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Pharmacokinetics of Extended-release Buprenorphine in Mongolian Gerbils (*Meriones unguiculatus*)

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Both the *Guide for the Care and Use of Laboratory Animals* and the *Animal Welfare Act and Regulations* require animals in research to receive adequate analgesia unless an exception can be scientifically justified and IACUC approved. Extended-release buprenorphine (BUP-XR) is a pharmaceutical-grade formulation that is FDA-indexed for use in mice and rats. However, this new formulation has not been evaluated in adult Mongolian gerbils (*Meriones unguiculatus*). Our goal was to determine whether the extrapolated dose (1 mg/kg SC) would achieve plasma buprenorphine concentrations above the murine therapeutic threshold (> 1.0 ng/mL) in male and female gerbils. We hypothesized that BUP-XR administered at 1 mg/kg would achieve the murine therapeutic threshold in both male and female gerbils until at least 48 h after injection. Gerbils received one injection of BUP-XR (1 mg/kg SC) and underwent 4 serial blood collections (0.5, 1, 2, and 4, or 0.5, 24, 48, and 72 h after injection). The average plasma buprenorphine concentrations were above 1 ng/mL within 30 min of administration for both males and females. Plasma buprenorphine concentrations remained above 1.0 ng/mL for 48 h after administration. In males, plasma buprenorphine concentrations were significantly higher at 1 h after injection as compared with females; no other significant differences were observed between sexes. Mild to moderate injection-site granulomas were observed in five of nine gerbils, presumably due to the lipid matrix of the BUP-XR formulation. Our findings demonstrate that a single BUP-XR dose (1 mg/kg SC) achieves plasma buprenorphine levels that remain above the murine therapeutic threshold of 1.0 ng/mL for up to 48 h in both sexes.

Abbreviations: AUC, area under the curve; BUP-XR, extended-release buprenorphine; PK, pharmacokinetic

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Introduction

Reducing and eliminating pain in research animals by providing analgesia is essential for both ethical and scientific reasons.[6,7,29,31,41] Buprenorphine is an opioid that is commonly used to provide analgesia for research animals.[22,28,34,45] The appropriate dosing frequency of the immediate-release formulation of buprenorphine in mice and rats is every 4 to 6 h based on recent literature.[12,22] Because the immediate-release formulation requires frequent repeated administration, providing adequate analgesia for long periods of time can be difficult. The injectable suspension Ethiqa XR (BUP-XR) is an extended-release formulation of buprenorphine that is now being used for analgesia in research animals. This formulation is pharmaceutical grade and FDA-indexed for use in both mice and rats to provide up to 72 h of analgesia with a single subcutaneous injection.[3,14,20] Recent literature has examined its utility in multiple species and models and has a demonstrated duration of analgesia efficacy ranging from 48 to 72 h.[34,39,43] Long-acting buprenorphine formulations, such as BUP-XR, have the advantage of providing analgesia while limiting stress due to frequent handling and repeated injections.

Gerbils provide important models for human health research on various physiologic and pathologic conditions. Gerbils are commonly used in research related to cardiovascular disease,[1,2,33] neurobiology,[11,13,25,44] immunology[46] and infectious diseases.[4,9,38] Surgical models have also been used for studies assessing learning and behavior.[11] However, providing gerbils with adequate pain management poses a challenge because specific analgesic doses are often based on data from mice. Traditionally, doses for novel species are extrapolated from both veterinary and human literature. While this method may be appropriate for some classes of drugs, it does not account for differences in physiology and behavior of some species.[24,35,36,42] Furthermore, because the proper administration of analgesics is a regulatory requirement[6,7,29] it is imperative that we collect empirical data to determine appropriate doses. Previous literature has demonstrated sex differences in response to analgesics,[32,41] including pharmacokinetic studies with BUP-XR,[34,43] indicating a need to address sex differences in analgesic dosing studies in gerbils.

