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Pharmacokinetics and Efficacy of a Long-lasting, Highly Concentrated Buprenorphine Solution in Mice

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Buprenorphine is a commonly used opioid for mitigating pain in laboratory mice after surgical procedures; however, the dosing interval necessary for standard buprenorphine may require treatment every 4 to 6 h to maintain an adequate plane of analgesia. An alternative formulation that provides prolonged plasma concentration with long-lasting effects would be beneficial in achieving steady-state analgesia. We evaluated a long-lasting and highly concentrated formulation of buprenorphine (Bup-LHC) in mice. Pharmacokinetic analysis was performed to assess plasma concentrations in male C57BL/6J (B6) and female CD1 mice after subcutaneous injection of 0.9 mg/kg. The Bup-LHC formulation provided plasma drug levels that exceeded the therapeutic level for at least 12 h in male B6 mice and was below therapeutic levels by 8 h in female CD1 mice. An experimental laparotomy model was used to assess analgesic efficacy. Female CD1 mice were treated with either Bup-LHC (0.9 mg/kg) or saline at 1 h before undergoing an ovariectomy via a ventral laparotomy. At 3, 6, 12, 24, and 48 h after surgery, pain was assessed based on the following behaviors: orbital tightness, grooming, wound licking, rearing, arched posture, ataxia, piloerection, nest building, and general activity. At 3 and 6 h after surgery, Bup-LHC–treated mice had significantly less wound licking and orbital tightness and considerably higher activity levels than did saline-treated mice. At 12 h, wound licking, orbital tightness and activity in Bup-LHC–treated mice were no longer significantly different from those of saline-treated mice. The results of this study suggest that Bup-LHC at 0.9 mg/kg provides sufficient plasma concentrations for analgesia in mice for 6 to 12 h after administration, as demonstrated behaviorally for at least 6 h after surgery.

Abbreviations: Bup-HCl, buprenorphine hydrochloride; Bup-LHC, long-lasting, highly concentrated buprenorphine

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The management of acute pain in animals is highly important in laboratory animal medicine. Because animals are sentient creatures that feel pain, together with the established link between animal welfare and quality science,[31] we are obligated to minimize pain and distress whenever possible. Mice are commonly used in biomedical research; however, pain recognition and assessment in mice is highly subjective and observer-dependent, relying on measurements of normal behaviors such as activity, grooming, rearing, facial grimace, and nesting activity.[15,18,20,30,32] Thus, assuring effective pain relief with minimal adverse effects in rodent pain models continues to be an important goal in laboratory animal medicine and research.[29]

Buprenorphine is a partial μ-agonist that is commonly used to reduce pain in mice in a variety of models.[4,12-14,18] The standard formulation of this drug is buprenorphine–HCl (Bup-HCl, 0.3 mg/mL), which is typically administered every 8 to 12 h.[8,9] This frequency requires additional handling and, according to previous efficacy studies[9] and pharmacokinetic analyses,[17] Bup-HCl may not maintain therapeutic concentrations during the entire dosing interval and may actually be as short as 4 h in some strains. The use of compounded sustained-release buprenorphine (ZooPharm, Laramie, WY) has increased in recent years and provides continuous analgesia for as long as 48 h after a single injection.[3,6,13,17] Although this drug is convenient, obtaining compounded controlled substances is becoming increasingly challenging with the emergence of state laws directed at opioid addiction. Bup-LHC (Simbadol, 1.8 mg/mL) is an FDA-approved veterinary drug labeled for 24-h analgesia in cats[7,42] and has demonstrated efficacy in other species as well.[23,40] Bup-LHC may be an option that is effective and avoids emerging issues with longer-acting compounded formulations yet still allows reduced handling in the postoperative period.

In this study, we determined the pharmacokinetics of Bup-LHC at 0.9 mg/kg in male C57BL/6J and female CD1 mice and the clinical analgesic efficacy in an experimental laparotomy model in female CD1 mice. We found that Bup-LHC results in sustained plasma concentrations that exceed the purported therapeutic value of 1 ng/mL[11] for as long as 12 h in male B6 mice but for less than 8 h in female CD1 mice. Analgesia was demonstrated during the first 6 h after surgery, but a clinically discernable difference in analgesic response was not observed after the 12-h time point. These results suggest with appropriate dosing intervals, Bup-LHC is a suitable alternative to Bup-HCl and sustained-release buprenorphine for abdominal surgical procedures.

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Materials and Methods

Mice. Male C57BL/6J (B6; weight, 20 to 26 g; age, 6 to 8 wk) were obtained from the Jackson Laboratory (Bar Harbor, ME),

and female Crl:CD1(ICR) (weight, 24 to 40 g; age, 6 to 8 wk) were obtained from Charles River Laboratories (Wilmington, MA). Mice were free of Sendai virus, mouse hepatitis virus, minute mouse virus, mouse parvovirus, mouse norovirus, Theiler murine encephalitis virus, rotavirus, *Mycoplasma pulmonis*, pinworms, and ectoparasites. For pharmacokinetic studies, male B6 and female CD1 mice were housed 3 per cage in IVC. B6 mice were housed in Super Mouse 750 cages (15.9 cm × 30.5 cm × 14 cm; Lab Products Seaford, DE), and CD1 mice were housed in Thoren no. 9 cages (19.5 cm × 30.9 cm × 13.3 cm; Thorne Caging Systems, Hazleton, PA). Mice had ad libitum access to Teklad Irradiated Diet 2918 (Envigo, Madison, WI) and filter-sterilized water. Mice were maintained on a 14:10-h light:dark cycle at a temperature of 21 to 24 °C. For the experimental laparotomy study, mice were identified via ear punch, singly housed in Thoren no. 9 cages, and moved to a static housing rack in a dedicated room. All experimental procedures were approved by the IACUC.

