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Assessment of the Safety and Efficacy of Pre-emptive Use of Extended-release Buprenorphine for Mouse Laparotomy

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Buprenorphine is commonly used to control postoperative pain in rodents. Short-acting formulations of buprenorphine (bup-HCl) require frequent handling and restraint of animals for appropriate dosing, which can be stressful and confound research outcomes. Ethiqa XR (bup-ER) is an FDA-indexed extended-release buprenorphine formulation that is an alternative to bup-HCl in mice and rats. In the current study, we first evaluated the pharmacokinetics of bup-ER in male C57BL/6J mice by sampling blood at 10 time points, ranging from 30 min to 72 h after administration (*n* = 3 mice per time point). Average plasma concentrations fell below therapeutic levels at 48 h after administration. We also evaluated the safety of bup-ER when administered prior to surgery in combination with common anesthetics and the efficacy of bup-ER in mouse laparotomy. Anesthetic safety was studied by measuring respiratory rate, rectal temperature, and recovery time in groups of mice (*n* = 8) given bup-HCl, bup-ER, or saline in combination with isoflurane or ketamine-xylazine anesthesia. No differences were seen between analgesic treatment groups with either of the general anesthetics. To evaluate efficacy, mice (*n* = 10) were randomly allocated to receive either bup-ER (3.25 mg/kg) once presurgically, bup-HCl (0.1 mg/kg) presurgically and then every 8 h, or saline once before surgery. Mice underwent a sham laparotomy and were assessed for pain based on changes in weight, cageside ethogram, nesting consolidation test, rearing frequency, and nociception to von Frey testing at 6, 12, 24, 48, and 72 h after surgery. Cageside ethogram, rearing frequency, and von Frey testing showed significant differences between bup-ER-treated mice and saline controls in the early postoperative period. No significant effects between treatment groups were seen in daily weights or nesting consolidation scores. This study demonstrates that bup-ER can be safely administered before surgery and provides analgesia for up to 48 h after administration based on pharmacokinetic and behavioral data.

Abbreviations: bup-ER, extended-release buprenorphine; bup-HCl, buprenorphine-hydrochloride

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Introduction

Biomedical research commonly requires performance of minor and major survival surgeries on mice, with major surgery generally understood to mean the removal of organs and other permanent alterations to the anatomy, or any procedure that requires the opening of a body cavity, such as a laparotomy. Various laparotomy protocols have been described in rodents, ranging from splenectomies to hepatectomies to aortic banding.[7,27,39] Due to the invasive nature and tissue trauma associated with any surgery, appropriate analgesics and their optimal dosing regimen must be identified to effectively mitigate postoperative pain in the animals. As outlined in the *Guide for the Care and Use of Laboratory Animals*,[31] pain can cause unacceptable levels of distress if not addressed, especially if central pain sensitization develops. Central sensitization is a process that enhances the pain transmission circuits in the CNS, ultimately resulting in the ability of innocuous stimuli to evoke behaviors indicative of pain (allodynia).[30] In addition, the area of injury itself becomes more sensitive to pain (primary hyperalgesia), and areas surrounding the injury, as well as distant sites, can also develop hypersensitivity (secondary hyperalgesia).[30] Moreover, induction of pain and the subsequent stress response can confound research results,[30] making the reduction of pain crucial to research requiring surgery in animals.

Buprenorphine-HCl (bup-HCl) is a partial agonist of the μ-opioid receptor and an antagonist of the κ-opioid receptor. Although not as potent as morphine, bup-HCl is preferred over other opioids due to its longer duration of action and its effective analgesic properties.[14,15,37] Current formularies suggest redosing bup-HCl every 12 h in mice and every 8 to 12 h in rats when administered subcutaneously.[12] However, pharmacokinetic analysis has revealed that plasma levels of bup-HCl are likely below therapeutic levels and thus not clinically relevant beyond 4 to 6 h after administration.[13,15,22] Although more frequent redosing may sustain plasma levels, it could also exacerbate the animal's stress response due to the higher frequency of handling, restraint, and injections.[28,29] In addition, more frequent dosing would require greater compliance from research personnel and would likely require at least one injection outside of regular working hours.

A veterinary compounding pharmacy offered a sustained-release formulation of buprenorphine for use in rodents prior to the availability of bup-ER. Studies comparing the efficacy of bup-HCl with the compounded sustained-release formulation have found that it does have a prolonged duration of action, with estimates ranging from 12 to 48 h in mice depending on the type of study.[5,20,22] However, clear guidelines on the appropriate dosage for this formulation in mice are not available, which

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creates some difficulty when comparing studies. Furthermore, institutional DEA licenses may not cover large-scale use of this product.