The current literature examining the pharmacokinetic (PK) parameters of extended-release buprenorphine has not measured plasma buprenorphine levels any earlier than 2 h after injection. However, knowing how long BUP-XR requires to reach the therapeutic threshold is essential to its proper use. Published data indicate that BUP-XR exceeds therapeutic threshold for 72 h in mice and rats,[34] but previous studies have not determined how long it takes the drug to reach therapeutic threshold. This information is crucial to providing adequate analgesia throughout the course of a painful event. The first goal of the current study was to determine whether gerbils that received a therapeutic dose of BUP-XR extrapolated from mouse

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and rat doses would achieve the therapeutic concentration (1.0 ng/mL) that has been associated with analgesic efficacy in mice and rats.[8,16,17,22,26,32,39,47] The second goal was to determine the time needed after dosing to reach therapeutic threshold and the duration that the concentration remained above the therapeutic threshold in gerbil plasma. The third goal was to compare the pharmacokinetics of the drug between male and female gerbils. We hypothesized that BUP-XR would achieve quantifiable plasma buprenorphine concentrations that exceed therapeutic threshold (1.0 ng/mL) for at least 48 h in both the male and female gerbils, thus supporting the use of this new formulation for pain management in this species.

Materials and Methods

Animals and husbandry. All animal procedures were performed in an AAALAC-accredited facility and approved by the Vanderbilt IACUC. Male (n = 10) and female (n = 10) adult Mongolian gerbils (*Meriones unguiculatus*) were purchased from Charles River Laboratories (Kingston, NY) for use in this study. Sample size was determined based on consultation with a statistician who recommended the use of descriptive statistics and a comparable number of animals to those used in similar buprenorphine studies of multiple species in the recent literature. The vendor tests the colony (serology, microbiology, and parasitology) quarterly and confirms gerbils to be free of lymphocytic choriomeningitis virus, *Clostridium piliforme* and endo- and ectoparasites.

At the onset of the study, male gerbils weighed 77 to 84 g (mean ± SD, 81 ± 2 g) and females weighed 67 to 79 g (72 ± 3 g). Prior to the experiment, all gerbils had been group-housed in individually ventilated cages (Allentown, LLC; 10.5 × 15 × 7 in.) on Alpha-Dri Plus bedding (Shepherd Specialty Papers, Watertown, TN). Each cage contained a stainless-steel hut as the enrichment item consistent with facility standard operating procedures. Gerbils were housed in an environmentally controlled room, maintained at temperatures from 68 to 79 °F and relative humidity between 30 and 70%. Gerbils had free access to a commercial rodent diet (Laboratory Rodent Diet; LabDiet 5001, St. Louis, MO) and autoclaved reverse osmosis water in plastic sipper bottles. The room was maintained on a 12:12-h light:dark cycle (0600:1800). All gerbils were acclimated to environmental conditions for a minimum of 1 wk prior to the study.

Method of dose selection. BUP-XR is labeled for use in both mice and rats. The label dose is 0.05 mL per 20 g for mice (3.25 mg/kg),[20] and 0.1 mL per 200 g for rats (0.65 mg/kg).[20] The average weight of gerbils used in our study (males, 81 ± 2 g; females, 72 ± 3 g) falls between the mouse and rat averages. We used extrapolation from mouse and rat doses to determine test dose for gerbils in our study. We extrapolated the BUP-XR gerbil dose to be 1 to 2 mg/kg based on a gerbil dose of 0.1 to 0.2 mg/kg Bup HCl.[21] Although BUP-XR is safe at up to 5 times the indicated dose in mice and 10 times the indicated dose in rats,[20,26] we used the low dose (1 mg/kg; 0.06 mL per 80 g gerbil) in our study.

