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# Pharmacokinetics of a Long-lasting, Highly Concentrated Buprenorphine Solution after Subcutaneous Administration in Rhesus Macaques (*Macaca mulatta*)

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**Opioids are essential for use in rhesus macaques (*Macaca mulatta*) that require multimodal analgesia or those unable to receive NSAID as part of their pain management plan. The current opioid epidemic has universally limited the availability of these vital analgesics, compelling clinicians to investigate other options including novel opioid formulations. A commercially available injectable, long-lasting, highly concentrated buprenorphine solution (HCBS) provides therapeutic plasma concentrations lasting 24 h after a single dose in cats (*Felis catus*). We hypothesized that this same HCBS would achieve therapeutic concentrations (≥ 0.1 ng/mL) for at least 24 h in rhesus macaques. In the current study, 6 healthy, adult rhesus macaques were included in a randomized, 2-period, 2-treatment crossover study. The low dose (0.24 mg/kg SC) achieved a peak plasma concentration of 19.1 ± 5.68 ng/mL at 0.308 ± 0.077 h, with an AUC of 236.4 ± 22.5 h/ng/mL and terminal elimination half-life of 19.6 ± 4.02 h; for the high dose (0.72 mg/kg SC), these parameters were 65.2 ± 14.7 ng/mL, 0.034 ± 0.004 h, 641.3 ± 79.4 h/ng/mL, and 20.6 ± 2.30 h, respectively. The mean plasma concentrations for the low and high doses in rhesus macaques significantly exceeded the therapeutic threshold for 48 and 72 h, respectively. One macaque showed mild somnolence at both doses, and another showed mild pruritus at both doses. These findings show that subcutaneous administration of HCBS provides prolonged and long-lasting therapeutic plasma levels for 48 to 72 h dosing without problematic adverse effects and thus represents a potential new analgesic alternative.**

**Abbreviations:** HCBS, highly concentrated buprenorphine solution; SRB, sustained-release buprenorphine

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Rhesus macaques (*Macaca mulatta*) are the most commonly used NHP in biomedical research.[19] Their physiologic similarity to humans makes them an appealing model to study a variety of human conditions such as aging, reproduction, development, and neurology.[28] NHP facilities, especially those with outdoor housing, may house 5000 macaques or more at once. Considering that rhesus macaques are an aggressive and agonistic species,[37] animals experience traumatic injuries relatively commonly, especially with high-density group housing. One study at a national primate research center calculated that the percentage of animals presenting to the facility's hospital with injuries requiring sutures ranged from 5% to 9% annually.[23] In addition to encounters with conspecifics resulting in trauma, many studies using rhesus macaques have the potential to cause temporary pain or discomfort; therefore, adequate analgesia should be provided unless scientifically justified otherwise, as required by the *Guide for the Care and Use of Laboratory Animals* and the Animal Welfare Act and Regulations.[2,3,21]

Opioids play an integral part when developing pain management plans for NHP in research. In NHP medicine, opioids are often combined with NSAID as multimodal analgesia for perioperative and postoperative care, in addition to providing adequate pain control for animals that have sustained moderate to severe injuries.[4] In addition, opioids are considered when NSAID are contraindicated, for example, during gestation, due to project needs, and in animals having compromised liver or kidney function.[27] Despite the significance of opioids in pain management, the current human opioid epidemic has caused withdrawal of these drugs from the market, thus severely affecting veterinary medicine.[10] This shortage has driven clinicians to explore other opioid options, such as new formulations and different dosing regimens.[9]

Buprenorphine is currently one of the most frequently prescribed opioid analgesics used by NHP facilities for the treatment of moderate acute and chronic pain.[32] It is a partial μ-agonist, κ-antagonist with high affinity for the μ-receptor and a slow dissociation rate.[25,34] These properties are responsible for the long half-life of buprenorphine and its ability to displace other μ-agonists, such as morphine, when given concomitantly. In addition, the ability of this analgesic to bind partially to the μ-receptor creates a ceiling effect; therefore, increasing

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the dose does not increase adverse effects, giving it a wide safety margin.[43] However, the high affinity of buprenorphine for the μ-receptor impedes μ-antagonists (that is, naloxone) and thus only partially reverses its effects.[33] Buprenorphine is approximately 30 times more potent than morphine and, due to its efficacy, long duration, safety, and injectable formulation,[12,25,33,34] it is a primary opioid used in NHP medicine. In light of the current opioid epidemic and commitment to the principles of the 3 Rs (replacement, reduction, and refinement),[35] it behooves clinicians to explore potential refinements which encompass alternative prescribing approaches to already established opioids and/or researching the use of novel opioids to improve animal welfare.

Currently, there are 3 different commercial buprenorphine formulations available and commonly used in veterinary medicine; 2 of these formulations have published pharmacokinetic data for rhesus macaques. Although therapeutic plasma concentrations of buprenorphine in rhesus macaques have not been established, several previous studies performed in humans suggested a minimal range of buprenorphine concentrations (0.1 to 0.5 ng/mL) to provide adequate analgesia.[14,15] In addition, 2 studies have cited using 0.1 ng/mL as the absolute minimal potential therapeutic threshold in rhesus macaques;[24,32] therefore, we chose this threshold as a minimum effective plasma concentration for our study. One study demonstrated that 0.03 mg/kg buprenorphine HCl IM remained above 0.1 ng/mL for 12 h in rhesus macaques.[24] This formulation with a concentration of 0.3 mg/mL and a vehicle consisting of anhydrous dextrose, water, and hydrochloric acid is currently in frequent use at many NHP facilities, including our own.[34] Another study involving a novel, sustained-release buprenorphine formulation (SRB; ZooPharm, Fort Collins, CO) determined that a single dose at 0.2 mg/kg SC remained above the therapeutic threshold for 5 d when given to rhesus macaques.[32] This compounded formulation contains 1.0 mg/mL of buprenorphine with a vehicle consisting of a patented sustained-release biodegradable liquid polymer matrix.[47] SRB is a viable alternative to buprenorphine HCl, but strict laws govern the acquisition of a compounded controlled substance from out of state, thus preventing its use at some NHP facilities.[1,5,16] The third formulation (highly concentrated buprenorphine solution [HCBS]) is very similar to buprenorphine HCl; however, it has a different concentration (1.8 mg/mL), dose, route of administration (subcutaneous), and vehicle, consisting of anhydrous dextrose, parabens, glacial acetic acid, water, and hydrochloric acid or sodium hydroxide to adjust pH.[46] This combination of concentration, route of administration, dose, and novel vehicle contribute to this formulation's long-lasting nature.[46] Lastly, this single-dose, injectable solution is FDA-approved for use in cats (*Felis catus*), providing therapeutic plasma concentrations lasting 24 h[11,46] and thus forming the basis for the current pharmacokinetic investigation.