Ethiqa XR (bup-ER) is currently the only pharmaceutical grade, FDA-indexed extended-release buprenorphine formulation indicated for the control of postprocedural pain in mice and rats.[10] Several studies have already assessed the duration of action and the safety margin of bup-ER.[4,26,32] The manufacturer's pharmacokinetic analysis for this product in an unspecified strain of mice revealed plasma levels ≥ 0.5 ng/mL at 3 d after administration in one trial and ≥ 1.0 ng/mL at 3 d in a second trial.[36] A subsequent independent study in C57BL/6 mice found that plasma levels of bup-ER remained above 1.0 ng/mL at 2 d after administration, but had fallen below this level at 3 d.[32] Currently, no pharmacokinetic studies have evaluated plasma levels during the first 4 h after administration. Furthermore, studies demonstrating safety during murine surgery evaluated this product only after postoperative administration.[36] Thus, the current study seeks to determine whether bup-ER can be safely administered in combination with anesthesia and the amount of time needed to reach therapeutic levels in plasma.

We sought to investigate the safety and efficacy of bup-ER for 72 h after surgery when administered before performing mouse laparotomy. This study had 3 objectives: 1) perform a pharmacokinetic analysis of bup-ER in C57BL/6J mice with early timepoints, 2) evaluate the safety of bup-ER when used in combination with common anesthetic agents, and 3) evaluate the analgesic efficacy of bup-ER when administered before laparotomy. We hypothesized that bup-ER could maintain therapeutic levels for at least 48 h after administration in mice, could be safely used in combination with common anesthetics, and would provide comparable analgesic efficacy to bup-HCl as compared with saline controls after laparotomy.

Methods

Animals. A total of 84 male and female C57BL/6J mice (6 to 8 wk old) were purchased from Jackson Laboratory (Bar Harbor, ME) and designated SPF for mouse hepatitis virus, minute virus of mice, mouse parvovirus, epizootic diarrhea of infant mice virus, ectromelia virus, Sendai virus, pneumonia virus of mice, Theiler murine encephalomyelitis virus, reovirus, lymphocytic choriomeningitis virus, mouse adenovirus, polyomavirus, *Mycoplasma pulmonis*, and pinworms. Mice were housed in microisolation cages on corncob bedding in ventilated racks under constant environmental conditions (68 to 72 °F [20 to 22.2 °C], 30% to 50% relative humidity, 12h:12h light/dark cycle). Mice were given food (Laboratory Rodent Diet 5001, PMI Lab Diet, St. Louis, MO) and water ad libitum. Mice were group housed in groups of 3 to 5 on receipt and remained in their original groups for the duration of the pharmacokinetic and anesthesia safety studies. Mice were individually housed during the analgesic efficacy study to allow accurate assessment of the nest consolidation test. All procedures were performed at an AAALAC International-accredited facility and were approved by the University of Michigan Institutional Animal Care and Use Committee in accordance with the *Guide for the Care and Use of Laboratory Animals*.

Buprenorphine formulations. Buprenorphine hydrochloride (bup-HCl), 0.3 mg/mL (Par Pharmaceutical, Chestnut Ridge, NY) was diluted in sterile saline to a concentration of 0.03 mg/mL prior to injection. Ethiqa XR (bup-ER) (Fidelis Pharmaceuticals, North Brunswick Township, NJ) was obtained from the manufacturer at a concentration of 1.3 mg/mL and was used undiluted.

Pharmacokinetic study. Male C57BL/6J mice were randomly allocated to one of the 10 timepoints (30 min, 60 min, 90 min, 2 h, 4 h, 6 h, 12 h, 24 h, 48 h, and 72 h after injection, $n = 3$ per timepoint) with 4 additional mice used for blank plasma controls. Mice were manually restrained, and 3.25 mg/kg of bup-ER was administered subcutaneously in the interscapular region. At their designated timepoint, mice were anesthetized with isoflurane and terminal blood collection was performed through the retro-orbital sinus by removing the eye and collecting blood passively as drops. Approximately 1 mL of blood was collected in EDTA microtainers and then centrifuged at 2,000*g* for 5 min. Plasma was separated and aliquoted into microfuge tubes before being stored at −80 °C. Samples from the same time point were pooled and pharmacokinetic analysis was performed by liquid chromatography-mass spectrometry at the University of Michigan Pharmacokinetic and Mass Spectrometry Core (Ann Arbor, MI). The limit of quantification was 0.5 ng/mL.