Experimental design. Ethiqa XR (1.3 mg/mL, 3-mL vial) is a proprietary pharmaceutical-grade extended-release buprenorphine formulation (Fidelis Pharmaceuticals, North Brunswick, NJ). All gerbils were weighed on the day of BUP-XR injection to calculate the volume to be administered to obtain a dose of 1 mg/kg. The same BUP-XR production lot was used for all gerbils. BUP-XR was stored at room temperature and briefly mixed by repeated vial inversion prior to subcutaneous administration in the interscapular region. The study was conducted in 2 groups. Blood samples were collected from Group 1 (n = 10, 5 M and 5 F) at 4 early time points (0.5, 1, 2, and 4 h after injection) to assess the time of onset of action of the drug. Based on the earliest time to reach therapeutic threshold in Group 1, blood samples from Group 2 (n = 10, 5 M and 5 F) were collected at 4 time points (0.5, 24, 48, and 72 h after injection) to assess duration of action of the drug. Animal health and appearance were monitored daily throughout experiment for signs of buprenorphine-associated adverse effects reported in other species, including pica, injection-site inflammation, over grooming and hyporexia.[34] At day 14 after injection, CO_2 asphyxiation euthanasia was performed followed by a full necropsy of all gerbils. Both the thoracic and abdominal cavities were assessed, and any tissue with gross lesions was collected, along with the injection-site skin tissue and preserved in 10% neutral buffered formalin for histopathologic evaluation. Tissues were processed routinely, embedded in paraffin, sectioned at 5 µm, stained with hematoxylin and eosin (HE), and evaluated microscopically for granulomatous inflammation and/or other cellular changes by a board-certified veterinary pathologist who was blind to the treatment group.

Blood collection. A 100-µL blood sample was obtained from the medial saphenous vein of each gerbil at 4 time points after BUP-XR injection (0.5, 1, 2, and 4; or 0.5, 24, 48, and 72 h). A 5.5-mm lancet was used to puncture the vein, and blood was then collected into a heparinized capillary tube attached to an EDTA tube. The blood tube was inverted several times and then placed on wet ice until it was centrifuged for plasma collection. Blood samples were centrifuged at 2,500 × g for 5 min. Plasma was then pipetted into individual microcentrifuge tubes and stored in -80 °C until shipment for measurement of buprenorphine concentration (ng/mL).

Pharmacokinetic (PK) analysis. Samples were submitted to and analyzed by the McWhorter School of Pharmacy at Samford University. Samples were assayed using reverse phase liquid chromatography-tandem mass spectrometry (Shimadzu LC-20 HPLC) with a triple quadrupole mass spectrometer (Applied Biosystems 5500 TRAP). Analyte quantifications were obtained using matrix-matched calibration standards with internal standardization along with QC samples in triplicate. Gerbil plasma that was free of any buprenorphine drug formulations was required for the QC samples. Blood was acquired by cardiocentesis from naïve Mongolian gerbils in a separate experiment after CO_2 asphyxiation euthanasia and was used as control. If experimental plasma sample volume was less than 50 µL (13 of 73 total samples), control gerbil plasma was added, and the plasma sample concentration (ng/mL) was corrected for dilution. If the quantified buprenorphine concentration was below the quantitation limit (< 0.1 ng/mL), a concentration was not reported.

Statistical analysis. PK parameters were calculated using PK Solver 2.0 with a noncompartmental analysis linear trapezoidal method. Average PK parameters included in the analysis were half-life, time to maximum concentration, peak concentration, and the area under the curve (AUC) for male and female gerbils. For statistical analysis, unpaired t tests were used to compare buprenorphine concentrations between males and females at the 0.5-, 1-, 2-, 4-, 24-, 48-, and 72-h sample time points (mean ± SEM). Significance was defined as a P value less than 0.05. All statistical analyses were completed by using Prism version 9.5.1 for Windows.

