fivefold higher than bupropion. Erythrohydrobupropion formation was minor, and in some subjects, erythrohydrobupropion was not even detected.

The relative bioavailability of bupropion was examined (Table III). The mean AUC_{0-inf} (%CV) associated with each formulation was calculated. There was a moderately large inter-subject variability observed within each formulation. The mean dose-normalized AUC was also calculated to compare exposure among formulations. The mean AUC of IR 75 mg formulation was used as a reference to calculate the relative bioavailability. For the IR and SR formulations, the mean relative bioavailability was similar (100–113%). However, for the ER formulation, the mean bioavailability was more than 20% less compared to the 75 mg of the IR formulation. This suggested that for the ER formulation, either less bupropion was getting into the systemic circulation or more bupropion was being metabolized.

The PK parameters of bupropion and the metabolites were estimated from a non-compartmental analysis for each subject and formulation. The mean ± SD for each formulation for bupropion is shown in Table IV (A). The apparent clearance (CL/F) and volume of distribution (V_z/F) for all the formulations were 169 to 288.5 L/h and 2109.5 to 4564.6 L. The IR and SR formulations CL/F and V_z/F were relatively similar; however, the ER formulation showed higher CL/F and V_z/F. The half-life was consistent among formulations, ranging from 10 to 14 h. The T_{max} was consistent with previous literature for each formulation with the IR, SR, and ER formulations showing ~1, 3, and 5 h, respectively (5).

Major metabolite PK parameters are shown in Table V. For erythrohydrobupropion, many subjects' PK parameters were not estimated due to lack of formation or not enough points in the terminal phase to estimate the half-life and $AUC_{0-\infty}$. For hydroxybupropion and threohydrobupropion, the T_{max} formed consistent with bupropion's formulation, where the IR, SR, and ER formulations were approximately 3–4, 6–7, and 10–12 h, respectively. The mean dose-normalized AUC decreased slightly for hydroxybupropion for the ER formulations, but this decrease is likely not significant. For threohydrobupropion and erythrohydrobupropion, there was practically no difference in dose-normalized concentrations among the various formulations.

Bupropion plasma concentrations and time data from all formulations were used for this analysis. Both a one-compartment model and two-compartment model were fitted to determine the optimum structure model. The two-compartment model described the data best. In addition to the objective function value (OFV) for model evaluation, goodness-of-fit plots, shrinkage, and standard errors were evaluated to confirm the two-compartment model (data not shown). The relative bioavailability (F) was calculated using the IR formulation as a reference (IR $F = 1$). The relative bioavailability estimated for the SR formulation was 95.5% and for the ER formulation was 68%. Both peripheral volume of distribution (V_3/F) and blood flow (Q) inter-individual variability (IIV) was fixed in the two-compartment model. The model showed that the typical clearance value was 154 L/h and the typical central volume of distribution was 1070 L for this population (Table IV (B)). The IIV for the absorption constant was high, likely due to the various formulations included in the model.

Pharmacogenomic Analysis

Genes encoding bupropion-metabolizing enzymes, *CYP2B6*, *CYP2C19*, and *ARK7A3*, were genotyped for the selected polymorphisms (Table VI). There were several SNPs in our population that had no variability and therefore were excluded from the individual SNP analysis (rs34223104, rs45482602, and rs4986893). PK measurements were then evaluated to determine the impact of each polymorphism on clearance or volume of distribution. Polymorphisms that showed zero variability in our subject cohort were excluded from our PK analysis. The covariate model was run in the forward method selection at a $p < 0.05$ and in the backward method of $p < 0.001$. However, no polymorphisms were statistically significant, suggesting that either pharmacogenomics had no impact on PK or our sample size was too small to measure the impact of these polymorphisms on bupropion metabolism.

To further confirm that these SNPs had no effect on PK in our cohort, the AUC(m)/AUC(p) ratio was calculated for each variant corresponding to the metabolite it produces (Figs. 2 and 3). Figure 2 shows the AUC(m)/AUC(p) of hydroxybupropion to bupropion grouped by *CYP2B6* and *CYP2C19* variant alleles. We found that CYP2B6 was not associated with variability in the ratio of AUC (hydroxybupropion)/AUC (bupropion). Figure 3 shows the AUC(m)/AUC(p) of threohydrobupropion to bupropion for AKR7A3 (Fig. 3a–d) and CYP2C19 (Fig. 3e, f). No differences were observed for the