Anesthesia safety study. Male and female C57BL/6J mice were randomly assigned in sex-balanced groups to either ketamine-xylazine ($n = 12$) or isoflurane ($n = 12$) anesthesia. The 12 mice in each group were then randomly assigned to receive either bup-ER (3.25 mg/kg), bup-HCl (0.1 mg/kg), or saline (0.05 mL) ($n = 4$ per analgesic treatment group). Mice receiving ketamine-xylazine anesthesia were induced with a single injection of a ketamine (VetOne, Boise, ID) and xylazine (Akorn Pharmaceuticals, Lake Forest, IL) cocktail IP (120 mg/kg ketamine, 10 mg/kg xylazine). Mice that received isoflurane anesthesia (VetOne, Boise, ID) were induced with 5% isoflurane until they no longer exhibited a righting reflex; they were then transferred to a nose cone at 2% isoflurane for maintenance of anesthesia. Once anesthetized, lubricating eye ointment (Bausch Health, Bridgewater, NJ) was administered to the eyes and the assigned analgesic treatment or saline was injected subcutaneously in the interscapular region. Mice were transferred to a heating pad set at 37 °C and a temperature probe was inserted in the rectum. Body temperature and respiratory rate were measured at the beginning of anesthesia and then 10 min afterward. After 10 min of isoflurane anesthesia, the inhalant was stopped, and mice were placed in dorsal recumbency in a recovery cage under a heat lamp. Mice receiving ketamine-xylazine remained on the heating pad for an additional 10 min with the temperature probe in place before being placed in a recovery cage in dorsal recumbency under a heat lamp. No reversal drugs were administered. Respiratory rate during recovery was recorded every 10 min until mice were able to reposition themselves to sternal recumbency. The time required to reposition into sternal recumbency and time to become fully ambulatory were measured.

After a washout period of 2 wk, mice were again randomly assigned to new groups in order to ensure that all mice received the alternate anesthetic from the first round and a different analgesic treatment. Procedures for analgesic administration, anesthesia, and recovery were the same between the first and second rounds.

Analgesic efficacy study. Male and female C57BL/6J mice were used in this study. Mice were randomly assigned in sex-balanced groups to one of 3 analgesic treatment groups: bup-ER ($n = 10$), bup-HCl ($n = 10$), or saline control ($n = 10$). Group 1 received bup-ER (3.25 mg/kg) subcutaneously once during surgical induction. Group 2 received bup-HCl (0.1 mg/kg) subcutaneously during surgical induction and then every 8 h for 72 h after surgery. Group 3 received a single 0.05 mL saline bolus subcutaneously before surgery. Mice were induced with 5% isoflurane and maintained at 2% on a nose cone once fully anesthetized. Lubricating eye ointment was applied. All mice

were then shaved to remove fur from the ventral abdomen and prepared aseptically for surgery with 3 alternating chlorhexidine and alcohol scrubs. A 1.0-cm midline incision was made through the abdominal skin and body wall to access the abdomen. A sterile cotton swab moistened with sterile saline was then inserted into the abdomen along the right side of the body wall, then swiped perpendicular to the incision for a minimum of 5 passes to simulate abdominal manipulation. The body wall was then sutured in a simple interrupted pattern with absorbable 4-0 poliglecaprone suture (DemeTECH, Miami, FL) and the skin closed with metal wound clips (Stoelting, Wood Dale, IL).

Mice were weighed daily and assessed for pain at 6, 12, 24, 48, and 72 h after surgery. The observer was blind to the treatment group for the entire period of assessment. Pain assessment was accomplished with a cageside ethogram, behavioral assays (nest consolidation score, rearing frequency), and changes in nociception based on von Frey testing. All mice were individually housed, and baseline measurements were made 24 h before surgery.

Cageside ethogram. Mice were scored cageside based on a visual assessment system as previously described.[3] Mice were scored based on real-time observation of eyes (0 to 2), hair coat (0 to 3), coordination and posture (0 to 5), and overall condition (0 to 3), with higher scores representing greater deviations from normal appearance and behavior. Individual scores for these characteristics were then summed to obtain a final pain index score for each mouse at each time point.