Results

Pharmacokinetics. The early time points tested in Group 1 (0.5, 1, 2, and 4 h) were assessed using 10 gerbils (5 M and 5 F) total. At 0.5 h, two female gerbil samples were excluded due

to a sampling error, leaving three of five female samples. At 4 h, four of five male gerbil samples were analyzed due to the death of one male prior to the final blood collection time point (the cause of death could not be determined at necropsy). The Group 2 time points (0.5, 24, 48, and 72 h) were assessed using nine gerbils (4 M and 5 F) total, as one male was excluded from experiment due to illness observed during the acclimation period. Two male gerbils had plasma buprenorphine concentrations below 0.1 ng/mL, which is below the quantitation limit (one at 0.5 h and one at 72 h). One female gerbil died prior to the 48-h collection, resulting in 48- and 72-h averages that included only the four remaining females. All gerbils, including those that died prior to study end point, were evaluated by full necropsy.

The average plasma buprenorphine concentrations for both male and female gerbils were greater than 1.0 ng/mL within 0.5 h after administration, and remained above this threshold for 48 h (Figure 1). Individual gerbil plasma buprenorphine concentrations are shown in Figure 2. The average plasma buprenorphine concentration was significantly ($P < 0.05$) higher in males than females at the 1-h time point (Figures 1 and 2). No significant differences were detected between male and female gerbils at all other time points (Figure 2).

PK parameters for BUP-XR-treated male and female gerbils are shown in Table 1. Buprenorphine concentrations peaked in both males and females at 4 h after drug administration, although the peak plasma concentration in males (14.2 ng/mL) was nearly twice as high in females (7.8 ng/mL, $P = 0.12$). Half-life and the area under the curve ($AUC_{0\text{-last}}$) were similar between the male (half-life, 19.8 h; $AUC_{0\text{-last}}$, 288.3 hxng/mL) and female (half-life, 17.0 h; $AUC_{0\text{-last}}$, 278.2 hxng/mL) gerbils.

Some gerbils exhibited fighting and aggression toward handlers and other gerbils, but no other adverse effects were observed.

Injection site histopathology. Nine gerbils (4 M and 5 F) were evaluated at necropsy for macroscopic lesions on day 14 after BUP-XR administration. Overall, no gross lesions were seen in either the thoracic or abdominal cavities of any gerbils. However, although all injection sites appeared normal on gross examination, five of the nine gerbils had microscopic pathology at the injection site. The histopathology was consistent with granulomas in which an empty space, presumed to be lipid material from the drug formulation,[34] was surrounded primarily by macrophages and multinucleated giant cells with fewer lymphocytes, rare plasma cells and multiple cholesterol clefts in the subcutaneous space (Figure 3).

Discussion

This study measured plasma buprenorphine concentrations for 72 h after administration of BUP-XR (1 mg/kg SC) to both male and female gerbils. Within 30 min after administration, the average plasma buprenorphine concentrations were greater than 1.0 ng/mL in both male and female gerbils. PK parameters (half-life, time to peak concentration, peak concentration, and AUC) were all similar in the male and female gerbils, and both sexes maintained plasma buprenorphine concentrations above the therapeutic threshold (1.0 ng/mL) for 48 h after administration. The results support our hypothesis that after administering