Pharmacokinetic study. Male B6 mice (n = 18) and female CD1 mice (n = 18) were used to assess pharmacokinetics. Three mice from each strain that did not receive buprenorphine were euthanized, and blood was collected to provide baseline plasma levels. The remaining 15 mice of each strain were manually restrained and injected subcutaneously in the interscapular region with a single dose of Bup-LHC at 0.9 mg/kg. The dose of administration of Bup-LHC was estimated based on allometric scaling of the labeled cat dose.[28] Three mice from each strain were euthanized by using carbon dioxide at 2, 4, 8, 12, and 24 h after treatment, and blood was collected via cardiocentesis (n = 3 per time point). Blood samples were placed in heparinized microcentrifuge tubes (Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 3200 × g for 15 min. Plasma was collected and stored at –80 °C until analyzed. Plasma buprenorphine concentration was determined by using liquid chromatography–tandem mass spectrometry as previously described.[17] Pharmacokinetic analysis was performed by using Phoenix WinNonlin software (Pharsight, Cary, NC).

Experimental laparotomy model. To determine whether the Bup-LHC formulation provided analgesia after surgery, mice underwent a laparotomy followed by behavioral pain scoring.[18] Female CD1 mice were randomly distributed into 4 groups (10 mice per group). Group 1 received anesthesia and surgery with saline treatment (saline group); group 2 received anesthesia and surgery with Bup-LHC treatment (Bup-LHC group); group 3 received anesthesia only (no surgery) with Bup-LHC treatment; and group 4 received anesthesia only (no surgery or Bup-LHC). Each mouse in surgery groups 1 and 2 was matched to a mouse from groups 3 and 4 (no surgery) that was induced and recovered from anesthesia at approximately the same time as the surgical counterpart. Procedures were performed between 0800 and 1000. Approximately 60 min before surgery, mice were treated subcutaneously with Bup-LHC (Zoetis, Parsippany, NJ) at 0.9 mg/kg or with 0.3 mL (approximate volume of Bup-LHC dose) of 0.9% saline (Baxter Health Care, Deerfield, IL). Mice were induced and anesthetized with isoflurane (Fluriso, VetOne, MWI Veterinary Supply, Boise, ID) and their abdomens shaved and prepared aseptically for surgery by using chlorhexidine surgical scrub. A 2.0-cm cutaneous incision was made along the abdominal midline, followed by a 1.5-cm incision through the abdominal wall. Both ovaries were removed by cauterizing each uterine horn with forceps heated in a microbead surgical sterilizer (Inotech Biosystems International, Rockville, MD). The abdominal wall incision was closed with 5-0 absorbable suture (Ethicon, Johnson and Johnson, New Brunswick, NJ) and the skin closed with surgical staples (Becton Dickinson). After the procedure, mice were returned to their cages and monitored until recovered. After completion of the behavioral assessments, mice were euthanized with CO_2 and necropsied to evaluate the surgical and injection sites.

Behavioral assessments. Baseline assessments were performed 24 h before anesthesia and at the time of surgery (time point 0), and indicators of pain were assessed postoperatively at 3, 6, 12, 24, and 48 h. Observers (BS, ALB) were trained regarding behaviors and were blind to treatments with Bup-LHC, saline, or anesthesia only. At each observation point, both observers scored each mouse in the 4 groups over a 5-min period, and the average score of the observers was recorded. Prior to observations and measurements, mice were placed in an ANY-maze (Stoelting, Wood Dale, IL) video tracking apparatus and given 10 min to acclimate. Episodes of grooming, wound licking, rearing, ataxia, and arched posture were tallied during the 5-min observation period after acclimation. Piloerection was scored as 0 (not present) or 1 (present). Orbital tightness scores were based on a modification of the facial grimace scale, which correlated to the level of pain experienced and was scored on a scale of 0 to 2 (0, no orbital tightening; 1, moderate orbital tightening; 2, severe orbital tightening).[1,20] Nesting activity was determined as previously described by using a modified time-to-integrate test by placing nesting material (Bed-r' Nest, The Andersons, Maumee, OH) on the opposite side of the cage during anesthetic recovery and assessing the amount of material that had been moved to the original nest (0, no material moved; 1, material moved part way to the nest; 2, moved completely to the nest).[18,32]

General activity was assessed by using ANY-maze video tracking software and Avisoft-SASLab Pro Sound analysis software (Avisoft Bioacoustics, Nordbahn, Germany). Briefly, mice are identified at the head, mid-region, and base of the tail by using the software, and the system tracks the whole body and identifies the head position. The software records the distance traveled. Activity bouts were identified by using the Avisoft-SASLab Pro program.[35,36] Ultrasonic recordings were collected for 5 min during the ANY-maze recording. Activity bouts (episodes of increased noise due to mouse movement) were assigned a section label according to a threshold of 4% of full scale and a hold time of 0.05 s, such that sound exceeding 4% of full scale and lasting at least 0.05 s was counted as an activity bout. Overloaded (saturated) events were excluded. Section labels were saved as a text file, and the total number of activity bouts was determined. These behavioral indicators were evaluated to assess pain: higher levels of orbital tightening, wound licking, arched posture, ataxia, piloerection were considered to indicate the presence of pain, as were reductions in grooming, rearing, nest building, and activity.

Statistical analysis. Data analysis was performed by using Prism 8.1.2 for Mac (GraphPad Software, San Diego, CA). Two-way ANOVA with Tukey multiple comparison tests were performed to compare all treatment groups at each time point. Two-way ANOVA with Dunnett multiple-comparison tests were performed to compare the postprocedural activity with the baseline within each group. Due to postoperative complications resulting in the death of 2 mice in group 1 and one mouse in group 2, the final sample size for this study was 37. Behavioral observation data were expressed as the average score or frequency of each specific behavior. Video tracking and sound analysis data were expressed as the average of total distance traveled and activity bouts recorded for a given group, respectively. For all tests, values are expressed as mean ± SD

P value less than 0.10 was considered statistically significant. The scoring between the 2 observers blinded to the treatment groups was assessed for agreement by using the Cohen κ statistic (idostatistics.com, Giacomo Scarpellini).