Long-lasting drugs are preferred in NHP medicine for many reasons, mainly because of the decrease in stress placed on animals when dosing as well as increased compliance. HCBS dosed at 0.24 and 0.72 mg/kg SC has been investigated and proven safe and effective in cats;[11,46] therefore, we chose to evaluate these 2 doses in rhesus macaques because of interspecies variation in drug absorption times and to determine a safe and effective dosing regimen. To this end, we designed a randomized, 2-period, 2-treatment crossover study. We hypothesized that HCBS would achieve therapeutic plasma concentrations (≥0.1 ng/mL) in rhesus macaques with minimal adverse effects for at least 24 h when dosed at 0.24 or 0.72 mg/kg SC as compared with published data from cats. We chose the 24-h time point for comparison in light of the lack of available pharmacokinetic data in cats and the extrapolated therapeutic plasma threshold of 0.1 ng/mL in rhesus macaques.[24,32] The objective of the current study was to evaluate the pharmacokinetic profiles of a single subcutaneous injection of HCBS in rhesus macaques at both doses. The lower dose was chosen because it is the FDA-approved dose in cats, and the higher dose was chosen because it was the highest dose studied that yielded minimal adverse effects in cats.[11,46]

## Materials and Methods

**Animals.** Three adult male (age [mean ± 1 SD], 7.3 ± 3.4 y; weight, 11.4 ± 2.1 kg) and 3 adult female rhesus macaques (age, 13.0 ± 2.0 y; weight, 9.1 ± 2.6 kg) were used to complete this study. All animals were determined healthy as assessed by a clinical history review, preproject physical examination, CBC, and serum chemistry analysis. Macaques were fed a commercial diet (LabDiet Monkey Diet 5047, Purina, St Louis, MO) supplemented with fresh produce twice weekly and edible forage daily; tap water was provided without restriction. Toys and coconuts were supplied as manipulanda. Macaques were pair-housed whenever compatible and appropriate or provided with visual and auditory contact with conspecifics during periods of observation for potential adverse effects. Macaques were maintained on a 12:12-h light:dark cycle. All procedures were conducted under an IACUC-approved protocol at the University of California, Davis. Animals were maintained in accordance with the Animal Welfare Act and Regulations, the *Guide for the Care and Use of Laboratory Animals*, and Public Health Service policy in an AAALAC-accredited, USDA-registered institution.[2,3,21,30]

**Study design and sample collection.** This study was a randomized, 2-period, 2-treatment crossover study. By using randomization software, 6 macaques (3 male, 3 female) were randomly assigned into 2 groups that received either 0.24 mg/kg SC ($n$ = 3) or 0.72 mg/kg SC ($n$ = 3) HCBS (Simbadol, Zoetis Kalamazoo, MI); this product is a clear, colorless to slightly yellow, sterile, injectable solution that contains 1.8 mg/mL buprenorphine (equivalent to 1.94 mg/mL buprenorphine HCl).[46]

Prior to dosing, the left shoulder of all animals was shaved to allow visualization of the injection, to ensure accurate dosing, and to facilitate observation of potential adverse effects at the dosing site. To ensure accurate dosing, body weights were obtained within 1 wk prior to the start of each treatment period. Dosing and blood sampling were performed in conscious animals, and blood samples were collected from the cephalic vein by using a cageside 'arm pull' technique. All macaques were acclimated to this technique for approximately 3 wk prior to study start. During this acclimation period, animals were rewarded for presenting their left arms for blood collection. At each time point, approximately 2 mL of blood was collected into 3-mL $K_3$-EDTA tubes; samples were collected prior to dosing (0 h, baseline) and at 0.5, 1, 2, 6, 12, 24, 48, 72, and 120 h afterward. After a 21-d washout period, the animals were reassigned to receive the other dose, and the study was repeated.

Blood tubes were placed immediately on ice until centrifuged. Blood samples were centrifuged at 3000 × $g$ at 4 °C for 10 min; plasma was aliquoted into 1.5-mL microcentrifuge tubes and stored at –80 °C until assayed.

**Selection of dose and dosing site.** Dose site selection was based on ease of administration with regard to animal restraint and positioning for conscious cageside manipulation. Although current data regarding dosing of buprenorphine HCl in macaques are available, none addressed dosing macaques with HCBS. Doses for macaques were selected according to

safe and effective doses in cats, with the hypothesis that effects would be similar in rhesus macaques and with consideration of the high therapeutic index of buprenorphine.[18] The low dose administered (0.24 mg/kg SC) was selected on the basis of the FDA-approved dose for cats.[11] The high dose (0.72 mg/kg SC) was chosen because it was the highest dose given to cats that yielded minimal adverse effects in a safety study conducted by the manufacturer.[11,46]

**Animal observation.** Macaques were monitored during all scheduled blood collections. In addition, animals were monitored for adverse effects continuously during the first 2 h after dosing and then every 2 to 4 h for the first 12 h, followed by 2 to 3 times daily for the remainder of the study. In most species, adverse effects associated with opioids include respiratory depression, sedation, constipation, and pruritus.[13,33,34]

**Plasma sample analysis.** Buprenorphine in NHP plasma was quantitated through HPLC–tandem mass spectrometry according to a previously published method.[45] Quantification of buprenorphine metabolites was not attempted because their relative biologic effects in NHP remain to be elucidated.[32] A partial validation was performed, with NHP plasma as the matrix. The response for buprenorphine was linear and gave correlation coefficients of 0.99 or better. Accuracy was reported as percentage nominal concentration, and precision was reported as percentage relative standard deviation. Accuracy was 96% for 0.15 ng/mL and 93% for 40 ng/mL. Precision was 9% for 0.15 ng/mL and 5% for 40 ng/mL. The technique was optimized to provide a limit of quantitation of 0.05 ng/mL and a limit of detection of approximately 0.025 ng/mL for buprenorphine.