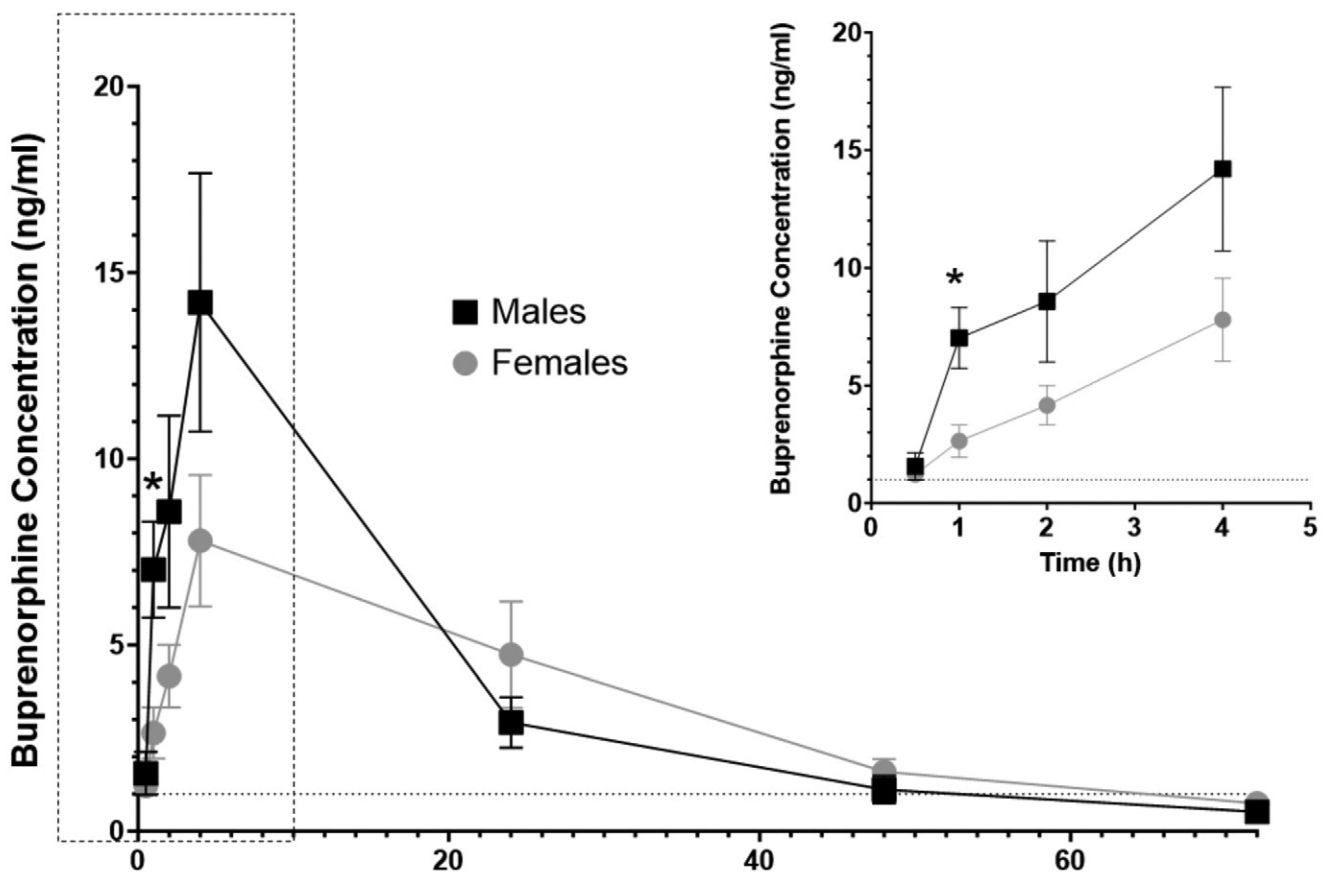

Figure 1. The average plasma buprenorphine concentrations (ng/mL) over 72 h after BUP-XR administration (1 mg/kg SC) in male and female gerbils. Data are presented as mean ± SEM. Boxed time points are displayed in insert, showing males had a significantly higher plasma buprenorphine concentration compared to females at 1 h after BUP-XR injection (*$P \leq 0.05$).