Results

Pharmacokinetics. Plasma buprenorphine concentrations were determined in male B6 and female CD1 female mice given Bup-LHC at 0.9 mg/kg SC over a 24-h period (n = 3 per time point). The highest plasma concentrations of buprenorphine (mean ± 1 SD) were detected at the 2-h time point (B6, 29.2 ± 8.5 ng/mL; CD1, 6.9 ± 3.5 ng/mL), with a stark decline by 24 h (Figure 1). The last time point at which plasma buprenorphine concentration exceeded 1 ng/mL was 12 h for male B6 mice (1.7 ± 1.3 ng/mL), and the plasma concentration was just below 1 ng/mL in female CD1 mice at the 8-h time point (0.7 ± 0.3 ng/mL).

Experimental laparotomy model and postoperative behavioral assessments. One mouse in the saline-treated group and 2 mice in the Bup-LHC-treated group died of unknown causes immediately after surgery and were not included in the analysis. The behavioral scores of the mice before and for 48 h after the procedures are summarized in Table 1. Interobserver agreement for all behavioral observations was substantial to excellent, with Cohen κ results between 0.77 and 0.94.

Baseline values did not differ between groups. Compared with the saline-treated group, Bup-LHC treatment resulted in fewer behaviors indicative of pain, including less orbital tightening (P = 0.06), and less piloerection (P = 0.08) at 3 h after surgery, with less wound licking at 3 and 6 h after surgery (P = 0.03 and 0.06). Compared with the nonsurgical groups (anesthesia only and combined Bup-LHC treatment and anesthesia only), Bup-LHC-treated mice that underwent surgery had more wound licking at 3 and 24 h after surgery (P = 0.03) and less rearing at 3 h (P = 0.03), indicating pain. Relative to nonsurgical groups, the saline-treatment mice that underwent surgery demonstrated behaviors consistent with pain throughout the 24-h time point. Specifically, saline-treated mice demonstrated more orbital tightening (P = 0.02), more grooming (P < 0.01), more wound licking (P = 0.01), less rearing (P < 0.01), more arching (P < 0.01), and less piloerection (P = 0.08) at 3 h after surgery; more wound licking (P < 0.02) and less rearing (P < 0.01) continued in the saline-treated surgical group at 6, 12, and 24 h after surgery. Neither the frequency of ataxia nor nest building differed among the groups.

Postprocedural activity was measured using ANY-maze software to determine the distance traveled and using sound measurements to determine activity bouts (Figure 2). Mice treated with Bup-LHC at 1 h prior to surgery traveled a significantly greater overall distance at 3 h compared with saline-treated mice treated (P = 0.09) and approached significance at 6 h (P = 0.12). The saline-treated mice that underwent surgery had traveled significantly less distance than did the nonsurgery control groups at 3 and 6 h (P < 0.04). Activity bouts were significantly higher in the Bup-LHC-treated mice at 3 and 6 h after surgery (P < 0.09 and P < 0.004, respectively), as compared with saline-treated mice. Saline-treated mice that underwent surgery had significantly fewer activity bouts than did the nonsurgery control groups at 3 and 6 h (P < 0.04). The distance traveled as measured by the ANY-maze correlated well with the activity bouts determined by using sound recording (Figure 3, r = 0.80). Compared to the baseline activity levels within their groups, the only significant differences were for the operated saline-treated mice at all time points, the Bup-LHC-treated group at 24 h after surgery, and the anesthesia-only group with Bup-LHC treatment at 24 and 48 h after injection.

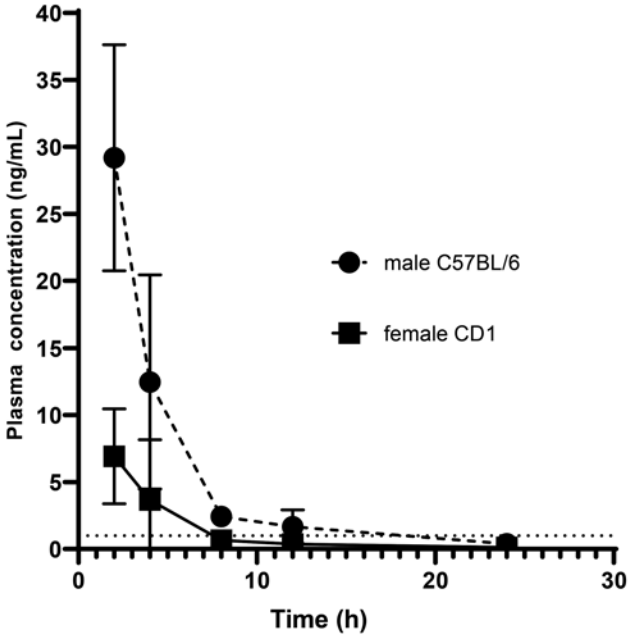

Figure 1. Pharmacokinetics of Bup-LHC in male C57BL/6 and female CD1 mice. The dotted line indicates 1 ng/mL.

Mice were euthanized after behavioral assessments, and gross necropsy was performed. No gross abnormalities were noted at the surgical and injection sites. There was no evidence of gastric dilation or impaction suggestive of pica.

Discussion

Previous studies demonstrated that SR-Bup administered at 0.6 mg/kg SC provided adequate plasma concentrations to support analgesia for at least 48 h in a surgical model of pain.[17,18,35] In the current study, we evaluated Bup-LHC as an alternative to SR-Bup for treating pain in mice. We found that Bup-LHC at 0.9 mg/kg SC resulted in a sufficient plasma concentration to provide analgesia in male B6 for at least 12 h. The plasma concentration in female CD1 mice was below the therapeutic level at the 8-h time point; however, this dose provided postoperative analgesia for at least 6 h in CD1 female mice after ventral midline ovariectomy.