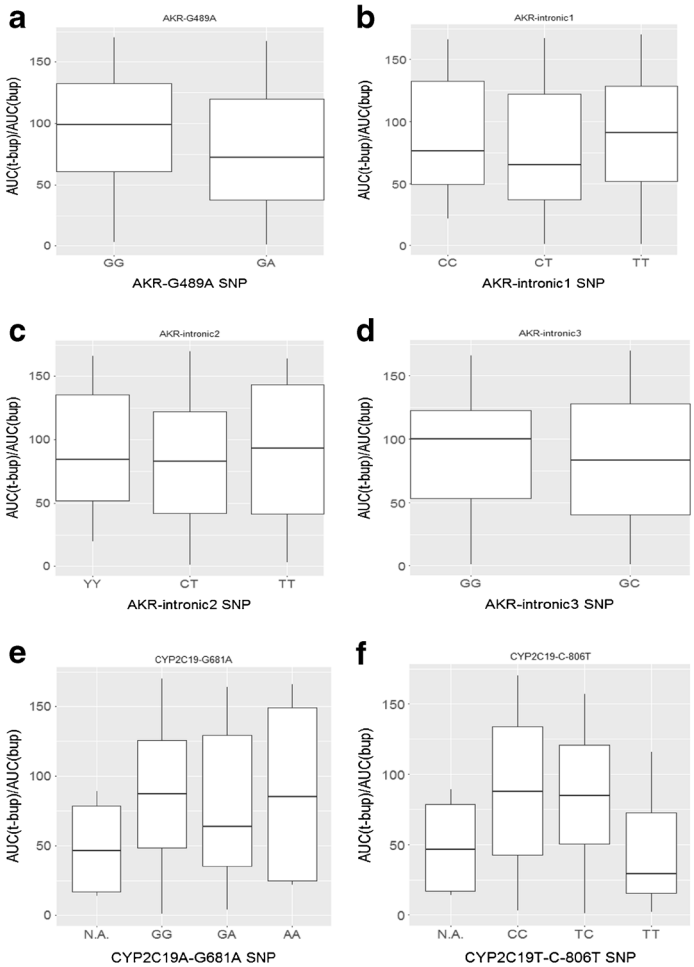

Fig. 3. Ratio of AUC(m)/AUC(p) for threohydrobupropion. The AUC(threohydrobupropion)/AUC(bupropion) is shown for AKR7A family **a** AKR7-G489A, **b** intronic 1, **c** intronic 2, and **d** intronic3. In addition, CYP2C19 **e** CYP2C19A-G681A phenotype and **f** CYP2C19T-C-806T phenotype. The height of each box corresponds to the interval between the first and the third quartiles, and the *bar* represents 1.5 times the interquartile range. *N.A.* not available

ratio of AUC (threohydrobupropion)/AUC (bupropion) for any variant allele. Similar results were observed for erythrohydrobupropion (data not shown, due to the low systemic concentrations).

Regional GI Metabolism Analysis

To further evaluate whether formulations could have regional GI metabolism rather than genetic metabolism differences, the ratio of the AUC(m) to that time point over the ratio of the AUC(p) was plotted for each formulation for all three metabolites (Fig. 4). For hydroxybupropion, there was no statistically significant difference among formulation for the AUC(m)/AUC(p) ratio, indicating that the higher exposure of hydroxybupropion was not formulation dependent. Likewise, this was also observed for threohydrobupropion and erythrohydrobupropion. For all three metabolites, there was no observable trend. For the modified release (SR and ER) products, a longer exposure to the GI tract may allow more metabolism to occur through GI metabolic enzymes. Therefore, one would expect to see higher ratio of AUC(m)/AUC(p) if this phenomenon was occurring. Since higher metabolite ratios were not observed for any of the three major metabolites, these data suggested that there was no difference in regional GI metabolism occurring with different formulations.

DISCUSSION

In this study, a total of 30 genotyped subjects completed all 6 periods. The ER formulation showed a lower relative bioavailability when compared to the IR formulation. The data suggest that the concentrations of bupropion and the major metabolites are variable, yet there is no significant difference between the ratio of AUC(m)/AUC(p) for all three major metabolites in any formulations. Population analysis was performed to analyze for differences in SNP and concentration, yet no statistically significant SNP was found to address concentration differences for bupropion or its metabolites.

This investigation critically analyzed bupropion HCl metabolites using various formulations. Since bupropion is

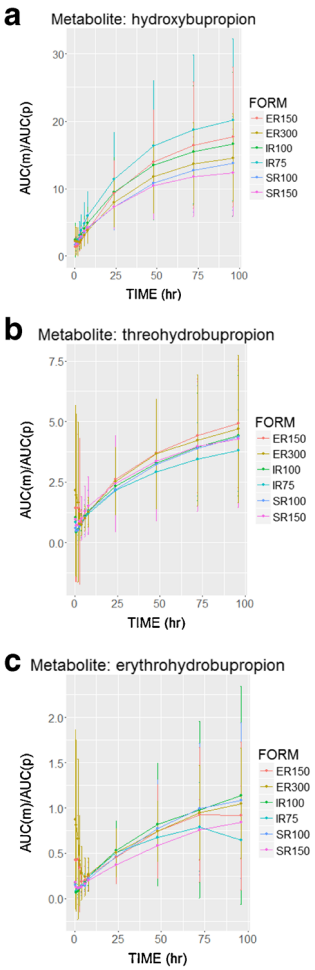

Fig. 4. Ratio of AUC(m)/AUC(p). The mean ± standard deviation of the ratio of AUC between each metabolite **a** hydroxybupropion, **b** threohydrobupropion, and **c** erythrohydrobupropion, and bupropion is shown over time for each formulation