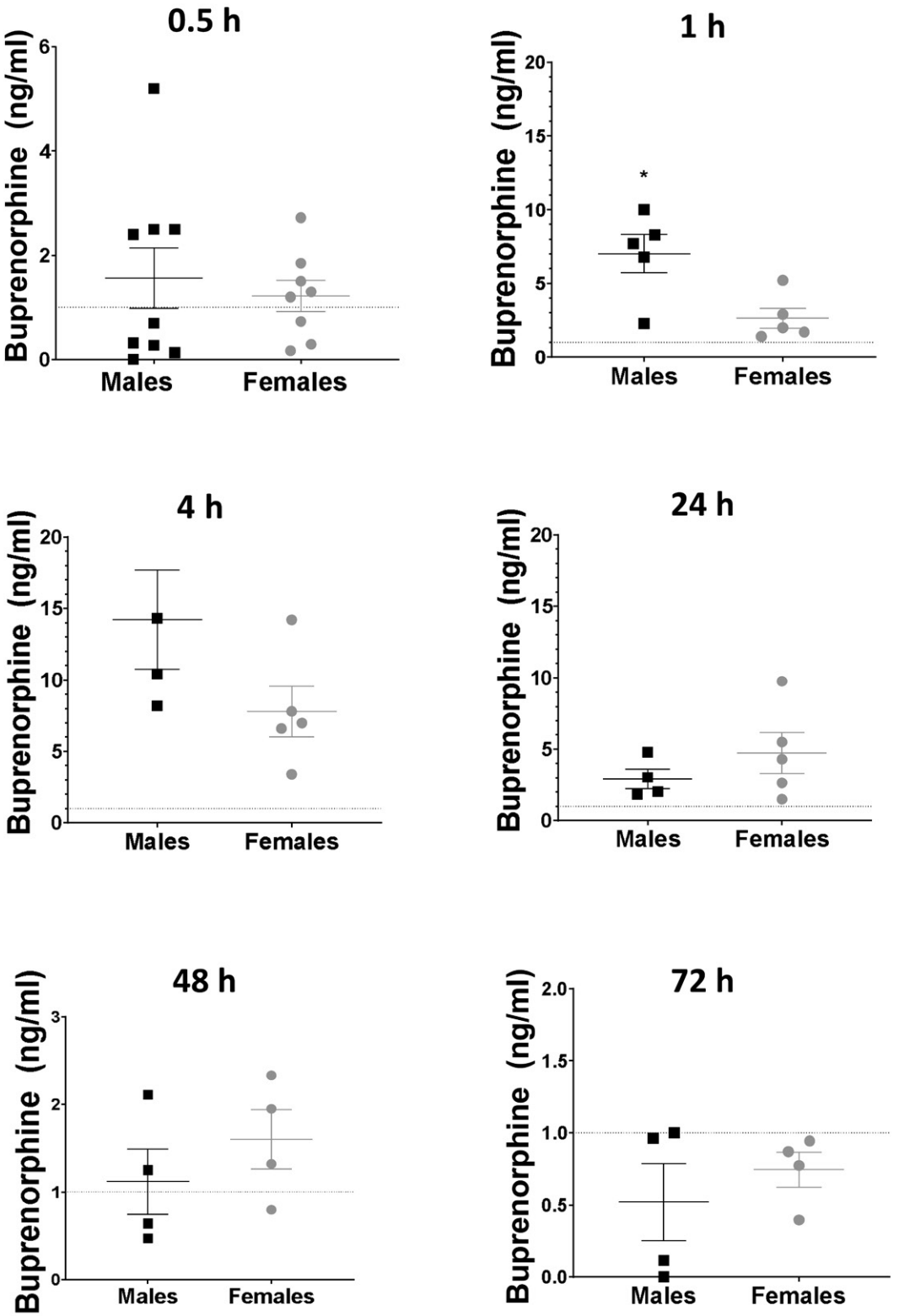

Figure 2. Individual gerbil plasma buprenorphine concentrations (ng/mL) at time points 0.5, 1, 4, 24, 48, and 72 h. 0.5-h time point includes groups 1 and 2 data. Time points 1 and 4 h includes group 1 data. Time points 24, 48 and 72 h include group 2 data. Data are presented as mean ± SEM. The dashed horizontal line shows 1.0 ng/mL. Males had a significantly higher plasma buprenorphine concentration compared to females at 1 h after injection (*$P \leq 0.05$).

BUP-XR at the extrapolated low dose (1 mg/kg SC), plasma buprenorphine levels will remain above the murine therapeutic level (1 ng/mL) for up to 48 h in both males and females.