The 4 experimental groups included mice that received Bup-LHC, anesthesia, and surgery and 3 control groups. Two controls had no surgery but received anesthesia alone or anesthesia with Bup-LHC administration. The anesthesia-only group served as a baseline control group to rule out the effects of anesthesia on behavioral responses, whereas mice that received anesthesia with Bup-LHC were included to rule out behavioral side effects of Bup-LHC. Neither the behavioral responses nor activity levels of the mice in either of these 2 groups differed, demonstrating the absence of behavioral side effects associated with Bup-LHC administration. The group that received saline treatment with anesthesia and surgery was used to demonstrate the degree and duration of pain and to validate the model. The saline-treated mice displayed behavioral signs indicative of pain for at least 12 h. These signs were reduced in mice that were treated with Bup-LHC, but this effect did not return the mice to the levels of the unoperated controls. Although our approach to assessing pain and analgesia may be controversial, these 3 control groups are essential for documenting analgesia effects.[21] This

Table 1. Postoperative behavioral scores (mean ± 1 SD) in mice treated with saline or Bup-LHC with anesthesia and surgery, and mice that received anesthesia and Bup-LHC, or anesthesia only.

	Treatment group			
Time (h) after procedure	Anesthesia and surgery with saline treatment (n = 9)	Anesthesia and surgery with Bup-LHC treatment (n = 8)	Anesthesia only with Bup-LHC treatment (n = 10)	Anesthesia only 10
Orbital tightening				
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
3	0.67 ± 0.7 [a,b,c]	0.13 ± 0.35	0 ± 0	0 ± 0
6	0.44 ± 0.73	0.13 ± 0.35	0 ± 0	0 ± 0
12	0.22 ± 0.67	0 ± 0	0 ± 0	0 ± 0
24	0.11 ± 0.33	0 ± 0	0 ± 0	0 ± 0
48	0.22 ± 0.67	0.31 ± 0.6	0 ± 0	0 ± 0
Grooming				
0	1.11 ± 1.43	1.88 ± 2.05	1.35 ± 2.12	0.90 ± 0.97
3	4.67 ± 6.35 [b,c]	0.75 ± 0.75	0.50 ± 0.75	6.20 ± 4.22
6	4.06 ± 5.02	0.81 ± 1.13	0.50 ± 0.67	3.40 ± 3.46
12	5.06 ± 5.09	1.63 ± 1.92	0.80 ± 1.21	3.20 ± 2.97
24	3.56 ± 3.11	1.44 ± 1.70	1.40 ± 2.49	1.45 ± 1.61
48	1.89 ± 2.49	2.38 ± 2.83	3.15 ± 3.01	1.85 ± 1.77
Wound licking				
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
3	4.83 ± 3.57 [a,b,c]	1.06 ± 0.94 [b,c]	0.0 ± 0.0	0.0 ± 0.0
6	6.06 ± 5.42 [a,b,c]	1.00 ± 1.93	0.0 ± 0.0	0.05 ± 0.16
12	4.61 ± 3.57 [b,c]	1.75 ± 2.83	0.0 ± 0.0	0.05 ± 0.16
24	2.28 ± 2.05 [b,c]	1.50 ± 1.75 [b,c]	0.0 ± 0.0	0.0 ± 0.0
48	0.89 ± 1.97	0.75 ± 1.31	0.0 ± 0.0	0.0 ± 0.0
Rearing				
0	23.5 ± 7.0	32.4 ± 18.1	28.8 ± 8.3	29.4 ± 6.4
3	6.5 ± 6.7 [b,c]	11.7 ± 9.8[c]	22.1 ± 9.3	24.5 ± 4.1
6	6.4 ± 6.1 [b,c]	16.2 ± 13.9	26.8 ± 14.1	26.0 ± 6.5
12	9.3 ± 5.8 [b,c]	16.9 ± 9.4	24.0 ± 12.6	23.2 ± 6.1
24	11.9 ± 6.4[c]	13.3 ± 11.9	21.5 ± 10.2	25.3 ± 5.6
48	13.8 ± 9.8	17.3 ± 12.9	24.3 ± 10.9	21.5 ± 2.4
Arched back				
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
3	0.61 ± 0.49 [a,b,c]	0.19 ± 0.37	0 ± 0	0 ± 0
6	0.22 ± 0.51	0.32 ± 0.46 [b,c]	0 ± 0	0 ± 0
12	0.22 ± 0.67	0.06 ± 0.18	0 ± 0	0 ± 0
24	0.22 ± 0.67	0.25 ± 0.46	0 ± 0	0 ± 0
48	0.11 ± 0.33	0.44 ± 0.82	0 ± 0	0 ± 0
Ataxia				
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
3	0.06 ± 0.17	0 ± 0	0 ± 0	0 ± 0
6	0.06 ± 00.17	0 ± 0	0 ± 0	0 ± 0
12	0 ± 0	0 ± 0	0 ± 0	0 ± 0
24	0 ± 0	0 ± 0	0 ± 0	0 ± 0
48	0.11 ± 0.33	0.13 ± 0.35	0 ± 0	0 ± 0
Piloerection				
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
3	0.44 ± 0.57 [a,b,c]	0 ± 0	0 ± 0	0 ± 0
6	0.22 ± 0.44	0 ± 0	0 ± 0	0 ± 0
12	0 ± 0	0 ± 0	0 ± 0	0 ± 0
24	0 ± 0	0 ± 0	0 ± 0	0 ± 0
48	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 1. Continued