extensively metabolized to form three major active metabolites both in the gut and liver, it was hypothesized that variations in metabolism of formulations with different GI release pattern may account for differences in PK. In addition, full pharmacogenomics were completed for these 30 subjects for ARK7A3, CYP2B6, and CYP2C19. Recent studies have suggested that CYP2C19 is important *in vivo* for bupropion's PK (16). For the modified release (MR) products, a longer exposure to the GI tract may allow more metabolism to occur through GI metabolic enzymes. Therefore, one would expect to see higher ratio of AUC(m)/AUC(p) if this phenomenon was occurring.

It was observed that the dose-normalized systemic exposure decreased for the ER formulation compared to the IR formulation. It is unknown whether this decrease is due to late release in the GI tract and therefore lack of absorption or whether there was a lack of release and the drug was excreted in the feces. As part of this investigation, we found little contribution of bupropion PK to the described polymorphisms in *ARK7A3*, *CYP2B6*, and *CYP2C19*. Previous work suggests a role for CYP2B6 and CYP2C19 in bupropion metabolism. CYP2B6 is known to metabolize bupropion into the pharmacologically active metabolite hydroxybupropion (28). While increased levels of hydroxybupropion are linked to clinical benefits such as increased success of smoking cessation, CYP2B6 is not associated with plasma levels of the parent drug suggesting that alternate mechanisms of metabolism are important (18). The most common *CYP2B6* variant in population studies and in our cohort is *CYP2B6*6*. This variant results in lower amounts of functional *CYP2B6* mRNA and, therefore, decreased enzymatic activity (29), which affects hydroxybuproprion AUC. Likewise, the variant *CYP2C19*2*, also a reduced activity allele, is associated with increased AUC of bupropion and its metabolites threohydrobupropion and erythrohydrobupropion (16). Unlike what is observed in the literature, we did not observe any PK differences associated with our genotyping analysis. Altogether, the PK analysis and graphical analysis suggest that genetic differences play little involvement in bupropion's metabolism in our cohort. A limitation to our analysis is a small study size, which may mask the contribution of pharmacogenetics to bupropion metabolism.

Likewise, various formulations were compared for the ratio of AUC(m)/AUC(p) for each metabolite. None of the three major metabolites showed a statistically significant difference in ratio nor trend, suggesting that GI regional metabolism was not causing the differences as well. Our previous *in vitro* metabolism study suggested that threohydrobupropion could be formed by intestinal carbonyl reductase (19). The plasma concentration data of parents and metabolites lumped the processes of intestinal metabolism, intestinal absorption, and liver metabolism. In order to have better insight of the metabolism in the GI tract, a mechanism-based absorption model that incorporated the gut metabolism, absorption, and liver metabolism could be helpful to understand the GI regional metabolism of different bupropion HCl formulations at different dose levels.

In the case of the two different strength BE studies conducted with generic bupropion HCl tablets against Wellbutrin XL, the establishment of BE with the lower strength (150 mg) and the failure to meet BE standards for the higher strength (300 mg) is not due to metabolic saturation in the GI lumen because there was no statistically significant difference in ratios of AUC(m)/AUC(p) among different formulations (Fig. 4). Other factors may have contributed to the unsuccessful BE study of the higher-strength (300 mg) bupropion HCl ER tablets.

The issue of whether monitoring metabolites in BE studies have been debated for quite some time (30–33). Our results suggest that GI metabolism does not clinically contribute to variation or decrease relative bioavailability. Instead, we observed that the release profile, lower absorption later in the GI track, or lack of release is the most significant contributor to relative bioavailability. These results also suggest that the reason for the BE failure at the 300 mg ER formulation was not due to metabolism. This is consistent with the pilot study performed by the FDA using Wellbutrin and Budeprion (failed BE generic drug product), where in the Budeprion arm, both bupropion and two of the three metabolites (hydroxybupropion and threohydroupropion) showed lower systemic exposure compared to the brand name drug product (34).

From this study, in combination with our previous findings (16), it is very unlikely that the establishment of BE with the lower strength (150 mg) and the failure to meet BE standards for the higher strength (300 mg) was due to metabolic saturation in the GI lumen. Additional research, including modeling and

simulation efforts, will be needed to investigate the root cause for the failure to meet BE standards for the higher strength based on a BE study on a lower strength.

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