The main aim of this study was to quantify plasma buprenorphine concentrations from 0.5 h to 72 h in both male and female gerbils. A plasma buprenorphine concentration of 1.0 ng/mL is

Table 1. Summary of average pharmacokinetic values after the subcutaneous injection of BUP-XR (1 mg/kg) in male (n = 9) and female (n = 10) gerbils

PK parameter	Males	Females
$T_{1/2}$ (h)	19.8	17.0
T_{max} (h)	4	4
C_{max} (ng/mL)	14.2	7.8
$AUC_{0\text{-last}}$ (h x ng/mL)	288	278

generally considered therapeutic in mice and rats,[8,16,17,22,26,32,39] thus, we used this value as our threshold when evaluating the PK in gerbils. However, the relationship between plasma buprenorphine concentrations and analgesic effects has not been well established for most species,[5,12,15,18,19,23,28,32,40,43] including gerbils. In fact, a recent publication reported that pain control after surgery in mice can be achieved with plasma buprenorphine concentrations as low as 0.5 ng/mL.[30] Future studies could examine whether a 1.0 ng/mL threshold is appropriate for gerbils or whether adequate analgesia can be achieved at a lower threshold.

Clinically, it is important to know how soon after administration a drug provides adequate analgesia to the animal. Our study demonstrated that the average plasma buprenorphine concentration for both male and female gerbils was greater than 1.0 ng/mL within 0.5 h after administration (Figure 1). This finding indicates rapid transfer of the drug into the bloodstream after subcutaneous administration. Therefore, when using BUP-XR as the exclusive method of analgesia in gerbils, it should be administered at a time that allows at least 30 min between administration and the occurrence of the painful stimulus.

The results support our hypothesis that both male and female gerbils receiving BUP-XR subcutaneously achieve average plasma buprenorphine concentrations that exceed the murine therapeutic threshold for as long as 48 h. At 24 h, 100% of the gerbils had plasma concentrations above 1.0 ng/mL. The majority of gerbils were still above 1.0 ng/mL at the 48-h time point. Only one gerbil maintained this concentration at 72-h time point. Based on our PK data, BUP-XR provides plasma buprenorphine concentrations above 1.0 ng/mL for most gerbils until 48 h after administration. Thus, using this formulation provides a refinement by requiring only one injection every 2 d, rather than the 3 to 4 injections every day for the immediate-release formulation. However, the variation observed between individual gerbils demonstrates the importance of assessing each animal to ensure they are receiving adequate analgesia.

Sex differences have been reported for a variety of physiologic systems, specifically with regard to sex differences in response to analgesia, and to BUP-XR in particular.[8,16,26,32,39] Our study did not identify any consistently significant differences between male and female gerbils. Both male and female gerbils reached their peak concentrations at the 4-h time point in our study. A recent publication reported that mice reached the peak concentration as soon as 2 h after injection.[14] In our study, the earliest time point we assessed was 30 min after injection. At the 30-min time point, the plasma buprenorphine concentrations were already above 1.0 ng/mL, and concentrations continued to increase until the 4-h time point. The AUC, which represents the overall systemic distribution of a drug after administration, was not significantly different between sexes. The similarity of the AUC in males and females indicates that both sexes metabolized the drug in a similar fashion.

We observed behavior changes in both male and female gerbils. Animals were initially group-housed by sex during both the acclimation period and the experiment. However, after receiving the BUP-XR injections, hyperactivity and aggression between gerbils and toward handlers were seen in both sexes. Therefore, all gerbils were individually housed for the remainder of the study. Hyperactivity has previously been reported after opioid administration in mice.[10,27,37,39,43] Whether the hyperactivity in this study is a direct result of BUP-XR in gerbils or is related to normal gerbil aggression secondary to hierarchy disturbance remains to be determined. A previous publication describes the use of calorimetry to assess differences in activity levels in mice treated with either sustained-release and extended-release buprenorphine before and after surgery.[43] Because our study focused on the PK analysis after BUP-XR administration, we did not assess the pharmacodynamic aspects of this drug, including behavior testing, and acknowledge this as a limitation to our study. However, neither a normal behavior ethogram