Time (h) after procedure	Treatment group: Anesthesia and surgery with saline treatment (n = 9)	Anesthesia and surgery with Bup-LHC treatment (n = 8)	Anesthesia only with Bup-LHC treatment (n = 10)	Anesthesia only 10
Nesting activity				
0	0.39 ± 0.48	0.31 ± 0.53	0.3 ± 0.42	0.35 ± 0.47
3	0.11 ± 0.33	0.50 ± 0.71	0.5 ± 0.67	0.35 ± 0.53
6	0 ± 0	0.44 ± 0.62	0.55 ± 0.83	0.35 ± 0.67
12	0 ± 0	0.06 ± 0.18	0 ± 0	0.15 ± 0.34
24	0 ± 0	0 ± 0	0.05 ± 0.16	0.1 ± 0.32
48	0 ± 0	0.19 ± 0.53	0.05 ± 0.16	0.25 ± 0.42

[a]Value significantly ($P < 0.10$) different from the anesthesia and surgery plus Bup-LHC treatment group
[b]Value significantly ($P < 0.10$) different from the anesthesia-only plus Bup-LHC treatment group
[c]Value significantly ($P < 0.10$) different from the anesthesia-only group

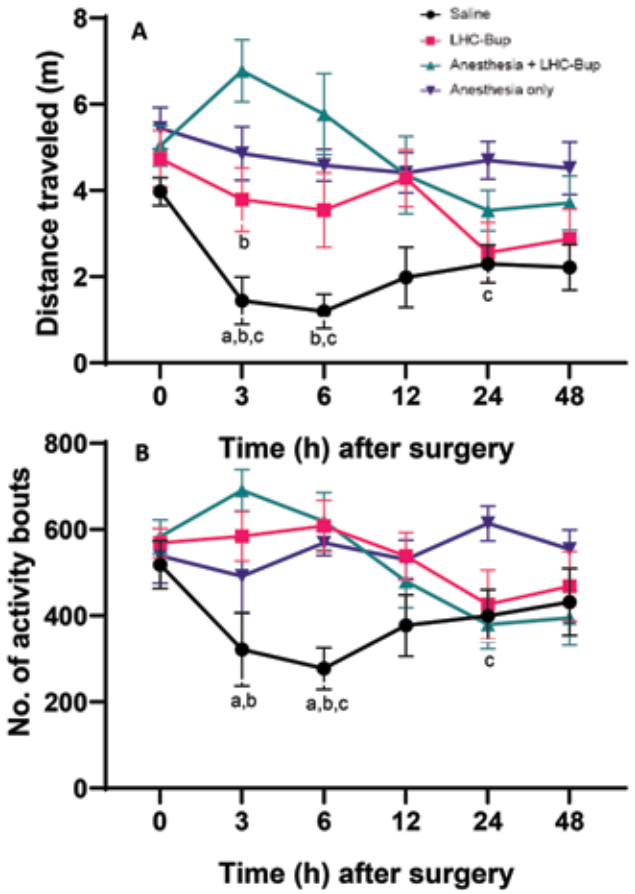

Figure 2. (A) Distance traveled postoperatively by female CD1 mice that underwent ovariohysterectomy. (B) Postoperative activity bouts in female CD1 mice that underwent ovariohysterectomy; a, significant difference ($P < 0.1$) compared with Bup-LHC-treated mice; b, significant ($P < 0.1$) difference compared with anesthetized Bup-LHC-treated mice; c, significant ($P < 0.1$) difference compared with anesthesia-only mice.

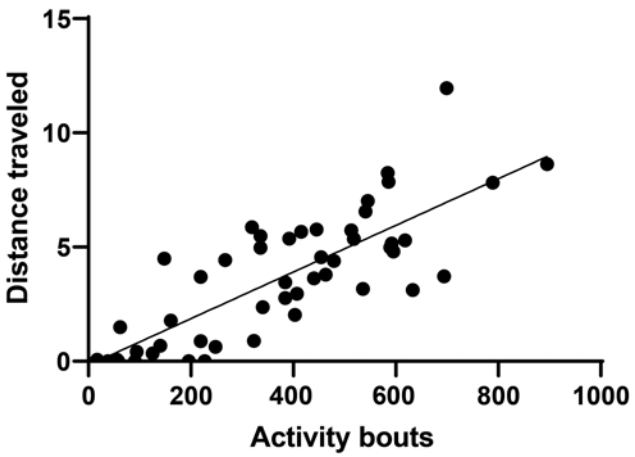

Figure 3. Correlation between distance traveled as determined by using ANY-maze and activity bouts as determined by using sound recordings: $r = 0.8$.

requirement is particularly true given the number of confounding factors that could influence the assessment of pain, including surgeon experience, surgery time, light cycle, bedding, sex of the observer, and duration of observations.[2,26,34,37]

Analgesiometric tests, such as von Frey filaments, the Hargreave test, or the Randall–Selitto test, are useful for assessing analgesic efficacy in response to thermal, chemical, and mechanical pain,[22] but they do not replace surgical models, which are more clinically relevant.[21,24] Therefore, we chose to conduct the study in a laparotomy model.

The cause of death of 3 mice after surgery was not determined. Two of the mice that died were in the Bup-LHC with anesthesia and surgery. Although buprenorphine might have contributed to their deaths, they more likely died due to a surgical complication or an anesthetic event.

Several studies have previously demonstrated the efficacy of Bup-LHC in cats and dogs. Bup-LHC subcutaneously administered to cats was present in plasma levels for as long as 72 h and a prolonged analgesic response to thermal nociception for more than 24 h.[7] In dogs treated postoperatively in combination with carprofen, pain scores did not differ from those of dogs treated with Bup-HCl and carprofen, and fewer dogs required rescue analgesics.[40] More recently, Bup-LHC was evaluated pharmacokinetically in NHPs.[23] The authors found that subcutaneous administration of Bup-LHC resulted in plasma concentrations greater than 0.1 ng/mL (the therapeutic level for humans) until 72 h.[23] The administration of Bup-LHC in mice resulted in significantly different pharmacokinetics than those reported in cats and macaques. Unlike those species, which demonstrated prolonged drug presence in plasma, mice had more rapid elimination, with plasma levels falling below purported therapeutic levels by 8 h after administration in female CD1 mice and after 12 h in B6 male mice.[11,17] The pharmacokinetic

profile of Bup-LHC appears to differ between the male B6 and the female CD1 mice. However, we did not perform a comprehensive comparison of strains and sex, thus hindering complete interpretation of the pharmacokinetics in different mouse strains or sexes. Nonetheless, our results suggest that dosing Bup-LHC at 0.9 mg/kg SC every 6 to 12 h achieves therapeutic plasma levels in mice. More comprehensive pharmacokinetic profiling, using both sexes and various strains given multiple doses 6 to 12 h apart, could extend our current findings.