Nest consolidation score. Mice were scored on their nesting ability based on a visual assessment scoring guide adapted and modified from a previous publication.[33] The test was prepared by placing one quarter of a cotton square in each corner of a standard mouse cage containing corncob bedding. Mice were then housed individually in the cage and the completeness of their nest was scored from 1 to 5 after 24 h, with higher scores representing a more elaborate and labor-intensive nest. Nest consolidation tests were set up 24 h before surgery and then scored on the day of surgery to establish a baseline score. After recovering from surgery, mice were housed individually in new cages and the nesting consolidation test was performed again.

Rearing frequency. Mice were placed individually in a clean, open-top container. Mice were allowed to explore the container and the number of times they reared during a 3-min period was counted. Rearing was defined as full extension of the animal's length upward against the wall of the container. Baseline measurements were recorded 24 h before surgery. All postoperative measurements were presented as a percentage of baseline rearing.

Von Frey testing. Mechanosensation was evaluated by measuring the hind paw withdrawal response to von Frey probe stimulation. Mice were individually placed in acrylic boxes with a wire grid bottom and given a minimum acclimation period of 5 min. The 90-g probe of an electronic von Frey reader (IITC Life Science, Woodland Hills, CA) was then applied perpendicular to the plantar surface of the left hind paw through the grid floor. Force was gradually applied until the paw was withdrawn, and the associated applied force was recorded. All mice were tested 3 times with a 2-min washout period between measurements to minimize sensitization between measurements. Each animal's nociceptive mechanical threshold (peak force in grams) was calculated as an average score of all 3 measurements. Mice were acclimated to the procedure once, 24 h before surgery, when baseline measurements were recorded.

Statistics. All statistics were performed using SPSS unless certain features were not available, in which case the alternative software is noted. Data are expressed as means ± SD. A *P* value less than 0.05 was considered significant. Data from all parameters were deemed normally distributed based on normal probability plots.

Data from the anesthesia safety study, except for respiratory rate in the ketamine-xylazine group, were analyzed with a one-way analysis of variance. Body temperature was analyzed as the difference from baseline after 10 min of anesthesia in the isoflurane group and after 10 min and 20 min of anesthesia in the ketamine-xylazine group. Respiratory rates were presented for the isoflurane group as the difference between values at baseline and after 10 min of anesthesia. Due to the prolonged and inconsistent anesthetic times in the ketamine-xylazine anesthesia group, respiratory rates were analyzed using a mixed effects model on GraphPad Prism to accommodate missing values and to incorporate time as a within-subjects effect.

Repeated measures of continuous data (weight, von Frey withdrawal force, percentage of baseline rearing frequency) and ordinal data (nest consolidation score, cageside ethogram score) were analyzed with a repeated measures general linear model. The within-subjects effect was defined as time and the between-subjects effect was defined as analgesic treatment (bup-ER, bup-HCl, or saline). No significant effects or interactions were associated with sex in any parameters; thus, sex was removed as a within-subjects effect in the final analysis. Posthoc pairwise comparisons of estimated marginal means were performed using the Bonferroni adjustment for multiple comparisons.

Results

Pharmacokinetic study. Plasma buprenorphine concentrations were measured over a 72-h period in mice given bup-ER at 3.25 mg/kg SC in the interscapular region. Plasma levels of bup-ER reached 1.68 ± 0.84 ng/mL at the earliest time point (30 min after administration) (Figure 1). Plasma levels did not consistently increase over time. One peak occurred at 2-h after administration (12.82 ± 2.89 ng/mL), then fell at the 4- and 6-h time points, and then peaked at 12 h after administration (13.15 ± 3.66 ng/mL). Mean plasma levels were above the purported effective dose of 1 ng/mL[6,41] at 24 h after administration (2.76 ± 0.96 ng/mL) but fell below this level while remaining above the ED50 of 0.5 ng/mL at 48 h after administration (0.52 ± 0.27 ng/mL).[17] Levels in all mice were below the limit of quantification at 72 h after administration.

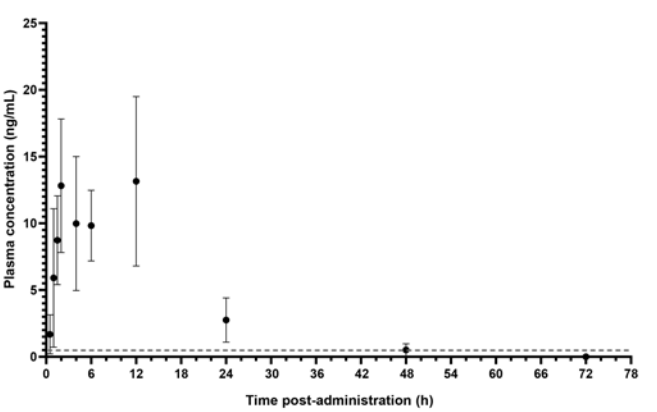

Figure 1. Pharmacokinetics of extended-release buprenorphine (bup-ER) in male C57BL/6 mice. Values that were below the limit of quantification were replaced with a value of 0 ng/mL to calculate means. Plasma concentrations are shown as a mean ± SD. The dotted line indicates a plasma concentration of 0.5 ng/mL.