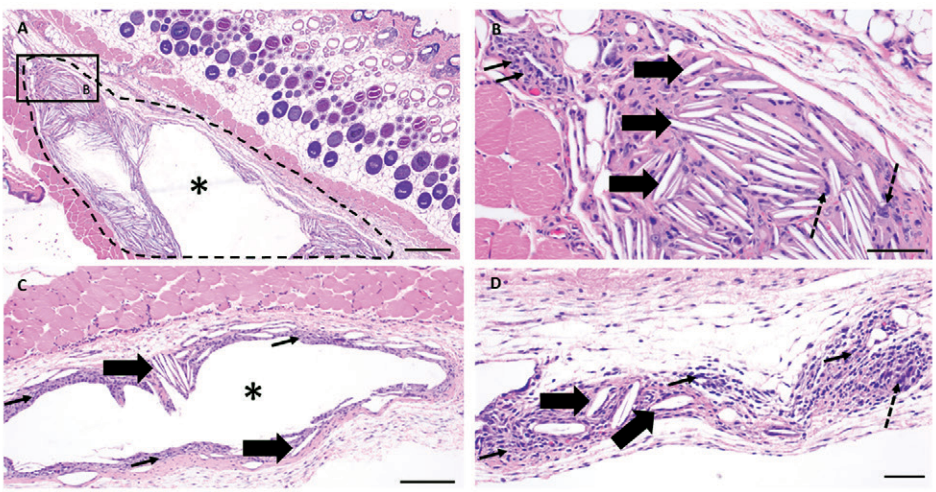

Figure 3. Representative photomicrographs of hematoxylin and eosin (HE) stained skin slides at the injection sites of gerbils on day 14 after administration of BUP-XR. (A) Low magnification and (B) higher magnification images of the injection site showing a subcutaneous accumulation of primarily macrophages (thin black arrows) with fewer multinucleated giant cells (thin dash arrows) surrounding empty space (*) and multiple cholesterol clefts (thick black arrows). (A) scale bar, 200 µm; (B) scale bar, 50 µm. (C) Sample from a different gerbil with similar histopathologic findings to A, B. Scale bar, 100 µm. (D) Sample from a third gerbil showing more abundant inflammatory cells and fewer cholesterol clefts. Scale bar, 50 µm.

nor a pain grimace scale has been established to allow reliable assessment of analgesic efficacy in gerbils. Future studies are necessary to further assess the relationship between hyperactivity and plasma buprenorphine concentrations in gerbils and in other rodents.

In this study we saw more variability in plasma buprenorphine levels at later time points (24, 48, and 72 h) after dosing at 1 mg/kg. Future studies could assess plasma buprenorphine levels in gerbils after administering the 2 mg/kg dose. However, higher doses of BUP-XR have been associated with subcutaneous hemorrhage and necrosis at the injection site in mice.[43] Another limitation of our study was the inability to sample the same gerbil at every time point due to animal size and maximum blood volume we could safely obtain. The study design was based on the 3Rs (reduction, refinement, replacement). To limit animal use, we performed multiple blood collections on each gerbil, rather than perform one terminal blood collection in which each gerbil would provide data for only one time point.

The management and alleviation of pain in research animals is an ethical imperative. Immediate-release buprenorphine formulations can be effective when used correctly; however, repeated injections are necessary, which can be difficult to provide in a research setting. The need for more frequent administration can cause stress due to handling, require more personnel time, and missed treatments, which increase variability and costs and can lead to animal welfare concerns and regulatory noncompliance. The results of the current study demonstrate that BUP-XR is a potential remedy for these issues in gerbils. Our results indicate that BUP-XR rapidly reaches plasma buprenorphine levels of 1.0 ng/ml within 30 min and remains above this level for up to 48 h in both male and female gerbils after a single 1 mg/kg subcutaneous injection. However, achieving the therapeutic threshold should not be the only parameter used when evaluating the utility of a drug in a new species. Future studies are needed to assess pharmacodynamic parameters and analgesic efficacy and to evaluate the relationship between plasma buprenorphine concentrations and aggression and hyperactivity.

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