The evaluation of pain in mice can be challenging because they are a prey species that typically masks signs of pain.[27,38] We used a compilation of several previous published mouse ethograms to assess analgesic efficacy,[1,16,20,25,33,41] which we have successfully demonstrated to be useful in assessing postoperative pain in rodents.[1,17] Similar to our prior study, the differences in many of the parameters we used to assess pain during our 5-min observation period were not evident until after statistical analysis was performed. For example, orbital tightening, used as a proxy for the mouse grimace score, was subtly but statistically higher in saline-treated mice compared with Bup-LHC-treated mice during the first 3 to 12 h after surgery. Assessing pain in mice is complicated by variation in the individual observer's interpretation of mouse pain behavior. Despite very good agreement between the observers in our study, the more objective measures, such as frequency of rearing, wound licking, and grooming, showed better agreement than the more subjective parameter of the amount of nesting material integrated to the nest. Based on statistical analysis, the 3 parameters indicative that the post-surgical pain was mitigated (or not) by analgesia were wound licking, orbital tightening, and activity (distance traveled and activity bouts). These measures showed significant effects of treatment over time and at specific time points, particularly early in the study.

Wound licking was highest in the saline-treated mice that underwent surgery, suggesting this group had the highest level of pain. The wound licking of the Bup-LHC-treated ovariectomized mice was reduced for the first 6 h after surgery as compared with the saline-treated surgical group and was higher than in the unoperated groups. This finding suggests that Bup-LHC provided analgesia in the acute postoperative period. A previous study found that wound licking was one of the more consistent indicators of postoperative pain in rodents after surgery.[18]

The mouse grimace scale has become a very popular and easily implemented way to assess mouse pain after surgery.[20,25] We chose to simplify the mouse grimace scale to orbital tightening because it is a readily visible sign in the mouse grimace scale, is easy to assess and to train personnel to recognize, and has been previously used to assess pain in mice.[1,18,35] The change in the orbital tightening scores between the saline- and Bup-LHC-treated groups emerged only after statistical analysis. This method is useful for pain assessment in mice but it requires astute observation of the mouse, as they may mask these clinical signs. If only a brief, single observation is performed, an observer could easily miss orbital tightening (and other facial expressions of pain) despite the presence of pain. This drawback can potentially be overcome by using remote video observation; however, this solution might be impractical for most institutions. Care should be taken to ensure that the observation time for assessing orbital tightening and other behaviors of pain is sufficient.

General activity can be an objective evaluation of mouse pain after a procedure and can be measured relatively quickly. The postprocedural findings should be compared with baseline data in the same animal to help control for interindividual variations that could occur if comparing behaviors between different groups of mice. The ANY-maze provides a means to track a mouse's movement and distance traveled. This software gives a more objective measure than an observer's assessment. Increased activity postoperatively has been identified as a positive correlate to analgesia in several mouse models.[5,10,18,19] In the current study, the distance traveled was significantly greater for Bup-LHC mice than for the saline-treated mice until 3 h after surgery and approached significance at 6 h. The number of activity bouts measured by using an acoustic recorder correlated with the distance traveled. We previously demonstrated the use of acoustic recordings to assess activity bouts in mice after LPS-induced inflammation and laparotomy.[35,36] Similarly, activity bouts were significantly more numerous after Bup-LHC administration as compared with saline treatment in mice for as long as 6 h. Unlike previous studies in which mice showed more activity after buprenorphine administration,[39] in our study mice that received Bup-LHC without surgery did not show increased activity. In addition, Bup-LHC-treated mice that underwent surgery did not have increased general activity compared with their baseline data. This result suggests that the activity of the surgical mice was not influenced by buprenorphine administration itself.

The clinical efficacy of Bup-LHC parallels its pharmacokinetics. The plasma concentration fell below the purported therapeutic level of 1.0 ng/mL prior to the 8-h time point, and the drug appeared to be clinically effective for at least 6 h after surgery. The concentrations detected at 2 and 4 h after Bup-LHC administration were 6.9 ± 3.5 ng/mL and 3.7 ± 4.5 ng/mL, respectively. These values are greater than the plasma concentrations reported in previous pharmacokinetic studies, in which Bup-HCl dosed at 0.1 mg/mL had a peak concentration of 19.1 ng/mL at 2 h after administration and was undetectable at 4 to 8 h[9,17] and in which sustained-release buprenorphine dosed at 0.6 mg/kg had a peak concentration of 14.5 ng/mL at 4 h after administration and declined to 4.2 ng/mL at 24 h.[17] Given the efficacy of Bup-LHC at 0.9 mg/kg and the duration of therapeutic plasma concentration, adjusting dosages to reduce the range of the Bup-LHC spike immediately after administration could minimize adverse effects associated with repeated administration if the drug is administered every 6 h. Although the duration of action of Bup-LHC appears to be shorter than for SR-Bup, Bup-LHC provides therapeutic levels for at least 6 h and thus provides an alternative analgesic. This approach offers a better dosing regimen than using Bup-HCl every 8 to 12 h, which resulted in undetectable plasma concentrations as early as 4 h after administration and was ineffective managing postoperative pain in several mouse models.[6,9,14,18]

The surgery performed in the current study was relatively brief, and the surgical pain appeared to subside by 24 h postoperatively, given that the indices of pain had improved in the saline-treated group at this time. This pattern is consistent with previous studies used to evaluate experimental laparotomy in female mice.[14,18] Mice in those studies returned to presurgical baseline behaviors by 24 h regardless of treatment. These studies suggest that the first 24 h after surgery are the most important in regard to pain mitigation in this mouse model. In the current study, the analgesic efficacy of Bup-LHC was most demonstrable in the acute postoperative period, from 3 to 12 h, as is sustained-release buprenorphine.

According to our pharmacokinetic analysis and efficacy study, Bup-LHC is an acceptable alternative analgesic for murine studies and provides analgesia that lasts longer than that of 0.1 mg/kg Bup-HCl. We found no complications such as heavy sedation,

pica, hyperactivity or injection site lesions in Bup-LHC-treated mice. Similar to our previous studies, evaluation of wound licking, orbital tightening, and activity levels were the most reliable indicators of pain. Assessment of these parameters indicates that analgesia is most critical during the first 24 h after mouse laparotomy, and this pain should be managed appropriately.

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References

1. **Adamson TW, Kendall LV, Goss S, Grayson K, Touma C, Palme R, Chen JQ, Borowsky AD.** 2010. Assessment of carprofen and buprenorphine on recovery of mice after surgical removal of the mammary fat pad. J Am Assoc Lab Anim Sci **49:**610–616.
2. **Akamine T, Koyanagi S, Kusunose N, Hashimoto H, Taniguchi M, Matsunaga N, Ohdo S.** 2015. Dosing time-dependent changes in the analgesic effect of pregabalin on diabetic neuropathy in mice. J Pharmacol Exp Ther **354:**65–72. https://doi.org/10.1124/jpet.115.223891.
3. **Carbone ET, Lindstrom KE, Diep S, Carbone L.** 2012. Duration of action of sustained-release buprenorphine in 2 strains of mice. J Am Assoc Lab Anim Sci **51:**815–819.
4. **Christoph T, Kögel B, Schiene K, Méen M, De Vry J, Friderichs E.** 2005. Broad analgesic profile of buprenorphine in rodent models of acute and chronic pain. Eur J Pharmacol **507:**87–98. https://doi.org/10.1016/j.ejphar.2004.11.052.
5. **Clark MD, Krugner-Higby L, Smith LJ, Heath TD, Clark KL, Olson D.** 2004. Evaluation of liposome-encapsulated oxymorphone hydrochloride in mice after splenectomy. Comp Med **54:**558–563.
6. **Clark TS, Clark DD, Hoyt RF JR.** 2014. Pharmacokinetic comparison of sustained- release and standard buprenorphine in mice. J Am Assoc Lab Anim Sci **53:**387–391.
7. **Doodnaught GM, Monteiro BP, Benito J, Edge D, Beaudry F, Pelligand L, Steagall P.** 2017. Pharmacokinetic and pharmacodynamic modelling after subcutaneous, intravenous and buccal administration of a high-concentration formulation of buprenorphine in conscious cats. PLoS One **12:**1–16. https://doi.org/10.1371/journal.pone.0176443.
8. **Flecknell P.** 2009. Laboratory animal anaesthesia, 3rd ed. London (United Kingdom): Academic Press.
9. **Gades NM, Danneman PJ, Wixson SK, Tolley EA.** 2000. The magnitude and duration of the analgesic effect of morphine, butorphanol, and buprenorphine in rats and mice. Contemp Top Lab Anim Sci **39:**8–13.
10. **Goecke JC, Awad H, Lawson JC, Boivin GP.** 2005. Evaluating postoperative analgesics in mice using telemetry. Comp Med **55:**37–44.
11. **Guarnieri M, Brayton C, DeTolla L, Forbes-McBean N, Sarabia-Estrada R, Zadnik P.** 2012. Safety and efficacy of buprenorphine for analgesia in laboratory mice and rats. Lab Anim (NY) **41:**337–343. https://doi.org/10.1038/laban.152.
12. **Hayes JH, Flecknell PA.** 1999. A comparison of pre- and post-surgical administration of bupivacaine or buprenorphine following laparotomy in the rat. Lab Anim **33:**16–23. https://doi.org/10.1258/002367799780578534.
13. **Healy JR, Tonkin JL, Kamarec SR, Saludes MA, Ibrahim SY, Matsumoto RR, Wimsatt JH.** 2014. Evaluation of an improved sustained-release buprenorphine formulation for use in mice. Am J Vet Res **75:**619–625. https://doi.org/10.2460/ajvr.75.7.619.
14. **Jirkof P, Tourvieille A, Cinelli P, Arras M.** 2014. Buprenorphine for pain relief in mice: repeated injections vs sustained-release depot formulation. Lab Anim **49:**177–187. https://doi.org/10.1177/0023677214562849.
15. **Jirkof P, Fleischmann T, Cesarovic N, Rettich A, Vogel J, Arras M.** 2013. Assessment of postsurgical distress and pain in laboratory mice by nest complexity scoring. Lab Anim **47:**153–161. https://doi.org/10.1177/0023677213475603.
16. **Jones CP, Carver S, Kendall LV.** 2012. Evaluation of common anesthetic and analgesic techniques for tail biopsy in mice. J Am Assoc Lab Anim Sci **51:**808–814.
17. **Kendall LV, Hansen RJ, Dorsey K, Kang S, Lunghofer PJ, Gustafson DL.** 2014. Pharmacokinetics of sustained-release analgesics in mice. J Am Assoc Lab Anim Sci **53:**478–484.
18. **Kendall LV, Wegenast DJ, Smith BJ, Dorsey KM, Kang S, Lee NY, Hess AM.** 2016. Efficacy of sustained-release buprenorphine in an experimental laparotomy model in female mice. J Am Assoc Lab Anim Sci **55:**66–73.
19. **Kolstad AM, Rodriguiz RM, Kim CJ, Hale LP.** 2012. Effect of pain management on immunization efficacy in mice. J Am Assoc Lab Anim Sci **51:**448–457.
20. **Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, LaCroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AMJM, Ferrari MD, Craig KD, Mogil JS.** 2010. Coding of facial expressions of pain in the laboratory mouse. Nat Methods **7:**447–449. https://doi.org/10.1038/nmeth.1455.
21. **Larson CM, Wilcox GL, Fairbanks CA.** 2019. The study of pain in rats and mice. Comp Med **69:**555–570. https://doi.org/10.30802/AALAS-CM-19-000062.
22. **Le Bars D, Gozariu M, Cadden SW.** 2001. Animal models of nociception. Pharmacol Rev **53:**597–652.
23. **Mackiewicz AL, Salyards GW, Knych HK, Hill AE, Christe KL.** 2019. Pharmacokinetics of a long-lasting, highly concentrated buprenorphine solution after subcutaneous administration in rhesus macaques (*Macaca mulatta*). J Am Assoc Lab Anim Sci **58:**501–509. https://doi.org/10.30802/AALAS-JAALAS-18-000115.
24. **Magalhães-Sant'Ana M, Sandøe P, Olsson I.** 2009. Painful dilemmas: the ethics of animal-based pain research. Anim Welf **18:**49–63.
25. **Matsumiya LC, Sorge RE, Sotocinal SG, Tabaka JM, Wieskopf JS, Zaloum A, King OD, Mogil JS.** 2012. Using the mouse grimace scale to reevaluate the efficacy of postoperative analgesics in laboratory mice. J Am Assoc Lab Anim Sci **51:**42–49.
26. **Moehring F, O'Hara CL, Stucky CL.** 2016. Bedding material affects mechanical thresholds, heat thresholds, and texture preference. J Pain **17:**50–64. https://doi.org/10.1016/j.jpain.2015.08.014.
27. **Mogil JS.** 2009. Animal models of pain: progress and challenges. Nat Rev Neurosci **10:**283–294. https://doi.org/10.1038/nrn2606.
28. **Morris TH.** 1999. Dose estimation among species, p 3–14. In: Hawk CT, Leary SL, editors. Formulary for laboratory animals. Ames (IA): Iowa State Press.
29. **National Research Council.** 2009. Recognition and alleviation of pain in laboratory animals. Washington (DC): National Academies Press.
30. **Oliver VL, Thurston SE, Lofgren JL.** 2018. Using cageside measures to evaluate analgesic efficacy in mice (*Mus musculus*) after surgery. J Am Assoc Lab Anim Sci **57:**186–201.
31. **Poole T.** 1997. Happy animals make good science. Lab Anim **31:**116–124. https://doi.org/10.1258/002367797780600198.
32. **Rock ML, Karas AZ, Rodriguez KGB, Gallo MS, Pritchett-Corning K, Karas RH, Aronovitz M, Gaskill BN.** 2014. The time-to-integrate-to-nest test as an indicator of wellbeing in laboratory mice. J Am Assoc Lab Anim Sci **53:**24–28.
33. **Roughan JV, Flecknell PA.** 2000. Effects of surgery and analgesic administration on spontaneous behaviour in singly housed rats. Res Vet Sci **69:**283–288. https://doi.org/10.1053/rvsc.2000.0430.
34. **Roughan JV, Flecknell PA.** 2003. Evaluation of a short duration behaviour-based post-operative pain scoring system in rats. Eur J Pain **7:**397–406. https://doi.org/10.1016/S1090-3801(02)00140-4.
35. **Smith BJ, Bruner KE, Hess AM, Kendall LV.** 2020. Female urine-induced male mice ultrasonic vocalization in C57BL/6J mice as proxy indicators for postoperative pain. J Am Assoc Lab Anim Sci **59:**204–211. https://doi.org/10.30802/AALAS-JAALAS-19-000059.
36. **Smith BJ, Bruner KE, Kendall LV.** 2019. Female- and intruder-induced ultrasonic vocalization in C57BL/6J mice as proxy indicators for animal wellbeing. Comp Med **69:**374–383. https://doi.org/10.30802/AALAS-CM-18-000147.

37. **Sorge RE, Martin LJ, Isbester KA, Sotocinal SG, Rosen S, Tuttle AH, Wieskopf JS, Acland EL, Dokova A, Kadoura B, Leger P, Mapplebeck JCS, McPhail M, Delaney A, Wigerblad G, Schumann AP, Quinn T, Frasnelli J, Svensson CI, Sternberg WF, Mogil JS.** 2014. Olfactory exposure to males, including men, causes stress and related analgesia in rodents. Nat Methods **11:**629–632. https://doi.org/10.1038/nmeth.2935.
38. **Stasiak KL, Maul D, French E, Hellyer PW, Vandewoude S.** 2003. Species-specific assessment of pain in laboratory animals. Contemp Top Lab Anim Sci **42:**13–20.
39. **Tubbs JT, Kissling GE, Travlos GS, Goulding DR, Clark JA, King-Herbert AP, Blankenship-Paris TL.** 2011. Effects of buprenorphine, meloxicam, and flunixin meglumine as postoperative analgesia in mice. J Am Assoc Lab Anim Sci **50:**185–191.
40. **Watanabe R, Monteiro BP, Evangelista MC, Castonguay A, Edge D, Steagall PV.** 2018. The analgesic effects of buprenorphine (Vetergesic or Simbadol) in combination with carprofen in dogs undergoing ovariohysterectomy: a randomized, blinded, clinical trial. BMC Vet Res **14:**304. https://doi.org/10.1186/s12917-018-1628-4.
41. **Winnicker C, Gaskill BN, Garner J, Pritchett-Corning KR.** 2012. A guide to the behavior and enrichment of laboratory rodents. Wilmington (MA): Charles River Laboratories.
42. **Zoetis**. 2014. SIMBADOL (package insert). Florham Park (NJ): Zoetis.