**Pharmacokinetic analysis.** Pharmacokinetic parameters were estimated through compartmental analysis in population mode using the NLME component (version 8.0, Phoenix WinNonlin, Certara, Princeton, NJ). Data were analyzed by using both 2- and 3-compartment models, and several error structures were assessed. The models were evaluated by using the first-order conditional estimation–extended least squares (FOCE-ELS) engine. Although the sample size was small ($n$ = 6), the coefficients of variation (CV) for the fixed effects were reasonable. Goodness of fit and the appropriate error term were selected through visual analysis of observed compared with predicted concentration graphs and residual plots, as well as CV, Akaike Information Criterion,[44] and % CV of parameter estimates.

**Statistical analysis.** HCBS concentration (in ng/mL) was measured at 0, 0.5, 1, 2, 6, 12, 24, 48, 72, and 120 h after dosing. The limit of detection was 0.025 ng/mL, and results that were below the limit of detection were recorded as 0.02 for statistical analysis. The hypothesis of interest was whether HCBS concentration (ng/mL) exceeded 0.1 ng/mL, the extrapolated minimal therapeutic concentration in rhesus macaques, at each time point and dose. A series of 1-sample $t$ tests were used (version 14.2, 1 Stata SE, StataCorp, College Station, TX) to compare the concentrations at each time point after dosing with the hypothesized mean of 0.1 ng/mL. Two-sample $t$ tests were used to compare HCBS concentrations in animals administered the high dose with those given the low dose. For each dosage, ANOVA was used to determine whether the concentration of HCBS was affected by both sex and time after dosing. Statistical significance was set as a $P$ value of less than 0.05.

## Results

All rhesus macaques remained healthy after subcutaneous administration of HCBS throughout the study, with the exception of mild adverse effects noted in 2 animals. One animal showed mild somnolence, and the other had mild pruritus at both doses, with the onset of clinical signs within 0.5 h after dosing and cessation by 2 h afterward. Clinical signs did not increase in severity or duration when animals received the high dose as compared with the low dose. No injection site reactions were noted in any of the macaques at either dose.

**Table 1.** Pharmacokinetic parameters for HCBS administered as a single subcutaneous injection in rhesus macaques ($n$ = 6; 3 male, 3 female)

| | 0.24 mg/kg | 0.72 mg/kg |
|---|---|---|
| $C_{max}$ (ng/mL) | 19.1 ± 5.68 | 65.2 ± 14.7 |
| $T_{max}$ (ng/mL) | 0.308 ± 0.077 | 0.491 ± 0.085 |
| A (ng/mL) | 16.1 (27.5) | 61.0 (4.36) |
| B (ng/mL) | 3.77 (127) | 11.5 (23.2) |
| α (1/h) | 0.123 (42.8) | 0.198 (35.4) |
| β (1/h) | 0.035 (75.0) | 0.034 (9.74) |
| $t_{1/2\alpha}$ (h) | 5.64 (42.8) | 3.49 (35.3) |
| $t_{1/2\beta}$ (h) | 19.6 (75.0) | 20.6 (9.74) |
| AUC (ng × h/mL) | 236.4 (163) | 641.3 (21.0) |

A and B are the intercepts at t = 0 for the model equation; α and β are the slopes for the modeled equation; t1/2α is the phase 1 half-life; and t1/2β is the phase 2 half-life.
Data for $C_{max}$ and $T_{max}$ are given as mean ± 1 SD; all other data are given as mean (%CV).

Pharmacokinetic parameters in rhesus macaques for both doses are shown in Table 1. A 2-compartment extravascular model ($C_p = A_e^{-}\alpha^t + B_e - \beta_t + C_e - ka \times t$) with clearance parameterization and a multiplicative weighting factor ($C \times [1 + C_{eps}]$) gave the best fit to buprenorphine concentration data points. Figures 1 through 8 illustrate the goodness of fit and the observed compared with predicted concentration graphs and residual plots from which the appropriate error term were selected through visual analysis, as described earlier. The average plasma concentrations of buprenorphine with regard to time are depicted in Figure 9. Plasma levels above the extrapolated minimal therapeutic plasma concentration of 0.1 ng/mL were reached within 0.5 h of dosing for both doses. Mean concentrations at 0.5, 1, 2, 6, 12, 24, and 48 h after dosing were significantly ($P < 0.05$) above the 0.1 ng/mL threshold for effective therapeutic concentration for both doses (Figure 9),[24] and the mean concentration at 72 h was significantly above the 0.1-ng/mL threshold ($P = 0.04$) for the high dose, but not the low dose ($P = 0.07$). At 120 h, neither dose resulted in concentrations above 0.1 ng/mL. At 0.5, 1, 2, 6, 12, and 24 h after dose, the HCBS concentration was significantly ($P < 0.05$) higher in macaques administered the high dose than in animals administered the low dose. At 48, 72, and 120 h, HCBS concentrations did not differ significantly according to dosage. HCBS concentration was significantly affected by sex at both the low ($P = 0.05$) and high ($P < 0.05$) dosages, with concentrations lower in female macaques than males at 0.5, 1, and 2 h after dosing (Figure 10).

## Discussion

Opioids are essential analgesics in veterinary medicine, but the current opioid crisis has made it progressively more challenging to obtain these analgesics for patient use. Manufacturer setbacks and government efforts to reduce opioid addiction in humans has led to a widespread shortage of injectable opioids.[22] This shortage not only affects human clinicians but has severely affected veterinary clinicians, because the use of these compounds crosses species. Injectable opioids previously frequently used perioperatively and for severe traumatic injuries

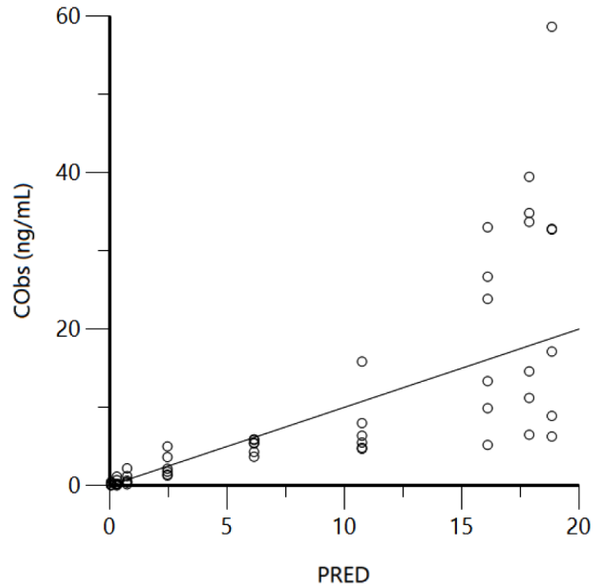


**Figure 1.** Diagnostic plots for NLME modeling of HCBS after administration of 0.24 mg/kg SC to rhesus macaques. Plots include the population predicted values (PRED) compared with the dependent variable (DV), CObs (ng/mL).

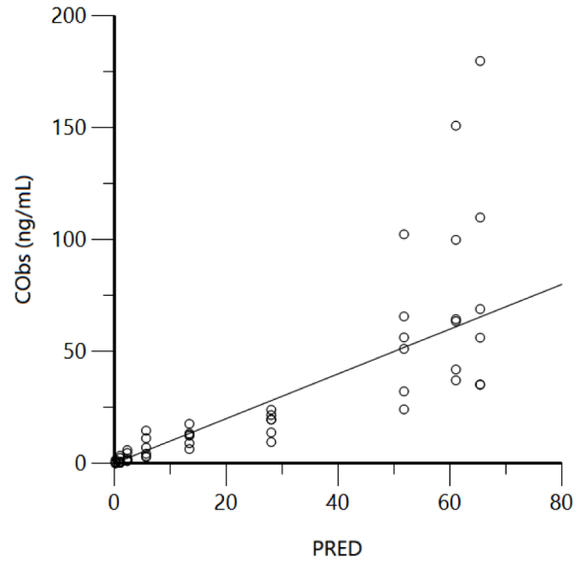


**Figure 3.** Diagnostic plots for NLME modeling of HCBS after administration of 0.72 mg/kg SC to rhesus macaques. Plots include the population predicted values (PRED) compared with the dependent variable, observed concentration (CObs, ng/mL).

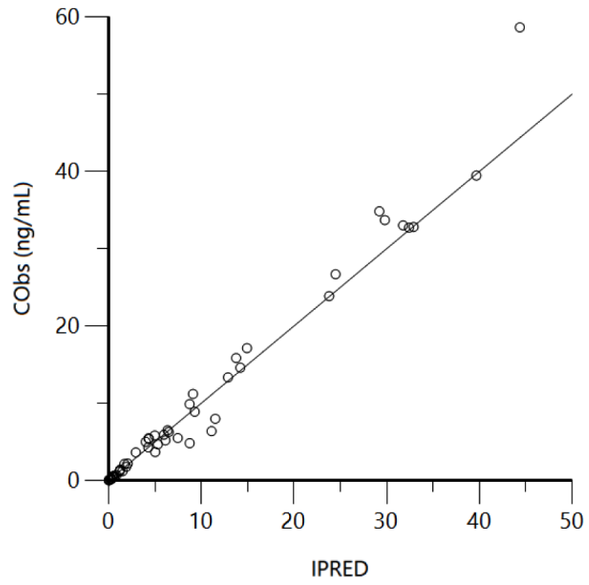


**Figure 2.** Diagnostic plots for NLME modeling of HCBS after administration of 0.24 mg/kg SC to rhesus macaques. Plots include the individual prediction (IPRED) compared with the DV, CObs (ng/mL).

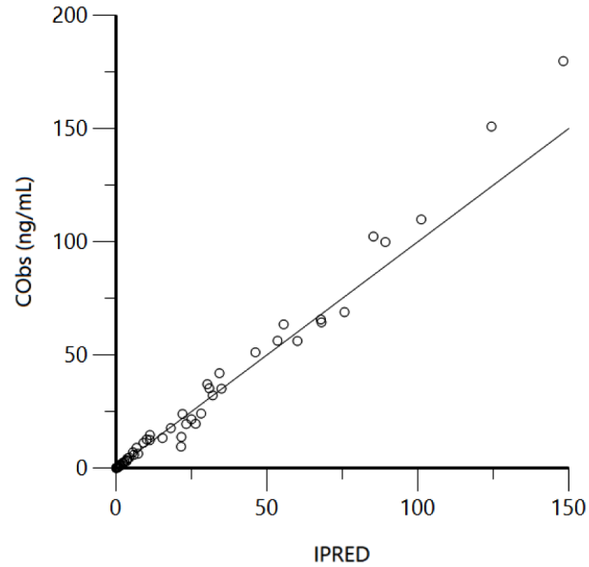


**Figure 4.** Diagnostic plots for NLME modeling of HCBS after administration of 0.72 mg/kg SC to rhesus macaques. Plots include the individual prediction (IPRED) compared with the dependent variable, observed concentration (CObs, ng/mL).

are no longer guaranteed to be in stock or available to hospitals.[22] This situation has left veterinarians with 2 options: find alternative opioids or replace opioids with a different class of analgesic. To ensure animal welfare and care, most veterinarians seek opioid alternatives. Multiple previous studies researched different formulations and drug-delivery methods of opioids with the goal of reducing animal stress by changing the dose, route, or frequency of administration.[11,17,32,38] Researching different opioid formulations and dosing protocols is important to provide alternative options in the event of a shortage of a specific, routinely used opioid.

This study reports the pharmacokinetic profiles of HCBS in adult male and female rhesus macaques (*Macaca mulatta*) dosed at 0.24 and 0.72 mg/kg SC. We chose to study HCBS because of its long-lasting analgesic effects that allow for a prolonged dosing interval compared with buprenorphine HCl and to find a suitable, obtainable opioid alternative during an injectable opioid shortage. We compared single subcutaneous injections of HCBS at 2 doses (0.24 and 0.72 mg/kg) in adult rhesus macaques by using an extrapolated minimal therapeutic plasma concentration of buprenorphine (0.1 ng/mL).[24,32] The threshold of 0.1 ng/mL represents the absolute minimal plasma

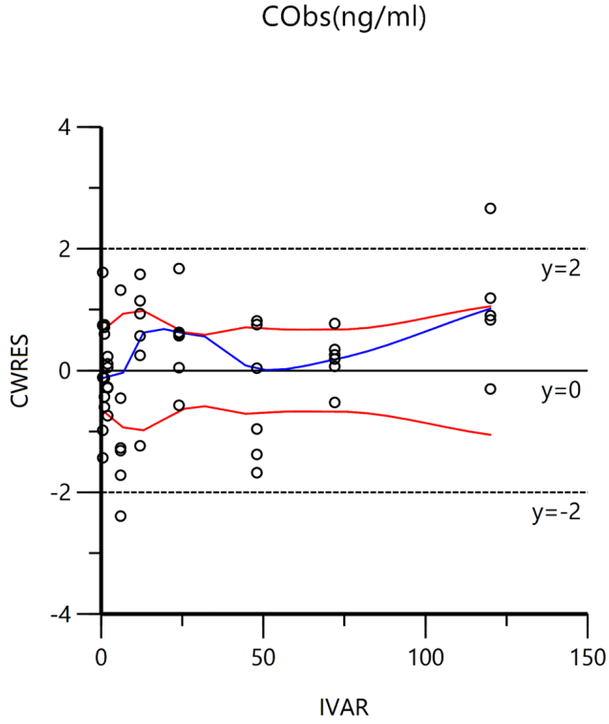


**Figure 5.** Diagnostic plots of the independent variable (time) compared with the conditional weighted residuals (CWRES) for NMLE modeling of HCBS in rhesus macaques after administration of 0.24 mg/kg SC. The red and blue lines represent trends of the data points.

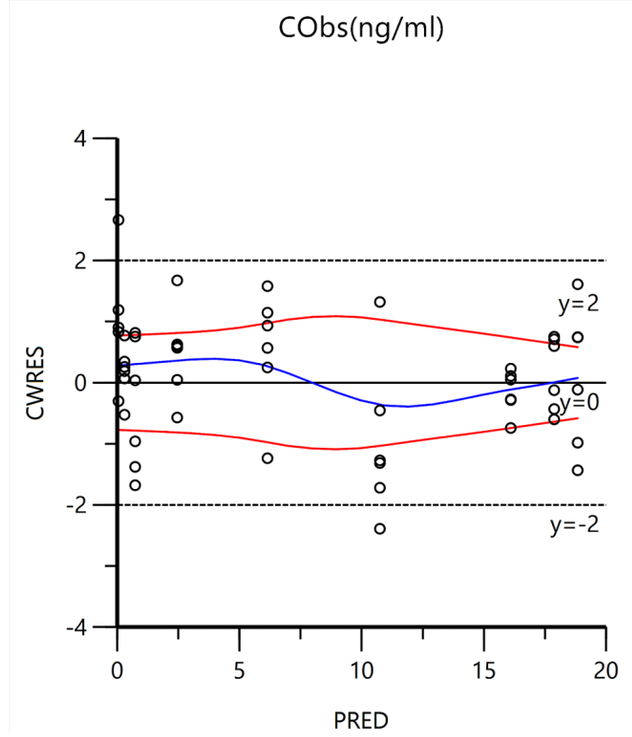


**Figure 6.** Diagnostic plots of the predicted value concentrations (PRED) compared with CWRES for NLME modeling of HCBS in rhesus macaques after administration of 0.24 mg/kg SC. The red and blue lines represent trends of the data points.

buprenorphine concentration that can be considered effective and was used in previous studies in rhesus macaques that investigated buprenorphine pharmacokinetics;[24,32] however, it is important to reiterate that the therapeutic plasma concentration of buprenorphine in rhesus macaques has yet to be determined. Considerable variation in the effective plasma concentration exists in the literature in terms of the species studied, metabolic and excretion pathways, assay techniques, study design, characterization and monitoring of analgesia, previous opioid treatment, subject's tolerance, type of noxious stimulus applied, and subject's activity level, to name a few.[26,32,38,40,42] The range of 0.1 to 0.5 ng/mL has been suggested as the minimal effective therapeutic concentration in humans;[14,15] however, other studies suggest that ranges in other species may be much higher.[26,40] Both doses of HCBS produced plasma buprenorphine levels that exceeded 0.5 ng/mL at 72 h; however, additional statistics were not performed to determine significance. The high buprenorphine concentration levels demonstrated support of the point that achieving the minimal effective therapeutic concentration in rhesus macaques requires further investigation. Pharmacodynamic studies investigating buprenorphine in rhesus macaque tail-withdrawal procedures have been performed but were not paired with pharmacokinetic analysis to fully define an effective therapeutic plasma concentration in this species.[36,41] Furthermore, performing pharmacodynamic analysis of HCBS was beyond the scope of this current study but should be considered in the future. The data presented herein can be applied within the range of reported effective plasma concentrations in domestic species and humans to establish effective dosing strategies in rhesus macaques that are coupled with necessary and invaluable cageside observations for adequate analgesia and to avoid the risk of breakthrough pain.[32]

The previous study in cats used combined pharmacokinetic–pharmacodynamic modeling.[11] The authors reported that a single subcutaneous injection of HCBS dosed at 0.24 mg/kg in cats provided thermal antinociception for 24 h or longer, with an elimination half-life of 12.3 h.[11] The elimination half-life in rhesus macaques were 19.6 h for the low dose (0.24 mg/kg SC) and 20.6 h for the high dose (0.72 mg/kg SC), which are 1.5 and 1.6 times longer, respectively, than in cats. Pharmacodynamics and efficacy of HCBS were beyond the scope of this study and therefore were not performed but can be considered for future studies of HCBS in rhesus macaques. The AUC increased nearly proportionally from the low dose to the high dose (the high dose was 2.7 times the low dose), suggesting linear kinetics in rhesus macaques.[13] Furthermore, the elimination half-life was comparable between doses. As expected, $C_{max}$ for the high dose was at least 3.4 times higher than the low dose and was reached within 0.5 h after administration for both doses.

Although not necessarily a limitation, the drug concentration curve did not reach 0 ng/mL at the final 120-h time point. Even though drug concentrations still exceeded the limit of detection at the termination of sample collection, HCBS never reached zero; therefore, extrapolation of the terminal portion of the curve is required, potentially affecting the values for AUC and half-life. However, this influence is likely to be of little clinical importance, given the wide therapeutic index of buprenorphine. Note that the low-dose group had large %CV values for β, β half-life, and AUC. Given that the study design and model fit were appropriate, this effect is most likely due to variation between animals. Although there are too few animals in the current study to determine the cause of this variation, a larger-scale study with more animals would likely allow for identification of one or more covariates that might explain a large portion of the variability.

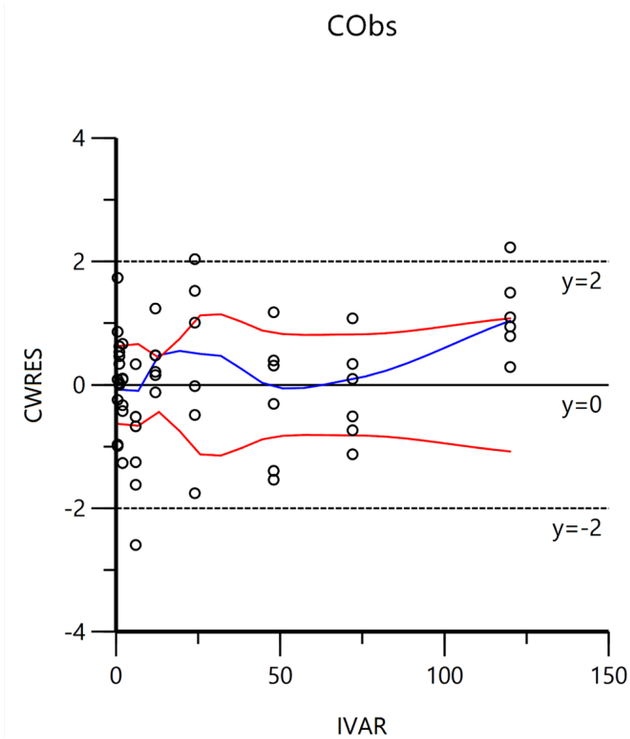


**Figure 7.** Diagnostic plots of the independent variable (time) compared with the conditional weighted residuals (CWRES) for NMLE modeling of HCBS in rhesus macaques after administration of 0.72 mg/kg SC. The red and blue lines represent trends of the data points.

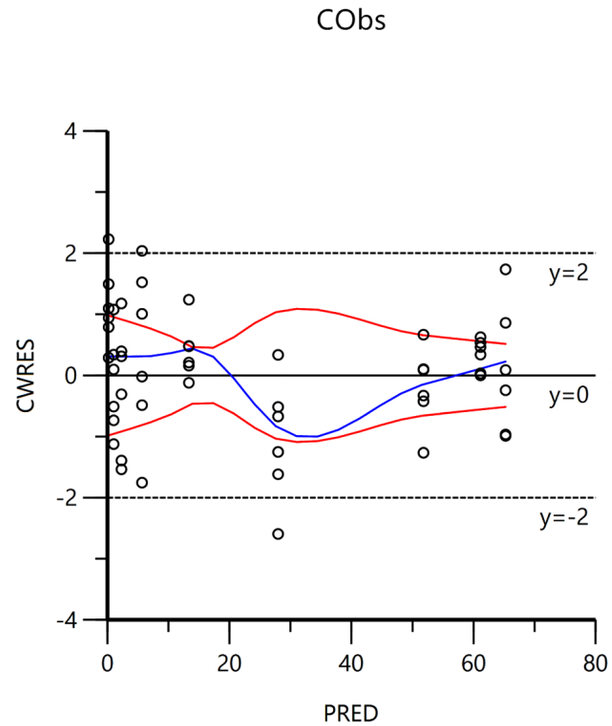


**Figure 8.** Diagnostic plots of the predicted value concentrations (PRED) compared with CWRES for NLME modeling of HCBS in rhesus macaques after administration of 0.72 mg/kg SC. The red and blue lines represent trends of the data points.

Historically, biomedical research has been conducted primarily on male subjects under the assumptions that physiology is the same between males and females and that data for females can be extrapolated from males.[29] This theory has been debunked over the last decade, and research has shown many biologic sex-related differences exist, particularly in pharmacokinetics.[7,39] Only in the past couple of years has NIH strictly required scientists to explain how they will address sex-associated differences in their research before acquiring a grant.[29] Given this knowledge, we made sure to have equal representation of both sexes in the current study. As a result, HCBS concentrations were lower in female macaques than males at 0.5, 1, and 2 h after dosing, but these values were still above the extrapolated therapeutic threshold of 0.1 ng/mL. Differences in drug metabolism in females have been attributed to circulating hormone levels.[39] In women, hormone levels fluctuate during times of estrous, pregnancy, and menopause, all of which could be responsible for the differences in drug metabolism and elimination.[8,39] At the time of the study, the female macaques were not in menses, pregnant, or menopausal, to the best of our knowledge. The lower HCBS levels in females may be due to other biologic sex-associated differences that are beyond the scope of this study.

We noted no severe adverse effects in our macaques. A single animal showed mild somnolence, and another had mild full-body pruritus; both clinical signs exhibited the same degree of effect and length of duration for both doses. These clinical signs are relatively common, mild, and nonlife-threatening adverse effects that are noted with opioid administration in general and are not specific to HCBS.[12,13,33,34] Although most opioids can be reversed easily by using naloxone, reversal of buprenorphine may not be possible due to its strong receptor affinity.[33] Increased doses of naloxone have been suggested to be used in reversal attempts, but it is unclear on how effective naloxone is at displacing buprenorphine from its receptors.[33] This situation should be acknowledged when using HCBS, given that it is more concentrated than buprenorphine HCl and may need a higher dose or frequent readministration of the reversal agent;[20] however, this likely is unnecessary given that the safety margin of buprenorphine is so wide. In addition, combined pharmacokinetic and pharmacodynamic studies have shown that HCBS is quickly absorbed when given subcutaneously; however, there is a delay between plasma concentration levels and the onset of analgesia of approximately 1 h after dosing.[11,46] This delayed onset should be taken into consideration when dosing HCBS if immediate analgesia is needed. Buprenorphine is highly bound to plasma proteins and metabolized in the liver by N-dealkylation and glucuronidation with excretion of its metabolites through feces and urine.[33,34,46] Safety studies performed by the manufacturer showed subacute liver inflammation in all dose groups (including controls); cats in the 0.72- and 1.2-mg/kg SC groups had a 33% higher incidence.[46] Although HCBS is dosed at a higher concentration than buprenorphine HCl and contrary to the risk in cats, we found no evidence of overt liver toxicity in rhesus macaques during this study.

Currently, SRB is only available as a compounded formulation with a patented biodegradable matrix.[47] This matrix breaks down over time, releasing appropriate levels of buprenorphine systemically over a 72-h period in rats.[17] Furthermore, SRB provided therapeutic levels of buprenorphine lasting 120 h in macaques after a single subcutaneous dose of 0.2 mg/kg.[32] Even though SRB was demonstrated to be a good alternative for buprenorphine HCl in rhesus macaques, a number of challenges preclude its use at some facilities. Compounded formulations do not necessarily have guarantees of potency, because they are not subject to FDA oversight, unlike FDA-approved

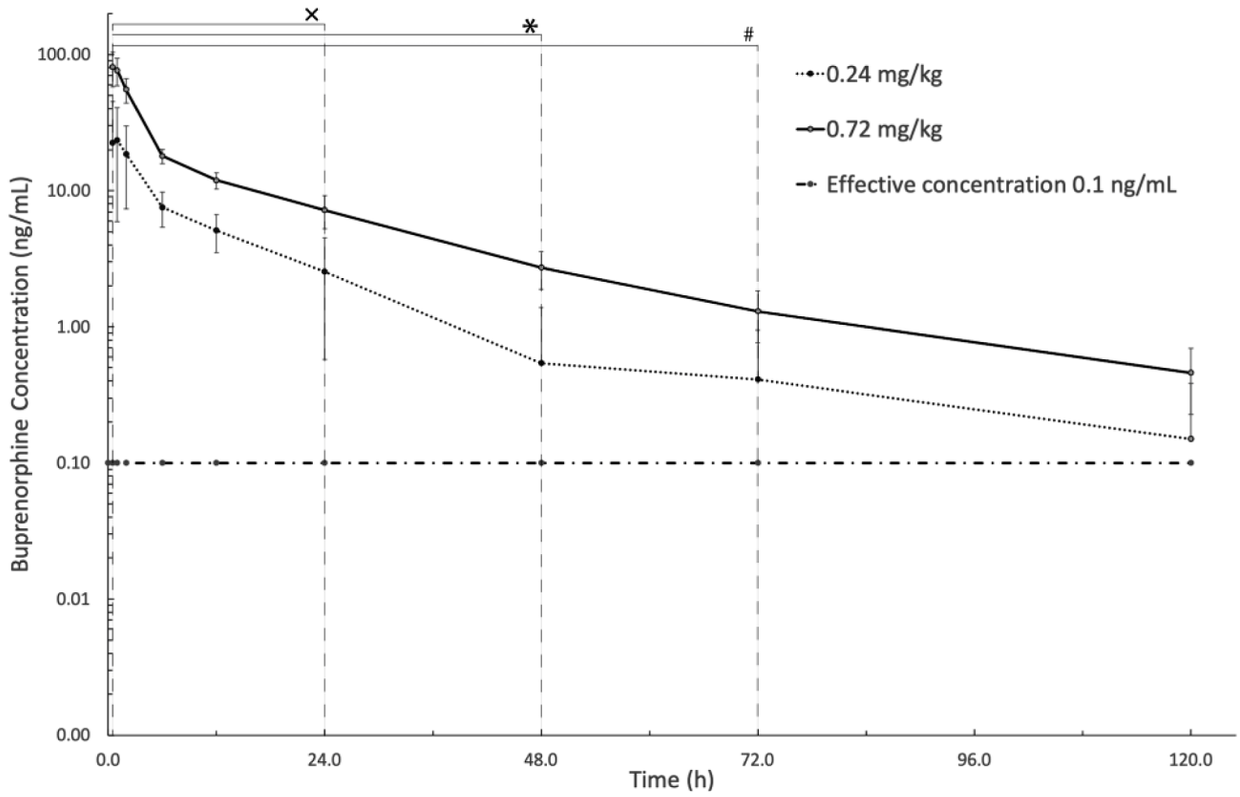


**Figure 9.** Semilog scale graph of mean plasma buprenorphine concentrations (ng/mL) over 120 h (5 d) in rhesus macaques given HCBS at a dose of either 0.24 or 0.72 mg/kg SC as compared with the minimal effective concentration (MEC, 0.1 ng/mL) in rhesus macaques. Error bars represent 1 SEM above and below the data point; *, time points at which buprenorphine concentrations for the 0.24-mg/kg dose were significantly ($P < 0.05$) above the MEC; #, time points at which buprenorphine concentrations for the 0.72-mg/kg dose were significantly ($P < 0.05$) above the MEC; ×, time points at which buprenorphine concentrations differed significantly ($P < 0.05$) between doses.

products;[16] therefore, SRB may be contraindicated in Good Laboratory Practice studies if its safety and efficacy cannot be verified. This situation also creates justifiable concern regarding analgesic efficacy in postoperative patients. In addition, the aforementioned pharmacokinetic study performed on macaques reported injection site reactions lasting approximately 5 to 30 d after dosing in 40% of animals.[32] The reason for these reactions is unknown; however, this effect is problematic and should be taken into consideration to avoid undue complications from the treatment itself. Furthermore, the prolonged duration of SRB can be excessive for some clinical cases and may be difficult to reverse with rescue analgesia, as previously discussed. Finally, strict state and university guidelines prevent a compounded controlled substance from being obtained out of state; therefore, the University of California, Davis is unable to obtain SRB because it is compounded in Colorado.[5,47]

There are several advantages to using HCBS at NHP facilities. To begin, using HCBS decreases drug wastage. The standard dosing regimen of buprenorphine HCl is 0.03 mg/kg IM twice daily for 3 d, with adjustments in duration evaluated on a case-by-case basis.[6] Buprenorphine is a Schedule III controlled substance listed by the Drug Enforcement Administration; therefore, strict requirements must be met to obtain the drug and keep it at the facility.[12] One of these requirements is to maintain detailed records for the storage and use of buprenorphine, which is commercially available in 1-mL glass ampules designed for single use in humans.[34] Given that rhesus macaques are dosed by body weight, the entire contents of a 1-mL ampule are not always used, unlike in humans. For example, the standard dose for a 5-kg macaque dosed at 0.03 mg/kg IM is 0.5 mL; the remaining contents (0.5 mL) must be measured for accuracy, recorded, and then discarded if not used within 24 h. Although many animals are treated with buprenorphine daily at our facility, there are many times when the entire contents of a vial are not used 24 h after opening, thus causing wastage. HCBS is available in a 10-mL vial that expires 56 d after the first puncture.[46] There is a significantly better chance of using the entire contents of a vial over 56 d than over 24 h.

Next, using HCBS will decrease the number and duration of human–animal interactions. Dosing will be decreased from 6 intramuscular injections to 1 subcutaneous injection over 3 d with the high dose or 2 subcutaneous injections over 4 d with the low dose. Limiting the amount of human–animal interactions decreases the stress placed on the macaques, because they are being restrained less often for dosing, and it decreases the risk of occupational hazards to employees, who will be giving half to a third fewer treatments.[30] In addition, the route of dosing has the potential to have fewer adverse effects; subcutaneous administration is preferred over intramuscular injection because subcutaneous administration reduces the risk of potential myositis and neuritis.[31] In addition, financial savings increase because there will be little to no wastage of drug based on drug stability after vial puncture. Labor and supply costs will be reduced due to the overall decrease in the frequency of buprenorphine treatment preparation and administration. Lastly, HCBS is FDA-approved, making it much easier to obtain from out of state, to use in Good Laboratory Practice studies, and to

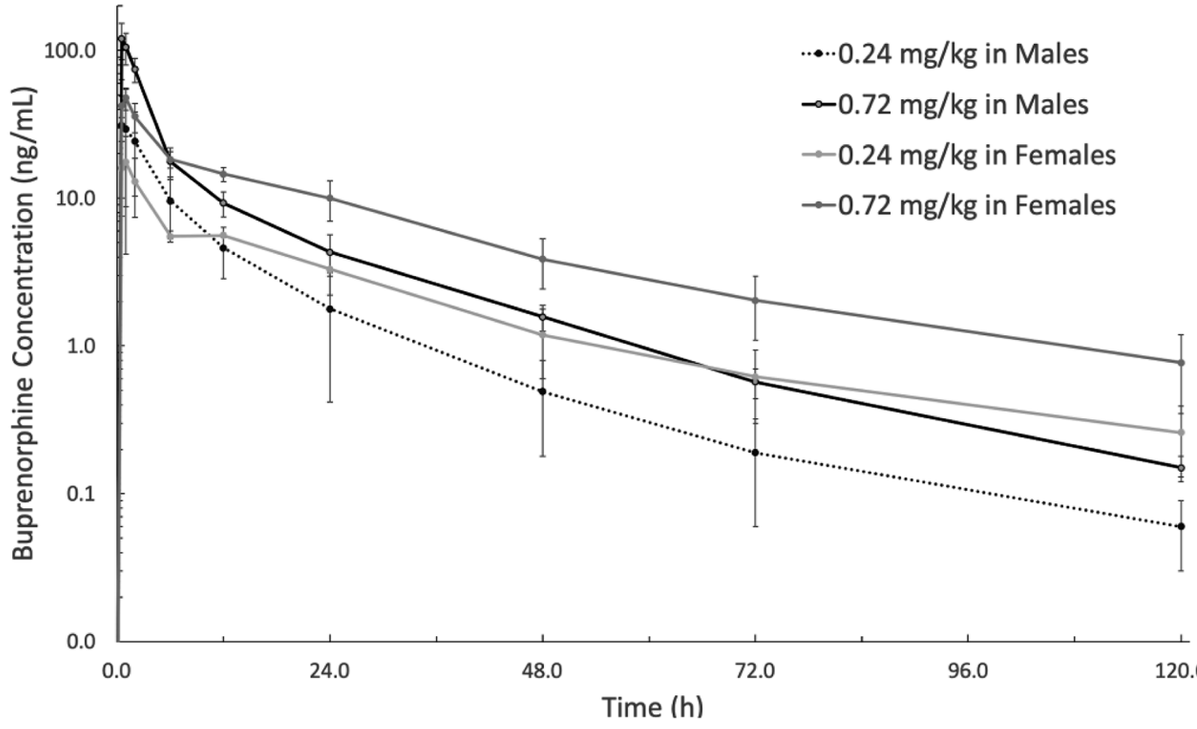


**Figure 10.** Semilog scale graph of mean plasma buprenorphine concentrations (ng/mL) according to sex (3 males, 3 females) over 120 h (5 d) in rhesus macaques administered HCBS at a dose of either 0.24 or 0.72 mg/kg SC. Error bars represent 1 SE above and below the data point.

ensure safety and efficacy from batch to batch. Furthermore, HCBS is labeled as a veterinary drug,[46] making it less likely to be affected by the human opioid epidemic shortage; opioids that have been affected thus far are specifically labeled for human use, even though they are used in veterinary medicine also.

In the current study, we evaluated the pharmacokinetic profiles of 2 doses of HCBS in rhesus macaques after a single subcutaneous administration. The manufacturer attributes the extended duration of this formulation to its novel combination of concentration, dose, and route of administration.[46] HCBS is approximately 6 times more concentrated than buprenorphine HCl and does not contain a liquid polymer matrix, as does SRB, thus avoiding potential injection site reactions.[46,47] Although previous pharmacokinetic studies have assessed buprenorphine HCl and SRB in rhesus macaques,[24,32] the current study is the first (to our knowledge) to investigate the use of HCBS in rhesus macaques. Plasma buprenorphine concentrations remained above the extrapolated minimal therapeutic concentration (>0.1 ng/mL) for 48 h when macaques were dosed with 0.24 mg/kg SC and for 72 h when dosed with 0.72 mg/kg SC. Adverse effects were minimal and consistent with common mild adverse effects occasionally observed with systemic opioid administration.[13,33] There were significant sex-associated differences in HCBS concentration levels during the first 2 h after dosing; however, the concentrations still exceeded 0.1 ng/mL and therefore will not change dosing recommendations. In conclusion, there are many benefits to the adoption and administration of HCBS to rhesus macaques at 0.24 mg/kg SC for 48 h or at 0.72 mg/kg SC for 72 h, given that this formulation represents a safe, effective, and available clinical alternative as well as animal welfare refinement.

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