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

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A list of non-standard abbreviations:

BUP, bupropion; OH-BUP, hydroxybupropion; TB, threohydrobupropion; EB, erythrohydrobupropion.
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 (mid-pregnancy), 142-156 days (late pregnancy), and 6 weeks postpartum. Blood and urine samples were collected for 12 and 24 hours, respectively. The concentrations of bupropion, hydroxybupropion (OH-BUP), threo hydrobupropion (TB), and erythrohydrobupropion (EB) in plasma were determined by LC-MS/MS. Relative to the postpartum period, the average mid-pregnancy clearance of BUP trended higher (3.6 ± 0.15 vs. 2.7 ± 0.28 L/h/kg) and the average C_max (294 ± 91 vs. 361 ± 64 ng/ml) and AUC_BUP values (288 ± 22 vs. 382 ± 42 h·ng/ml) trended lower. The area under the curve of OH-BUP also tended to be lower mid-pregnancy compared to postpartum (AUC_OH-BUP = 194 ± 76 vs. 353 ± 165 h·ng/ml). While 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 CYP2B.
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 pro-gestational hormones that may alter the activity of hepatic metabolizing enzymes (Jeong, 2010), increased hepatic blood flow, and increased renal plasma flow. Since 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, 1981) 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 (Dickmann and Isoherranen, 2013; Choi et al., 2013). Data obtained from rodent
studies also demonstrated that estradiol can induce the expression of CYP enzymes (Nemoto and Sakurai, 1995). Furthermore, the increased clearance of efavirenz and methadone during pregnancy as compared to the postpartum period (Cressey et al., 2012; Pond et al., 1985; Wolff et al., 2005), two drugs which are mainly metabolized by hepatic CYP2B6, also suggest increased activity of the enzyme. However, to date, the effect of pregnancy on the hydroxylation of bupropion—which is considered a CYP2B6 probe-substrate—has not been reported.

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 non-primate 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 non-human 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 bupropion 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, 11β-hydroxysteroid dehydrogenases were identified as the major
carbonyl-reducing enzymes responsible for the reduction of bupropion 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.

Since 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 Chemical Co. (St. Louis, MO); hydroxybupropion (OH-BUP), erythrohydrobupropion (EB), threohydrobupropion (TB), and the deuterium labeled internal standards $d_9$-BUP hydrochloride, $d_6$-OH-BUP, $d_9$-EB, and $d_9$-TB from Toronto Research Chemicals Inc. (North York, Canada); LC-MS-grade methanol, acetonitrile, 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 approximately two 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 approximately one week. One week prior to drug administration, a long term indwelling catheter was surgically placed in the femoral vein and trocarred to an exit site mid-back. A tether jacket was placed on the animal, and she was returned to her cage and placed on the tether pump via a coil. The intravenous dose of bupropion was administered to a
pregnant animal by a veterinarian. Each dose was flushed through the line with at least 10 ml saline. After the last blood draw for each PK sample set, the animal’s catheter was locked with heparin, obturated, knotted, coiled, and was 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, checked for a clear flush and draw, and the animal was reattached to the tether pump. All surgical procedures including catheter implant, caesarian 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 (*i.v.*) 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 4.62 mg/kg (FDA, 2005) and consequent correction for oral bioavailability to an intravenous dose of 1 mg/kg, assuming a similar extent of bioavailability for BUP around 20% (GlaxoSmithKline. Wellbutrin SR). BUP was injected to the animal during the second term (between 94-108 days of gestation), third term (between 142-156 days of gestation), and 6 weeks postpartum (representing the non-pregnant 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 post-dosing. An additional *i.v.* dose of BUP hydrochloride (1 mg/kg) was injected to the pregnant baboon one hour before C-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 0-2, 2-4, 4-8, and 8-24-hour intervals 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 approximately 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 $d_9$-BUP hydrochloride, 10 ng/ml $d_6$-OH-BUP, 7.3 ng/mL $d_9$-EB, and 8.0 ng/ml $d_9$-TB) were acidified by 50 µL of 2% (w/v) trichloroacetic acid (TCA) and extracted with 800 µL of acetonitrile. The solution was vortexed for 30 sec and centrifuged at 12000 x g for 15 min 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 µL of the mobile phase (methanol and 0.04% formic acid aqueous solution (v/v)) and filtered using a 0.45 µm syringe filter. An aliquot of 25 µL of each sample was analyzed by LC-MS/MS. The concentration of creatinine in baboon plasma was determined by the Texas Biomedical Research Institute as a part of routine healthcare for each animal.

**Urine sample preparation**

Urine samples (200 µL) containing the deuterium-labeled internal standard (10 ng/ml $d_9$-BUP hydrochloride) were acidified by 10 µL of 10% (w/v) trichloroacetic acid (TCA) and extracted with 800 µL of acetonitrile. The solution was vortexed for 30 sec and centrifuged at 12000 x g for 15 min 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 µL of the mobile phase and filtered using a 0.45 µm syringe filter. An aliquot of 25 µL of each sample was analyzed by
high-performance liquid chromatography-mass spectrometry (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 clearance (ml/min) was determined as follows: (Urine concentration of creatinine in mg/dl) x (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 µL aliquot of baboon urine was incubated with 50 µL of β-glucuronidase (2000 units/mL in PBS buffer, 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 (FDA, 2001). 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). Intra-day accuracy of the method ranged between 99% and 106% with precision < 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 < 13%. The matrix factor of BUP and its
metabolites in five individual plasma samples ranged between 94% and 110% with variation < 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 HPLC system consisted of a degasser (G1379B), binary pump delivery system (G1312A), Hip-ALS auto-sampler (G1376B) and column compartment (G1316A) controlled by Analyst™1.5 Software (MDS INC. and Applera Corporation, USA). Separation of analytes was achieved by a Waters Symmetry C\textsubscript{18} column (150 × 4.6 mm, 5 \textmu m) connected to a Phenomenex C\textsubscript{18} guard (4 × 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 min at flow rate 1 mL/min. The API 4000 triple quadrupole mass spectrometer is equipped with a Turbo (V) ion source (ESI) and was operated in positive mode. Multiple reaction monitoring (MRM) mode was applied for the quantification of analytes. The source/gas dependent MS parameters were as follows: IonSpray 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; Collision Gas 5 L/h. The MRM transition was setup as follows: \textit{m/z} 240→184 for BUP; \textit{m/z} 256→238 for OH-BUP; \textit{m/z} 242→168 for TB and EB; \textit{m/z} 249→185 for \textit{d}_9\text{-BUP}; \textit{m/z} 262→244 for \textit{d}_6\text{-OH-BUP}; \textit{m/z} 251→169 for \textit{d}_9\text{-EB} and \textit{d}_9\text{-TB} (Table 1).

\textbf{Pharmacokinetic analysis}

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

\textbf{Statistical analysis}

Standard descriptive statistics were used to summarize the data. All reported values are expressed as mean ± s.d. Comparisons of pharmacokinetic parameters between pregnant and non-pregnant states (using postpartum data for each animal) were performed by repeated measures ANOVA and considered significant if $P < 0.05$, followed by Tukey-Kramer multiple comparison tests using NCSS statistical software (version 9.0.13, Kaysville, UT).
Results

The mean age of the pregnant baboons (n=5) was 8.8 ± 1.9 years. The average baboon pre-pregnancy weight was 15.8 ± 1.2 kg (13.9 – 17.8). The average baboon weight was 15.3 ± 0.5 kg (14.5 – 15.6) at mid-pregnancy, 15.7 ± 0.9 kg (14.9 – 16.7) in late pregnancy, 16.1 ± 0.91 kg (15.1 -17.4) at the time of C-section, and 14.8 ± 0.8 kg (13.8 – 15.6) 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 two of the fifteen studies, data were excluded from PK analysis due to deviations from the experimental protocol (animal #1, late pregnancy and animal #2, mid-pregnancy). Since the same site was used for drug dosing and blood sampling, in order 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, mid-pregnancy), an error precluded the determination of the plasma sample at the 5-minute time point. Therefore, the Cmax value is not reported in this case, but all other PK parameters from that study were calculated. Plasma concentrations of TB and EB during gestation as well as in the 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.

Bupropion. 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 t1/2, Cmax and AUC0-∞ determined in baboons during pregnancy were lower than in the postpartum period. The mean value of AUC0-∞ during mid-pregnancy (288 ± 22 h·ng/ml) was lower than the AUC0-∞ 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 3 animals. Although paired t-tests comparing mid-pregnancy to postpartum (n=4) demonstrated significant differences (P<0.05) in both BUP clearance and \( \text{AUC}_{0-\infty} \), 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 clearance and \( \text{AUC}_{0-\infty} \), respectively). Relative to the non-pregnant state (postpartum), the average values of BUP clearance during mid-pregnancy 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 non-pregnant baboons, respectively (Figure 2).

**Hydroxybupropion.** 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 non-pregnant baboons, peak plasma concentrations of OH-BUP occurred approximately 0.6 hours after bolus injection of the drug. As was observed for the parent drug, the mean values of \( C_{\text{max}} \) and \( \text{AUC}_{0-\infty} \) trended lower in pregnant than in non-pregnant baboons. Relative to the postpartum period, the average \( C_{\text{max}} \) and \( \text{AUC}_{0-\infty} \) values during mid-pregnancy 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 non-pregnant and pregnant baboons ranged from 2.7- to 7.9-fold higher than the excretion of unchanged BUP (Figure 2). Furthermore, during late pregnancy, the mean cumulative urinary excretion of non-glucuronidated OH-BUP was significantly higher than the excretion of bupropion or glucuronidated OH-BUP (3.9- and 6.9-fold higher, respectively, \( P < 0.05 \)). With the exception of animal #4, the ratio of \( \text{AUC}_{\text{OH-BUP}} \) to \( \text{AUC}_{\text{BUP}} \) in pregnant animals was lower than in the non-pregnant state. Relative to the postpartum period, the mean ratios of \( \text{AUC}_{\text{OH-BUP}} \) to \( \text{AUC}_{\text{BUP}} \).
during mid-pregnancy and late pregnancy were decreased by 28% and 20%, respectively (Table 4).

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

**Neonatal exposure to bupropion 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 inter-animal 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 i.v. bolus dose of BUP during mid-pregnancy, 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 non-pregnant baboons was approximately 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 i.v. route and at a similar concentration (Kiptoo et al., 2009). However, the formation of reduced metabolites of bupropion (TB and EB) was very low (Tables 5 & 6). In some cases, the concentrations of TB and EB in baboon plasma were below the lower limit of quantification, 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 7-10- and 5-fold higher, respectively, than that of the parent compound, while the concentration of EB is similar to that of BUP (Schroeder et al, 1981). While the observed lower formation of OH-BUP, TB, and EB in baboons following i.v. administration of bupropion vs. their formation in humans following oral dosing could be route-dependent, interspecies differences cannot be excluded. Suckow et al.
reported differences in the biotransformation of bupropion among rats, mice and guinea pigs following intraperitoneal (i.p.) administration—a route which involves the first-pass effect and thus the PK of substances are more similar to those seen after oral administration (Suckow et al., 1986). In rats, the formation of OH-BUP and TB was very low. In mice, although the AUC of OH-BUP was approximately 5-fold higher, the AUC of TB was 2-fold lower than the AUC of bupropion. 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 clearance during baboon pregnancy trended higher than in the postpartum period (Table 2). Most of the individual plasma 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. Since in baboons BUP is extensively metabolized primarily to OH-BUP, one might expect that the elevated clearance 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$_{\text{max}}$ and AUC$_{0-\infty}$ values of OH-BUP in pregnant baboons trended lower than the postpartum values (Table 3). Furthermore, the AUC$_{\text{OH-BUP}}$/AUC$_{\text{BUP}}$ ratio was not significantly different when comparing pregnant versus non-pregnant baboons (Table 4), as might be expected if the increased clearance of BUP was due to increased activity of the enzyme catalyzing the hydroxylation. Bupropion is a drug with a high extraction ratio (bioavailability ≈ 20% in humans). Therefore, its hepatic clearance would be dependent on hepatic blood flow. Pregnancy-related changes in hepatic blood flow in baboons could thus affect the clearance of bupropion. 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 (non-pregnant) 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 bupropion 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 clearance (as a
surrogate marker for glomerular filtration rate) was higher during mid-pregnancy compared to
postpartum (30.9 ± 3.9 mL/min vs. 23.9 ± 2.9 mL/min, n=3, \(P<0.05\)).

The ratios of \(\text{AUC}_{\text{OH-BUP}}\) to \(\text{AUC}_{\text{BUP}}\) trended lower in pregnant baboons as compared to non-
pregnant, except for one animal (Table 4). It is possible that the hepatic clearance of BUP
increases due to an increase in hepatic arterial blood flow. Nevertheless, the plasma \(\text{AUC}_{\text{OH-BUP}}\)
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 bupropion are approximately 1.5-times that of OH-BUP, 14-
times that of TB, and 90-times that of EB, suggesting primarily intrauterine fetal exposure to
bupropion and hydroxybupropion. The mean plasma BUP concentration in the neonate after
delivery was 55 ± 2 ng/ml. This represents approximately 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 ± 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 while the maternal plasma concentration of BUP in baboons after cesarean section (approximately 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 (Burchrdi 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 one hour after BUP administration, the main component of its total clearance 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. 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 to BUP (Kiptoo et al., 2009).

Due to the wide inter-individual variability between baboons in the biotransformation of bupropion 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. While the observed increase in the clearance of BUP during baboon pregnancy could be caused by a pregnancy-induced increase in its biotransformation, the trend towards 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 clearance 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 clearance of BUP (Wang et al., 2011).
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Authorship Contributions

Participated in research design: Tatiana N. Nanovskaya

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Contributed new reagents or analytic tools: Xiaoming Wang, Daria I. Vernikovskaya, Ying Zhang

Performed data analysis: Erik Rytting, Tatiana N. Nanovskaya

Wrote or contributed to the writing of the manuscript: Erik Rytting, Susan M. Abdel-Rahman, Mahmoud S. Ahmed, Tatiana N. Nanovskaya
References


Metabolism of 17 α-hydroxyprogesterone caproate by hepatic and placental

Kinetics of glyburide metabolism by hepatic and placental microsomes of human and
Footnotes

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Legends for Figures

**Figure 1.** Mean (± s.d.) plasma concentrations of bupropion (A) and hydroxybupropion (B) in baboons during mid-pregnancy (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 bupropion (n=4-5).

**Figure 2.** Cumulative 24-hour urinary excretion of bupropion, hydroxybupropion (OH-bupropion), and glucuronidated hydroxybupropion in baboons during mid-pregnancy, late pregnancy, and postpartum following an i.v. bolus dose of bupropion (1 mg/kg). Data are presented as mean ± s.d. (n=3). The amount of glucuronidated bupropion detected accounted for less than 1% of excreted bupropion, and is not presented. * ANOVA followed by Tukey-Kramer post hoc analysis showed statistically significant differences between the amount of hydroxybupropion excreted in late pregnancy compared to bupropion and glucuronidated hydroxybupropion ($P < 0.05$).
Table 1. The compound dependent parameters for the MRM transition of bupropion and its metabolites.

<table>
<thead>
<tr>
<th>Compound dependent parameters</th>
<th>BUP</th>
<th>OH-BUP</th>
<th>EB</th>
<th>TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time of analyte (min)</td>
<td>7.7</td>
<td>6.7</td>
<td>8.5</td>
<td>9.5</td>
</tr>
<tr>
<td>Analyte MRM transition (m/z)</td>
<td>240→184</td>
<td>256→238</td>
<td>242→168</td>
<td>242→168</td>
</tr>
<tr>
<td>Retention time of internal standard (min)</td>
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<td>6.6</td>
<td>8.4</td>
<td>9.3</td>
</tr>
<tr>
<td>Internal standard MRM transition (m/z)</td>
<td>249→185</td>
<td>262→244</td>
<td>251→169</td>
<td>251→169</td>
</tr>
<tr>
<td>Declustering Potential (v)</td>
<td>40</td>
<td>40</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Entrance Potential (v)</td>
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<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Collision energy (v)</td>
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<td>18</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Collision Cell Exit Potential (v)</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
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</tbody>
</table>
Table 2. Individual pharmacokinetic parameters of bupropion in pregnant and postpartum baboons following a 1 mg/kg i.v. bolus dose of bupropion. (*P*-values from repeated measures ANOVA: CL, 0.060; \(C_{\text{max}}\), 0.208; \(\text{AUC}_{0-\infty}\), 0.078.)

<table>
<thead>
<tr>
<th>Study Animal</th>
<th>Mid-pregnancy</th>
<th></th>
<th></th>
<th>Late pregnancy</th>
<th></th>
<th></th>
<th>Postpartum</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CL (L/h/kg)</td>
<td>(C_{\text{max}}) (ng/ml)</td>
<td>(\text{AUC}_{0-\infty}) (h·ng/ml)</td>
<td>CL (L/h/kg)</td>
<td>(C_{\text{max}}) (ng/ml)</td>
<td>(\text{AUC}_{0-\infty}) (h·ng/ml)</td>
<td>CL (L/h/kg)</td>
<td>(C_{\text{max}}) (ng/ml)</td>
<td>(\text{AUC}_{0-\infty}) (h·ng/ml)</td>
</tr>
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<td>3.5</td>
<td>375.4</td>
<td>318.2</td>
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<td>383.0</td>
<td>364.3</td>
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</tr>
<tr>
<td>2</td>
<td>3.4</td>
<td>333.9</td>
<td>308.1</td>
<td>2.8</td>
<td>420.3</td>
<td>356.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.6</td>
<td>311.3</td>
<td>275.4</td>
<td>3.5</td>
<td>440.0</td>
<td>292.6</td>
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<td>269.4</td>
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<td>263.0</td>
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<td>287.8</td>
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<td>344.8</td>
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<tr>
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<td>3.48</td>
<td>330.4</td>
<td>302.1</td>
<td>2.68</td>
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<td>381.7</td>
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<td>s.d.</td>
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<td>91.1</td>
<td>21.7</td>
<td>0.41</td>
<td>82.2</td>
<td>34.1</td>
<td>0.31</td>
<td>64.3</td>
<td>42.0</td>
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Table 3. Individual Pharmacokinetic parameters of hydroxybupropion in pregnant and postpartum baboons following a 1 mg/kg i.v. bolus dose of bupropion. (P-values from repeated measures ANOVA: $C_{\text{max}}$, 0.373; $t_{\text{max}}$, 0.751; $\text{AUC}_{0-\infty}$, 0.102.)

<table>
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<tr>
<th>Study Animal</th>
<th>Mid-pregnancy</th>
<th>Late pregnancy</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_{\text{max}}$</td>
<td>$t_{\text{max}}$</td>
<td>$\text{AUC}_{0-\infty}$</td>
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<tr>
<td>1</td>
<td>85.5</td>
<td>0.5</td>
<td>211.0</td>
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<tr>
<td>2</td>
<td></td>
<td>99.1</td>
<td>0.75</td>
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<td>3</td>
<td>62.5</td>
<td>0.5</td>
<td>109.6</td>
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<tr>
<td>4</td>
<td>108.0</td>
<td>0.5</td>
<td>288.3</td>
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<tr>
<td>5</td>
<td>82.8</td>
<td>0.75</td>
<td>165.3</td>
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<tr>
<td>mean</td>
<td>84.7</td>
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<td>194</td>
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<tr>
<td>s.d.</td>
<td>18.6</td>
<td>0.1</td>
<td>76</td>
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Table 4. Ratio of $\text{AUC}_{\text{hydroxybupropion}}$ to $\text{AUC}_{\text{bupropion}}$ following a 1 mg/kg i.v. bolus dose of bupropion to baboons during pregnancy and the postpartum period. ($P$-value from repeated measures ANOVA: 0.959.)

<table>
<thead>
<tr>
<th>Study animal</th>
<th>Mid-pregnancy</th>
<th>Late pregnancy</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.72</td>
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<td>0.92</td>
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<tr>
<td>2</td>
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<td>1.21</td>
<td>1.93</td>
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<td>3</td>
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<td>0.26</td>
<td>0.54</td>
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<tr>
<td>4</td>
<td>1.16</td>
<td>1.20</td>
<td>0.73</td>
</tr>
<tr>
<td>5</td>
<td>0.62</td>
<td>0.63</td>
<td>1.00</td>
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<tr>
<td>mean</td>
<td>0.73</td>
<td>0.82</td>
<td>1.02</td>
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<tr>
<td>s.d.</td>
<td>0.31</td>
<td>0.46</td>
<td>0.53</td>
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Table 5. Individual pharmacokinetic parameters of threohydrobupropion in pregnant and postpartum baboons following a 1 mg/kg i.v. bolus dose of bupropion.

<table>
<thead>
<tr>
<th>Study Animal</th>
<th>Mid-pregnancy</th>
<th>Late pregnancy</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>t&lt;sub&gt;max&lt;/sub&gt;</td>
<td>AUC&lt;sub&gt;0→∞&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>ng/ml</td>
<td>h</td>
<td>h-ng/ml</td>
</tr>
<tr>
<td>2</td>
<td>1.52</td>
<td>2.00</td>
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<td>1.74</td>
<td>0.75</td>
<td>5.90</td>
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<td>1.98</td>
<td>0.75</td>
<td>6.8</td>
</tr>
<tr>
<td>s.d.</td>
<td>0.34</td>
<td>0.00</td>
<td>1.3</td>
</tr>
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</table>
Table 6. Individual pharmacokinetic parameters of erythrohydrobupropion in pregnant and postpartum baboons following a 1 mg/kg i.v. bolus dose of bupropion.

<table>
<thead>
<tr>
<th>Study Animal</th>
<th>Mid-pregnancy</th>
<th>Late pregnancy</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_{max}$</td>
<td>$t_{max}$</td>
<td>$AUC_{0-\infty}$</td>
</tr>
<tr>
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<td>1.05</td>
<td>1.00</td>
<td>4.71</td>
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<td>5</td>
<td>1.27</td>
<td>1.00</td>
<td>4.17</td>
</tr>
<tr>
<td>Mean</td>
<td>1.27</td>
<td>1.00</td>
<td>4.17</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.11</td>
<td>0.18</td>
<td>0.70</td>
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</table>
**Table 7.** Concentrations of bupropion and its metabolites in baboon maternal and neonatal plasma at the time of delivery by C-section, 1 hour after a 1 mg/kg i.v. bolus dose of bupropion to the pregnant baboon.

<table>
<thead>
<tr>
<th>Study Animal</th>
<th>Maternal Plasma (ng/ml)</th>
<th>Neonatal Plasma (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OH-BUP</td>
<td>BUP</td>
</tr>
<tr>
<td>1</td>
<td>213</td>
<td>74</td>
</tr>
<tr>
<td>2</td>
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<td>52</td>
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<td>mean</td>
<td>164</td>
<td>63</td>
</tr>
<tr>
<td>s.d.</td>
<td>46</td>
<td>11</td>
</tr>
</tbody>
</table>
Figure 1

(A) Bupropion in plasma (ng/ml) over time (hours) for mid-pregnancy, late pregnancy, and postpartum conditions.

(B) Hydroxybupropion in plasma (ng/ml) over time (hours) for mid-pregnancy, late pregnancy, and postpartum conditions.