Anesthesia safety study. ***Isoflurane anesthesia.*** No significant differences were seen in rectal temperatures or respiratory rate after 10 min of anesthesia between analgesic groups. During recovery, no significant differences were found in time-to-sternal or time-to-ambulatory between analgesic groups. All mice became ambulatory within 10 min of being removed from anesthesia and thus respiration was not measured during the recovery period. All mice recovered normally with no signs of morbidity or mortality.

Ketamine-xylazine anesthesia. Anesthetic durations ranged from 20 to 70 min. The change in rectal temperature at 10 and 20 min after the start of anesthesia was not significantly different between analgesic groups. During recovery, no significant differences in time-to-sternal or time-to-ambulatory were detected between analgesic groups. Analgesia did not have a significant effect on respiratory rates during ketamine-xylazine anesthesia between the time of administration and the time of sternal recumbency. All mice recovered normally with no signs of morbidity or mortality.

Analgesic efficacy study. ***Weight.*** Time after surgery had a significant effect on weight over the course of the experiment ($P < 0.001$). No significant differences were found between analgesic groups overall ($P = 0.849$) (Figure 2).

Nest consolidation score. Analgesia did not significantly affect nest consolidation scores ($P = 0.955$) (Figure 3A). No differences were found between any analgesic group at any time point. Time had a significant effect ($P < 0.001$) and a significant interaction with analgesia ($P = 0.012$). Saline control scores were significantly below baseline at 6 h ($P = 0.018$) and 12 h ($P = 0.008$) after surgery but did not differ from baseline at 24 h after surgery. The bup-ER group never fell significantly below baseline during the first 24 h after surgery ($P = 1.000$, 0.331, 1.000 for 6, 12, and 24 h, respectively). The bup-HCl group had significantly lower nest scores at 6 h after surgery ($P = 0.036$), returned to baseline at 12 h, and remained at baseline at 24 h. Average scores continued to increase in all groups at 48- and 72-h timepoints.

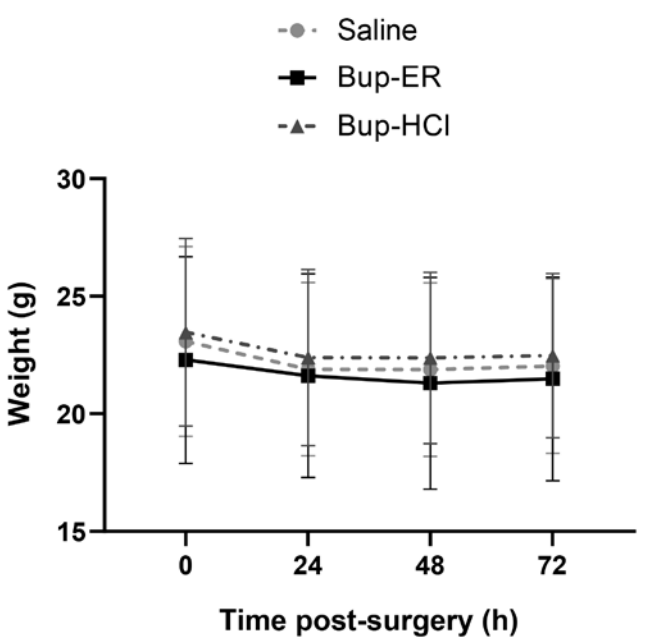

Figure 2. Daily body weights from the time of surgery (t = 0 h) until 72 h postoperatively of mice that had received extended-release buprenorphine (bup-ER), buprenorphine-HCl (bup-HCl), or saline. Weights are shown as a mean ± SD.

Rearing frequency. Mice had an average baseline rearing frequency of 23 ± 5. Baseline scores were not different among groups. Rearing frequency is presented as a percentage of baseline rearing; therefore, values less than 100% would indicate that rearing was lower as compared with preoperative values (Figure 3B). