Metabolism and Disposition of Bupropion in Pregnant Baboons (*Papio cynocephalus*)

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ABSTRACT

Recent *in vitro* data obtained in our laboratory revealed similarities between baboons and humans in the biotransformation of bupropion (BUP) by both hepatic and placental microsomes. These data supported the use of baboons to study BUP biotransformation during pregnancy. The aim of this investigation was to determine the pharmacokinetics of BUP in baboons during pregnancy and postpartum, as well as fetal exposure to the drug after intravenous administration. Pregnant baboons ($n = 5$) received a single intravenous bolus dose of bupropion hydrochloride (1 mg/kg) at gestational ages 94–108 days (midpregnancy), 142–156 days (late pregnancy), and 6 weeks postpartum. Blood and urine samples were collected for 12 and 24 hours, respectively. The concentrations of BUP, hydroxybupropion (OH-BUP), threohydrobupropion, and erythrohydrobupropion in

plasma were determined by liquid chromatography–tandem mass spectrometry. Relative to the postpartum period, the average midpregnancy clearance of BUP trended higher (3.6 ± 0.15 versus 2.7 ± 0.28 l/h per kg) and the average $C_{\max}$ (294 ± 91 versus 361 ± 64 ng/ml) and the area under the curve (AUC) of BUP values (288 ± 22 versus 382 ± 42 h·ng/ml) trended lower. AUC_{OH-BUP} also tended to be lower midpregnancy compared with postpartum (194 ± 76 versus 353 ± 165 h·ng/ml). Whereas the observed trend toward increased clearance of BUP during baboon pregnancy could be associated with a pregnancy-induced increase in its biotransformation, the trend toward increased renal elimination of OH-BUP may overshadow any corresponding change in the hydroxylation activity of CYP2B6.

Introduction

Bupropion (BUP) is an antidepressant used for the treatment of women at increased risk of developing depressive episodes during pregnancy or postpartum, as well as an aid for smoking cessation in non-pregnant patients. Despite the widespread use of BUP during pregnancy to treat depression, it has not yet been approved in this group of patients as an aid for smoking cessation.

It is well recognized that the onset of pregnancy is accompanied by changes in maternal physiology that affect the absorption, distribution, metabolism, and elimination of several medications (Loebstein et al., 1997). These changes include, but are not limited to, increased body water, increased gastric and intestinal emptying time, decreased concentration of plasma albumin, increased production of progestational hormones that may alter the activity of hepatic metabolizing enzymes (Jeong, 2010), increased hepatic blood flow, and increased renal plasma flow. Because BUP binding to plasma proteins is relatively

low (80%) (Findlay et al., 1981) and its high lipophilicity suggests preferential distribution into the tissue over the water compartment, changes in body water and concentrations of plasma albumin are not likely to significantly affect BUP disposition. On the other hand, BUP is extensively metabolized (Schroeder, 1983), and pregnancy-induced increases in hepatic blood flow, along with the changes in hepatic enzyme activity, may alter BUP metabolism, thus affecting maternal plasma drug concentration and, consequently, fetal exposure. Although the effect of pregnancy on the activity of CYP2B6—the primary hepatic enzyme catalyzing hydroxylation of BUP (Hesse et al., 2000)—had not been reported, analysis of the data obtained from *in vitro* and some *in vivo* investigations suggests increased activity of CYP2B6 during pregnancy (Dickmann and Isoherranen, 2013). For example, it was demonstrated that estradiol—one of the steroid hormones secreted during pregnancy by the placenta—induces CYP2B6 mRNA activity and expression in human hepatocytes (Choi et al., 2013; Dickmann and Isoherranen, 2013). Data obtained from rodent studies also demonstrated that estradiol can induce the expression of cytochrome P450 enzymes (Nemoto and Sakurai, 1995). Furthermore, the increased clearance (CL) during pregnancy as compared with the postpartum period of efavirenz and methadone (Pond et al., 1985; Wolff et al., 2005; Cressey et al., 2012), two drugs that are metabolized mainly by hepatic CYP2B6, also suggests increased activity of the enzyme. However, to date, the effect of pregnancy on the hydroxylation of BUP—which is considered a CYP2B6 probe-substrate—has not been reported.

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ABBREVIATIONS: ANOVA, analysis of variance; AUC, area under the curve; BUP, bupropion; CL, clearance; EB, erythrohydrobupropion; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LLOQ, lower limit of quantification; OH-BUP, hydroxybupropion; TB, threohydrobupropion; TCA, trichloroacetic acid.

Due to ethical and safety concerns associated with drug development for the pregnant patient, an alternative approach is the use of an animal model that best simulates drug metabolism and placentation in humans. The use of nonprimate animal models to study drug disposition in vivo has certain advantages (e.g., short gestation and lower expense). Nevertheless, distinct differences in placental development, structure, and functions, as well as revealed interspecies differences in the biotransformation of BUP between rats, mice, and humans (Suckow et al., 1986), may present some limitations.

During the last 7 years, we have been studying drug disposition during pregnancy using baboons as a nonhuman primate animal model (Zharikova et al., 2007; Yan et al., 2008) because of similarities to humans in placental structure and function (Houston, 1969), as well as in fetal development (Enders et al., 1997). Furthermore, recent in vitro data obtained in our laboratory revealed similarities between baboons and humans in the biotransformation of BUP by both hepatic and placental microsomes (Wang et al., 2010, 2011). The major metabolites formed by human and baboon hepatic and placental microsomes were hydroxybupropion (OH-BUP), threohydrobupropion (TB), and erythrohydrobupropion (EB). OH-BUP was the main metabolite formed by human and baboon hepatic microsomes, and hydroxylation of BUP was catalyzed by CYP2B6 and CYP2B, respectively (Hesse et al., 2000; Wang et al., 2011). On the other hand, in human and baboon placental microsomes, the main metabolites formed were TB and EB. Furthermore, 11 β -hydroxysteroid dehydrogenases were identified as the major carbonyl-reducing enzymes responsible for the reduction of BUP to TB and EB in human and baboon microsomal fractions (Wang et al., 2010, 2011). These data support, in part, the use of baboons to study BUP disposition during pregnancy.

Because maternal drug concentration is one of the major determinants of fetal drug concentration, it is important to investigate the relationship between maternal exposure to BUP and its active metabolites during different gestational periods. Therefore, the goal of this investigation was to determine the biotransformation of BUP in baboons during pregnancy and postpartum, as well as fetal exposure to the drug and its metabolites after intravenous administration.

Materials and Methods

Chemicals. Chemicals were purchased from the following companies: BUP hydrochloride, from Sigma-Aldrich (St. Louis, MO); OH-BUP, EB, TB and the deuterium-labeled internal standards BUP- d_9 hydrochloride, OH-BUP- d_6 , EB- d_9 , and TB- d_9 , from Toronto Research Chemicals Inc. (North York, ON, Canada); and liquid chromatography–mass spectrometry -grade methanol, acetonitrile, and formic acid and analytical-grade trichloroacetic acid (TCA), from Fisher Scientific (Fair Lawn, NJ).

Subjects. All animal procedures were performed in accordance with accepted standards of humane animal care, approved by the Texas Biomedical Research Institute, formerly Southwest Foundation for Biomedical Research, and University of Texas Health Science Center at San Antonio Institutional

Animal Care and Use Committees, and conducted in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care. The female animals were harem housed and naturally mated by a single male in the cage. Female baboons (*Papio cynocephalus*; $n = 5$) were observed for perineal skin swelling to identify onset of pregnancy. Cycle readings were conducted and pregnancy with estimated date of conception was determined by monitoring the perineal skin and by ultrasound (typically accurate to within 3 days). Animals remained group housed ~ 2 weeks prior to drug injection. At gestational day 75, the baboons were examined by ultrasound to confirm gestational age and were placed in a single cage, fitted with a tether jacket and coil, and acclimated to the tether apparatus for ~ 1 week. One week prior to drug administration, a long-term indwelling catheter was surgically placed in the femoral vein and trocared to an exit site midback. A tether jacket was placed on the animal, and the animal was returned to its cage and placed on the tether pump via a coil. The intravenous dose of BUP was administered to a pregnant animal by a veterinarian. Each dose was flushed through the line with at least 10 ml of saline. After the last blood draw for each pharmacokinetic sample set, the animal's catheter was locked with heparin, obturated, knotted, coiled, and buried subcutaneously in the back until the next scheduled blood draw. At this time, the animal was removed from the tether apparatus and jacket. Approximately 1 week before the next phase of dosing, the animal's catheter was exteriorized and checked for a clear flush and draw and the animal was reattached to the tether pump. All surgical procedures, including catheter implant, cesarean section, and explants of catheters after the last blood draw, were performed under sedation with ketamine (10 mg/kg, intramuscular) and anesthesia by inhalation of isoflurane. At the end of the experiments, catheters were explanted and the adult animals were returned to regular life.

Study Protocol. Pregnant baboons received a single intravenous bolus dose of BUP hydrochloride (1 mg/kg). This dose was calculated by the conversion of a human oral dose of 150 mg to the equivalent oral baboon dose of 4.62 mg/kg (<http://www.fda.gov/downloads/Drugs/Guidances/UCM078932.pdf>) and consequent correction for oral bioavailability to an intravenous dose of 1 mg/kg, assuming a similar extent of bioavailability for BUP around 20% (<https://www.gsksource.com/gskprmt/htdocs/documents/WELLBUTRIN-SR-PI-MG.PDF>). BUP was injected into the animal during the second term (between 94 and 108 days of gestation), during the third term (between 142 and 156 days of gestation), and 6 weeks postpartum (representing the nonpregnant animal). Serial blood samples were collected prior to dosing and at 5, 15, 30, and 45 minutes and 1, 2, 3, 4, 6, 8, 10, and 12 hours postdosing. An additional intravenous dose of BUP hydrochloride (1 mg/kg) was injected into the pregnant baboon 1 hour before cesarean section on gestational day 165. Immediately after delivery, single blood samples from the mother and the newborn were collected. The blood samples were centrifuged immediately, and the plasma was stored at -70°C until analysis.

Urine samples were collected over 24 hours between hours 0 and 2, 2 and 4, 4 and 8, and 8 and 24 and stored at -70°C until analysis. All urine output was collected, the volume was noted, and a 10-ml sample was taken and frozen for later analysis.

Neonatal and maternal blood samples were collected for three mother-neonate pairs immediately after newborn delivery by cesarean section ~ 1 hour after the BUP injection. All blood was centrifuged immediately, and plasma was stored at -70°C until analysis.

Plasma Sample Preparation. Plasma samples (100 μl) containing the deuterium-labeled internal standards (10 ng/ml BUP- d_9 hydrochloride, 10 ng/ml OH-BUP- d_6 , 7.3 ng/ml EB- d_9 , and 8.0 ng/ml TB- d_9) were acidified by 50 μl of

TABLE 1
Compound-dependent parameters for the multiple reaction monitoring (MRM) transition of BUP and its metabolites

Compound-Dependent Parameter	BUP	OH-BUP	EB	TB
Retention time of analyte (min)	7.7	6.7	8.5	9.5
Analyte MRM transition (m/z)	240 $\rightarrow$ 184	256 $\rightarrow$ 238	242 $\rightarrow$ 168	242 $\rightarrow$ 168
Retention time of internal standard (min)	7.6	6.6	8.4	9.3
Internal standard MRM transition (m/z)	249 $\rightarrow$ 185	262 $\rightarrow$ 244	251 $\rightarrow$ 169	251 $\rightarrow$ 169
Declustering potential (V)	40	40	50	50
Entrance potential (V)	6	6	6	6
Collision energy (V)	18	18	25	25
Collision cell exit potential (V)	14	14	14	14

TABLE 2

Individual pharmacokinetic parameters of BUP in pregnant and postpartum baboons following a 1-mg/kg i.v. bolus dose of BUP

P values from repeated-measures ANOVA: CL, 0.060; *C*_{max}, 0.208; AUC_{0-∞}, 0.078.

	Midpregnancy			Late Pregnancy			Postpartum		
	CL	<i>C</i> _{max}	AUC _{0-∞}	CL	<i>C</i> _{max}	AUC _{0-∞}	CL	<i>C</i> _{max}	AUC _{0-∞}
	l/h/kg	ng/ml	h-ng/ml	l/h/kg	ng/ml	h-ng/ml	l/h/kg	ng/ml	h-ng/ml
Study animal									
1	3.5	375.4	318.2				3.0	383.0	364.3
2				3.4	333.9	308.1	2.8	420.3	356.9
3	3.6	311.3	275.4	3.5	440.0	292.6	2.4	369.4	399.6
4	3.8	195.7	269.4	4.0	243.5	263.0	2.3	250.9	446.4
5	3.5		288.2	3.0	304.0	344.8	2.9	379.8	341.4
Mean	3.6	294.1	287.8	3.48	330.4	302.1	2.68	360.7	381.7
S.D.	0.14	91.1	21.7	0.41	82.2	34.1	0.31	64.3	42.0

2% (w/v) TCA and extracted with 800 μ l of acetonitrile. The solution was vortexed for 30 seconds and centrifuged at 12,000g for 15 minutes at 4°C. The supernatant was transferred to a tube and dried under a stream of air at 40°C. The dried residues were reconstituted with 120 μ l of the mobile phase [methanol and 0.04% formic acid aqueous solution (v/v)] and filtered using a 0.45- μ m syringe filter. An aliquot of 25 μ l of each sample was analyzed by LC-tandem MS (LC-MS/MS). The concentration of creatinine in baboon plasma was determined by the Texas Biomedical Research Institute as a part of routine health care for each animal.

Urine Sample Preparation. Urine samples (200 μ l) containing the deuterium-labeled internal standard (10 ng/ml BUP-*d*₉ hydrochloride) were acidified by 10 μ l of 10% (w/v) TCA and extracted with 800 μ l of acetonitrile. The solution was vortexed for 30 seconds and centrifuged at 12,000g for 15 minutes at 4°C. The supernatant was transferred to a tube and dried under a stream of air at 40°C. The dried residues were reconstituted with 100 μ l of the mobile phase and filtered using a 0.45- μ m syringe filter. An aliquot of 25 μ l of each sample was analyzed by high-performance LC-MS/MS. The concentration of creatinine in baboon urine was determined with a urinary creatinine assay kit (Cayman Chemical Company, Ann Arbor, MI). Creatinine CL (ml/min) was determined as follows: (urine concentration of creatinine in mg/dl) $\times$ (urine flow rate in ml/min)/(plasma concentration of creatinine in mg/dl).

Determination of Glucuronides of BUP and OH-BUP in Urine Samples.

The concentrations of glucuronidated metabolites of BUP in urine samples were calculated from the difference between the concentrations of nonconjugated (free) and total drug. The total concentrations of BUP and OH-BUP were determined by enzymatic hydrolysis of glucuronidated conjugates as follows: a 100- μ l aliquot of baboon urine was incubated with 50 μ l of β -glucuronidase (2000 units/ml in phosphate-buffered saline; pH 5.0) at 37°C for 4 hours. After incubation, each sample was processed according to the method described above and analyzed by LC-MS/MS to determine the total drug concentration.

Instrumental and Analytical Conditions. Plasma concentrations of BUP, OH-BUP, TB, and EB were determined using a validated LC-MS/MS method previously described from our laboratory (Wang et al., 2012). The method was partially validated using baboon plasma for specificity, matrix effect, linearity, sensitivity, precision, and accuracy according to the US Food and Drug Administration guidelines (<http://www.fda.gov/downloads/Drugs/GuidanceCompliance-RegulatoryInformation/Guidances/UCM070107.pdf>). The calibration curve exhibited linearity within the tested ranges ($r^2 > 0.99$), and the lower limit of quantification (LLOQ) was set as the lowest concentration of the calibration curves (for BUP and OH-BUP, 0.8 ng/ml; for EB and TB, 0.25 ng/ml). Intraday accuracy of the method ranged between 99 and 106% with precision of <10% for high, medium, low, and LLOQ quality control standards. The extraction efficiency of BUP and its metabolites from baboon plasma samples ranged between 90 and 98% with variation of <13%. The matrix factor of BUP and its metabolites in five individual plasma samples ranged between 94 and 110% with variation of <5%.

The analysis of BUP and its three metabolites was achieved by an Agilent HPLC 1200 series system coupled with an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA). The high-performance LC system consisted of a degasser, binary pump delivery system, Hip-ALS autosampler, and column compartment controlled by Analyst 1.5 Software (AB

Sciex, Framingham, MA). Separation of analytes was achieved by a Symmetry C₁₈ column (150 $\times$ 4.6 mm, 5 μ m) (Waters Corporation, Milford, MA) connected to a Phenomenex (Torrance, CA) C₁₈ guard (4 $\times$ 3.0 mm) at 15°C. The mobile phase was made of methanol and 0.04% (v/v) formic acid aqueous solution. Elution in 31% methanol was isocratic for 12 minutes at a flow rate 1 ml/min. The API 4000 triple quadrupole mass spectrometer is equipped with a Turbo (V) ion source (electrospray ionization) and was operated in positive

Fig. 1. Mean ($\pm$ S.D.) plasma concentrations of BUP (A) and OH-BUP (B) in baboons during midpregnancy (94–108 days of gestation), late pregnancy (142–156 days of gestation), and 6 weeks postpartum following a 1-mg/kg i.v. bolus dose of BUP ($n = 4$ –5).

mode. Multiple reaction monitoring mode was applied for the quantification of analytes. The source/gas-dependent MS parameters were as follows: ion spray voltage, 4000 V; curtain gas, 15 l/h; ion source gas 1, 40 l/h; ion source gas 2, 20 l/h; temperature, 300°C; and collision gas, 5 l/h. The multiple reaction monitoring transition was set up as follows: m/z 240 $\rightarrow$ 184 for BUP; m/z 256 $\rightarrow$ 238 for OH-BUP; m/z 242 $\rightarrow$ 168 for TB and EB; m/z 249 $\rightarrow$ 185 for BUP- d_9 ; m/z 262 $\rightarrow$ 244 for OH-BUP- d_6 ; and m/z 251 $\rightarrow$ 169 for EB- d_9 and TB- d_9 (Table 1).

Pharmacokinetic Analysis. Pharmacokinetic analyses were conducted using Kinetica software version 5.0 (Thermo Scientific, Waltham, MA). All data were evaluated using a model-independent approach. Maximum plasma drug concentration (C_{max}) and time to C_{max} (t_{max}) were determined by visual inspection of the plasma concentration–time profile. The area under the plasma concentration–time curve ($AUC_{0-\infty}$) was determined using the log-linear trapezoidal rule. CL was calculated as the dose divided by $AUC_{0-\infty}$. The in vivo activity of baboon enzymes responsible for biotransformation of BUP was described by the ratio of $AUC_{metabolite}$ to $AUC_{parent\ drug}$.

Statistical Analysis. Standard descriptive statistics were used to summarize the data. All reported values are expressed as mean $\pm$ S.D. Comparisons of pharmacokinetic parameters between pregnant and nonpregnant states (using postpartum data for each animal) were performed by repeated-measures analysis of variance (ANOVA) and considered significant if $P < 0.05$, followed by Tukey-Kramer multiple-comparison tests using NCSS statistical software version 9.0.13 (NCSS, LLC, Kaysville, UT).

Results

The mean age of the pregnant baboons ($n = 5$) was 8.8 ± 1.9 years. The average baboon prepregnancy weight was 15.8 ± 1.2 kg (range, 13.9–17.8 kg). The average baboon weight was 15.3 ± 0.5 kg (range, 14.5–15.6 kg) at midpregnancy, 15.7 ± 0.9 kg (range, 14.9–16.7 kg) in late pregnancy, 16.1 ± 0.91 kg (range, 15.1–17.4 kg) at the time of cesarean section, and 14.8 ± 0.8 kg (range, 13.8–15.6 kg) postpartum. Thus, the body weight of pregnant baboons remained relatively stable throughout the pregnancy. The plasma concentrations of BUP and OH-BUP were determined in all five baboons during mid- and late pregnancy as well as in the postpartum period. However, in 2 of the 15 studies, data were excluded from pharmacokinetic analysis due to deviations from the experimental protocol (animal #1, late pregnancy; animal #2, midpregnancy). Because the same site was used for drug dosing and blood sampling, to avoid specimen contamination by residual drug concentration after each injection the line was flushed with at least 10 ml of saline. However, in the two aforementioned cases, incomplete flushing led to residual retention of BUP in the sampling line. In a third study (animal #5, midpregnancy), an error precluded the determination of the plasma sample at the 5-minute time point. Therefore, the C_{max} value is not reported in this case, but all other pharmacokinetic parameters from that study were calculated. Plasma concentrations of TB and EB during gestation as well as in the

Fig. 2. Cumulative 24-hour urinary excretion of BUP, OH-BUP, and glucuronidated OH-BUP in baboons during midpregnancy, late pregnancy, and postpartum following an intravenous bolus dose of BUP (1 mg/kg). Data are presented as mean $\pm$ S.D. ($n = 3$). The amount of glucuronidated BUP detected accounted for $<1\%$ of excreted BUP and is not presented. * $P < 0.05$ vs. BUP and glucuronidated OH-BUP excretion in late pregnancy, based on ANOVA followed by Tukey-Kramer post hoc analysis.

postpartum period could be determined only in three and two baboons, respectively. In the other animals, plasma concentrations of TB and EB were below the LLOQ, which precluded full analysis.

BUP. The individual pharmacokinetic parameters of BUP in pregnant and postpartum baboons are presented in Table 2. Figure 1 shows the mean plasma concentrations of BUP and OH-BUP during the three experimental periods. Most of the individual plasma levels of BUP as well as the mean values of half-life, C_{max} , and $AUC_{0-\infty}$ determined in baboons during pregnancy were lower than in the postpartum period. The mean value of $AUC_{0-\infty}$ during midpregnancy (288 ± 22 h·ng/ml) was lower than the $AUC_{0-\infty}$ in the postpartum period (382 ± 42 h·ng/ml). Due to the missing data as a result of the aforementioned deviations from the experimental protocol, repeated-measures ANOVA was limited to analysis of the data from three animals. Although paired t tests comparing midpregnancy to postpartum ($n = 4$) demonstrated significant differences ($P < 0.05$) in both BUP CL and $AUC_{0-\infty}$, ANOVA analysis of the data for the three animals for which data were available in all three gestational periods did not reveal any significant differences ($P = 0.060$ and 0.078 for CL and $AUC_{0-\infty}$, respectively). Relative to the nonpregnant state (postpartum), the average values of BUP CL during midpregnancy and late pregnancy were 25 and 22% higher, respectively. The mean cumulative urinary excretion of unchanged BUP within 24 hours during mid- and late pregnancy was 2.9- and 5.8-fold that of nonpregnant baboons, respectively (Fig. 2).

TABLE 3

Individual pharmacokinetic parameters of OH-BUP in pregnant and postpartum baboons following a 1-mg/kg i.v. bolus dose of BUP

P values from repeated-measures ANOVA: C_{max} , 0.373; t_{max} , 0.751; $AUC_{0-\infty}$, 0.102.

	Midpregnancy			Late Pregnancy			Postpartum		
	C_{max}	t_{max}	$AUC_{0-\infty}$	C_{max}	t_{max}	$AUC_{0-\infty}$	C_{max}	t_{max}	$AUC_{0-\infty}$
	ng/ml	h	h·ng/ml	ng/ml	h	h·ng/ml	ng/ml	h	h·ng/ml
Study animal									
1	85.5	0.5	211.0				110.7	0.5	310.1
2				99.1	0.75	344.7	232.1	0.5	636.9
3	62.5	0.5	109.6	26.3	0.75	71.3	72.3	0.25	201.0
4	108.0	0.5	288.3	98.7	0.5	292.5	83.5	1	302.7
5	82.8	0.75	165.3	66.1	1	200.2	177.9	0.5	315.9
Mean	84.7	0.6	194	72.6	0.8	227	135	0.6	353
S.D.	18.6	0.1	76	34.5	0.2	120	68	0.3	165

TABLE 4

Ratio of AUC_{OH-BUP} to AUC_{BUP} following a 1-mg/kg i.v. bolus dose of BUP to baboons during pregnancy and the postpartum period

P value from repeated-measures ANOVA: 0.959.

	AUC _{OH-BUP} /AUC _{BUP} Ratio		
	Midpregnancy	Late Pregnancy	Postpartum
Study animal			
1	0.72		0.92
2		1.21	1.93
3	0.43	0.26	0.54
4	1.16	1.20	0.73
5	0.62	0.63	1.00
Mean	0.73	0.82	1.02
S.D.	0.31	0.46	0.53

OH-BUP. The individual pharmacokinetic parameters of OH-BUP calculated at different stages of baboon pregnancy and in the postpartum period are presented in Table 3. Following a single dose of BUP in pregnant and nonpregnant baboons, peak plasma concentrations of OH-BUP occurred ~0.6 hour after bolus injection of the drug. As was observed for the parent drug, the mean values of $C_{\max}$ and AUC_{0-∞} trended lower in pregnant than in nonpregnant baboons. Relative to the postpartum period, the average $C_{\max}$ and AUC_{0-∞} values during midpregnancy were reduced by 38 and 46%, respectively, and during late pregnancy by 47 and 36%, respectively.

The mean cumulative urinary excretion of total OH-BUP within 24 hours in nonpregnant and pregnant baboons ranged from 2.7- to 7.9-fold higher than the excretion of unchanged BUP (Fig. 2). Furthermore, during late pregnancy, the mean cumulative urinary excretion of nonglucuronidated OH-BUP was significantly higher than the excretion of BUP or glucuronidated OH-BUP (3.9- and 6.9-fold higher, respectively; $P < 0.05$). With the exception of animal #4, the ratio of AUC_{OH-BUP} to AUC_{BUP} in pregnant animals was lower than in the nonpregnant state. Relative to the postpartum period, the mean ratios of AUC_{OH-BUP} to AUC_{BUP} during midpregnancy and late pregnancy were decreased by 28 and 20%, respectively (Table 4).

TB and EB. The individual pharmacokinetic parameters of TB and EB in pregnant and postpartum baboons are presented in Tables 5 and 6. In several instances, the plasma concentrations of TB and EB determined in baboons were below the LLOQ, suggesting that the formation of these reduced metabolites in baboons after an intravenous bolus dose of BUP is minimal.

Neonatal Exposure to BUP and Its Metabolites. The mean ratios of neonatal/maternal plasma concentrations of BUP and OH-BUP were 0.89 ± 0.18 and 0.24 ± 0.10 , respectively (Table 7). The ratios for TB and EB fell within this same range (0.43 and 0.21, respectively);

however, the degree of interanimal variability was markedly higher for these minor metabolites.

Discussion

Recognizing that pregnancy can alter the pharmacokinetics of medications and that BUP is extensively metabolized, it is important to compare maternal exposure to BUP during different terms of gestation and postpartum. Therefore, a goal of the current investigation was to determine the biotransformation of BUP in baboons during pregnancy. To minimize the impact of pharmacogenetic variations on the findings, the study was conducted in a longitudinal fashion with each baboon serving as its own control.

In this investigation, pregnant baboons received a single 1-mg/kg intravenous bolus dose of BUP during midpregnancy, late pregnancy, and 6 weeks postpartum. The major metabolite determined in the baboons' plasma was OH-BUP. Previously we reported that in baboons CYP2B was the major hepatic enzyme responsible for the hydroxylation of BUP in vitro (Wang et al., 2011). Following intravenous administration of 1 mg/kg of BUP, the peak plasma concentration of OH-BUP in nonpregnant baboons was ~3-fold less than the peak level of the parent drug. The observed peak plasma concentration of OH-BUP relative to BUP in this study was comparable to its formation in guinea pigs following administration of the drug by the same intravenous route and at a similar concentration (Kiptoo et al., 2009). However, the formation of reduced metabolites of BUP (TB and EB) was very low (Tables 5 and 6). In some cases, the concentrations of TB and EB in baboon plasma were below the LLOQ, suggesting that after an intravenous bolus dose of BUP, the reduction pathway plays a minor role in the baboon's biotransformation of BUP. In humans, for whom BUP is always administered orally, the plasma concentrations of OH-BUP and TB are 7- to 10-fold and 5-fold higher, respectively, than that of the parent compound, whereas the concentration of EB is similar to that of BUP (Schroeder, 1983). Although the observed lower formation of OH-BUP, TB, and EB in baboons following intravenous administration of BUP versus their formation in humans following oral dosing could be route-dependent, interspecies differences cannot be excluded. Suckow et al. (1986) reported differences in the biotransformation of BUP among rats, mice, and guinea pigs following intraperitoneal administration; this route involves the first-pass effect, and thus the pharmacokinetics of substances are more similar to those seen after oral administration. In rats, the formation of OH-BUP and TB was very low. In mice, although the AUC of OH-BUP was ~5-fold higher, the AUC of TB was 2-fold lower than the AUC of BUP. However, in guinea pigs, the AUCs of both OH-BUP and TB were higher than the AUC of the parent drug (Suckow et al., 1986).

We observed that BUP CL during baboon pregnancy trended higher than in the postpartum period (Table 2). Most of the individual plasma

TABLE 5

Individual pharmacokinetic parameters of TB in pregnant and postpartum baboons following a 1-mg/kg i.v. bolus dose of BUP

	Midpregnancy			Late Pregnancy			Postpartum		
	$C_{\max}$	$t_{\max}$	AUC _{0-∞}	$C_{\max}$	$t_{\max}$	AUC _{0-∞}	$C_{\max}$	$t_{\max}$	AUC _{0-∞}
	ng/ml	h	h-ng/ml	ng/ml	h	h-ng/ml	ng/ml	h	h-ng/ml
Study animal									
2				1.52	2.00	9.83	1.65	1.00	6.57
3	1.74	0.75	5.90	1.35	1.00	5.48	1.75	1.00	6.47
5	2.22	0.75	7.67	3.24	0.75	11.51	1.84	0.50	6.02
Mean	1.98	0.75	6.8	2.04	1.25	8.9	1.75	0.83	6.4
S.D.	0.34	0.00	1.3	1.05	0.66	3.1	0.10	0.29	0.3

TABLE 6

Individual pharmacokinetic parameters of EB in pregnant and postpartum baboons following a 1-mg/kg i.v. bolus dose of BUP

	Midpregnancy			Late Pregnancy			Postpartum		
	$C_{\max}$	$t_{\max}$	$AUC_{0-\infty}$	$C_{\max}$	$t_{\max}$	$AUC_{0-\infty}$	$C_{\max}$	$t_{\max}$	$AUC_{0-\infty}$
	ng/ml	h	h-ng/ml	ng/ml	h	h-ng/ml	ng/ml	h	h-ng/ml
Study animal									
2				1.05	1.00	4.71	1.89	0.50	4.16
5	1.27	1.00	4.17	1.20	0.75	3.74	1.85	0.50	3.30
Mean	1.27	1.00	4.17	1.13	0.88	4.2	1.87	0.50	3.7
S.D.				0.11	0.18	0.7	0.03	0.00	0.6

levels as well as the average $AUC_{0-\infty}$ values of BUP in pregnant baboons trended lower during pregnancy, suggesting lower maternal exposure to the parent drug. Because in baboons BUP is extensively metabolized primarily to OH-BUP, one might expect that the elevated CL of the parent drug may result from its increased hydroxylation during pregnancy. However, most of the individual plasma values as well as the average $C_{\max}$ and $AUC_{0-\infty}$ values of OH-BUP in pregnant baboons trended lower than the postpartum values (Table 3). Furthermore, the AUC_{OH-BUP}/AUC_{BUP} ratio was not significantly different when comparing pregnant versus nonpregnant baboons (Table 4), as might be expected if the increased CL of BUP was due to increased activity of the enzyme catalyzing the hydroxylation. BUP is a drug with a high extraction ratio (bioavailability $\approx 20\%$). Therefore, its hepatic CL would be dependent on hepatic blood flow. Pregnancy-related changes in hepatic blood flow in baboons could thus affect the CL of BUP. Although we do not have any data regarding hepatic blood flow in baboon pregnancy, Nakai et al. (2002) have reported a tendency for hepatic arterial blood flow to increase with gestation in healthy pregnant women, from 0.57 ± 0.31 l/min (nonpregnant) to 0.58 ± 0.13 l/min (first trimester), 0.70 ± 0.41 l/min (second trimester), and 1.06 ± 0.55 l/min (third trimester). (Whereas portal vein flow would be significant for drugs administered orally, the hepatic artery is the main route of entry to the liver for drugs administered intravenously.)

Another factor affecting the pharmacokinetics of BUP is urinary excretion of the parent drug and its metabolites during late pregnancy. Indeed, the mean urinary excretion of OH-BUP during late pregnancy was 6.7-fold higher than postpartum. The urinary excretion of unchanged BUP also trended higher during pregnancy, being 2.9- and 5.8-fold greater in mid- and late pregnancy, respectively. The increased rates of excretion of BUP and OH-BUP suggest the influence of pregnancy-induced increases in the glomerular filtration rate. In humans, increases in renal plasma flow and glomerular filtration rate also occur as early as the middle of the first trimester (Davison, 1984). In baboons, we observed that the mean creatinine CL (as a surrogate marker for glomerular filtration rate) was higher during midpregnancy compared with postpartum (30.9 ± 3.9 ml/min versus 23.9 ± 2.9 ml/min; $n = 3$; $P < 0.05$).

The ratios of AUC_{OH-BUP} to AUC_{BUP} trended lower in pregnant baboons as compared with nonpregnant, except for one animal (Table 4). It is possible that the hepatic CL of BUP increases due to an increase in hepatic arterial blood flow. Nevertheless, the plasma AUC_{OH-BUP} may not be higher due to enhanced renal elimination of this metabolite during pregnancy. The increased elimination of OH-BUP during baboon pregnancy may consequently overshadow any pregnancy-induced increase in the hydroxylation of BUP. We also cannot rule out the possible formation of other baboon-specific metabolites beyond OH-BUP, EB, and TB.

BUP and its three metabolites were determined in neonatal plasma (Table 7). The neonatal plasma concentrations of BUP are ~ 1.5 times

that of OH-BUP, ~ 14 times that of TB, and ~ 90 times that of EB, suggesting primarily intrauterine fetal exposure to BUP and OH-BUP. The mean plasma BUP concentration in the neonate after delivery was 55 ± 2 ng/ml. This represents $\sim 20\%$ of the peak BUP concentration in the maternal circulation determined 5 minutes after the injection. The neonatal/maternal ratios of BUP and OH-BUP after 1 hour of BUP injection were 0.89 ± 18 and 0.24 ± 0.10 , respectively. Previously, we reported the transfer of BUP and OH-BUP across the dually perfused human placental lobule (Earhart et al., 2010; Hemauer et al., 2010). After 1 hour of BUP perfusion, the concentration of BUP in the fetal circuit in vitro was $24 \pm 3\%$ of its initial concentration in the maternal circuit and the fetal-to-maternal ratio was 0.68 ± 0.15 , which agrees with the data obtained in this investigation.

It should be noted here that although the maternal plasma concentration of BUP in baboons after cesarean section (~ 1 hour after drug administration) was comparable to its concentration determined during the same time interval in late pregnancy, maternal plasma concentrations of OH-BUP, TB, and EB were at least 2-fold higher. This observed difference in maternal concentrations of metabolites could be explained by the reduced renal excretion of more polar compounds due to the use of the inhalation anesthetic isoflurane during surgery (Burchardi and Kaczmarczyk, 1994). This could lead to an overestimation of maternal concentrations of OH-BUP, TB, and EB and, consequently, an underestimation of the neonatal-to-maternal concentration ratios. On the other hand, the plasma concentration of BUP was less affected because 1 hour after BUP administration, the main component of its total CL would be biotransformation. Furthermore, despite higher maternal concentrations of OH-BUP than BUP 1 hour after the dose, the concentration of OH-BUP in the neonatal plasma was lower than that of BUP, suggesting lower permeability of OH-BUP across baboon placenta. In part, these data could be explained by differences in physicochemical properties between the parent drug and its metabolite.

TABLE 7

Concentrations of BUP and its metabolites in baboon maternal and neonatal plasma at the time of delivery by cesarean section, 1 hour after a 1-mg/kg i.v. bolus dose of BUP to the pregnant baboon

	Concentration of Drug or Metabolite in:							
	Maternal Plasma				Neonatal Plasma			
	OH-BUP	BUP	TB	EB	OH-BUP	BUP	TB	EB
	ng/ml							
Study animal								
1	213	74	2.4	0.3	29	56	2.9	0.5
2	156	63	16.1	4.8	53	52	4.4	0.5
3	121	52	8.7	3.6	32	56	4.2	0.9
Mean	164	63	9.1	2.9	38	55	3.8	0.6
S.D.	46	11	6.8	2.3	13	2	0.8	0.3

Thus, the presence of the hydroxyl group and consequent hydrogen bonding can lead to a decreased diffusivity of OH-BUP across the placenta as compared with BUP (Kiptoo et al., 2009).

Due to the wide interindividual variability between baboons in the biotransformation of BUP and the number of animals involved in this study, the power of the statistical analysis of the data was limited. Nevertheless, it appears that maternal exposure to BUP and its major active metabolite OH-BUP is decreased during baboon pregnancy. Although the observed increase in the CL of BUP during baboon pregnancy could be caused by a pregnancy-induced increase in its biotransformation, the trend toward increased renal elimination of OH-BUP may overshadow any corresponding change in the hydroxylation activity of CYP2B. The transplacental transfer of BUP and its metabolites also contributes to the total CL of BUP from maternal plasma, as BUP, OH-BUP, TB, and EB were all detected in neonatal plasma after delivery. Based on previous in vitro data, the metabolism of BUP to TB and EB within baboon placenta is expected to be only a minor factor affecting the overall CL of BUP (Wang et al., 2011).

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References

- Burchardi H and Kaczmarczyk G (1994) The effect of anaesthesia on renal function. *Eur J Anaesthesiol* **11**:163–168.
- Choi SY, Koh KH, and Jeong H (2013) Isoform-specific regulation of cytochromes P450 expression by estradiol and progesterone. *Drug Metab Dispos* **41**:263–269.
- Cressey TR, Stek A, Capparelli E, Bowonwatanuwong C, Prommas S, Srivatanapa P, Yuthavisuthi P, Neungton C, Huo Y, and Smith E, et al.; IMPAACT P1026s Team (2012) Efavirenz pharmacokinetics during the third trimester of pregnancy and postpartum. *J Acquir Immune Defic Syndr* **59**:245–252.
- Davison JM (1984) Renal haemodynamics and volume homeostasis in pregnancy. *Scand J Clin Lab Invest Suppl* **169**:15–27.
- Dickmann LJ and Isoherranen N (2013) Quantitative prediction of CYP2B6 induction by estradiol during pregnancy: potential explanation for increased methadone clearance during pregnancy. *Drug Metab Dispos* **41**:270–274.
- Earhart AD, Patrikeeva S, Wang X, Abdelrahman DR, Hankins GD, Ahmed MS, and Nanovskaya T (2010) Transplacental transfer and metabolism of bupropion. *J Matern Fetal Neonatal Med* **23**:409–416.
- Enders AC, Lantz KC, Peterson PE, and Hendrickx AG (1997) From blastocyst to placenta: the morphology of implantation in the baboon. *Hum Reprod Update* **3**:561–573.
- Findlay JW, Van Wyck Fleet J, Smith PG, Butz RF, Hinton ML, Blum MR, and Schroeder DH (1981) Pharmacokinetics of bupropion, a novel antidepressant agent, following oral administration to healthy subjects. *Eur J Clin Pharmacol* **21**:127–135.
- Hemauer SJ, Patrikeeva SL, Wang X, Abdelrahman DR, Hankins GD, Ahmed MS, and Nanovskaya TN (2010) Role of transporter-mediated efflux in the placental biotransformation of bupropion and its metabolite, OH-bupropion. *Biochem Pharmacol* **80**:1080–1086.
- Hesse LM, Venkatakrishnan K, Court MH, von Moltke LL, Duan SX, Shader RI, and Greenblatt DJ (2000) CYP2B6 mediates the in vitro hydroxylation of bupropion: potential drug interactions with other antidepressants. *Drug Metab Dispos* **28**:1176–1183.
- Houston ML (1969) The development of the baboon (*Papio sp.*) placenta during the fetal period of gestation. *Am J Anat* **126**:17–29.
- Jeong H (2010) Altered drug metabolism during pregnancy: hormonal regulation of drug-metabolizing enzymes. *Expert Opin Drug Metab Toxicol* **6**:689–699.
- Kiptoo PK, Paudel KS, Hammell DC, Pinninti RR, Chen J, Crooks PA, and Stinchcomb AL (2009) Transdermal delivery of bupropion and its active metabolite, hydroxybupropion: a prodrug strategy as an alternative approach. *J Pharm Sci* **98**:583–594.
- Loebstein R, Lalkin A, and Koren G (1997) Pharmacokinetic changes during pregnancy and their clinical relevance. *Clin Pharmacokinet* **33**:328–343.
- Nakai A, Sekiya I, Oya A, Koshino T, and Araki T (2002) Assessment of the hepatic arterial and portal venous blood flows during pregnancy with Doppler ultrasonography. *Arch Gynecol Obstet* **266**:25–29.
- Nemoto N and Sakurai J (1995) Glucocorticoid and sex hormones as activating or modulating factors for expression of Cyp2b-9 and Cyp2b-10 in the mouse liver and hepatocytes. *Arch Biochem Biophys* **319**:286–292.
- Pond SM, Kreek MJ, Tong TG, Raghunath J, and Benowitz NL (1985) Altered methadone pharmacokinetics in methadone-maintained pregnant women. *J Pharmacol Exp Ther* **233**:1–6.
- Schroeder DH (1983) Metabolism and kinetics of bupropion. *J Clin Psychiatry* **44**:79–81.
- Suckow RF, Smith TM, Perumal AS, and Cooper TB (1986) Pharmacokinetics of bupropion and metabolites in plasma and brain of rats, mice, and guinea pigs. *Drug Metab Dispos* **14**:692–697.
- Wang X, Abdelrahman DR, Fokina VM, Hankins GD, Ahmed MS, and Nanovskaya TN (2011) Metabolism of bupropion by baboon hepatic and placental microsomes. *Biochem Pharmacol* **82**:295–303.
- Wang X, Abdelrahman DR, Zharikova OL, Patrikeeva SL, Hankins GD, Ahmed MS, and Nanovskaya TN (2010) Bupropion metabolism by human placenta. *Biochem Pharmacol* **79**:1684–1690.
- Wang X, Vernikovskaya DI, Abdelrahman DR, Hankins GD, Ahmed MS, and Nanovskaya TN (2012) Simultaneous quantitative determination of bupropion and its three major metabolites in human umbilical cord plasma and placental tissue using high-performance liquid chromatography-tandem mass spectrometry. *J Pharm Biomed Anal* **70**:320–329.
- Wolff K, Boys A, Rostami-Hodjegan A, Hay A, and Raistrick D (2005) Changes to methadone clearance during pregnancy. *Eur J Clin Pharmacol* **61**:763–768.
- Yan R, Nanovskaya TN, Zharikova OL, Mattison DR, Hankins GD, and Ahmed MS (2008) Metabolism of 17 α -hydroxyprogesterone caproate by hepatic and placental microsomes of human and baboons. *Biochem Pharmacol* **75**:1848–1857.
- Zharikova OL, Ravindran S, Nanovskaya TN, Hill RA, Hankins GDV, and Ahmed MS (2007) Kinetics of glyburide metabolism by hepatic and placental microsomes of human and baboon. *Biochem Pharmacol* **73**:2012–2019.

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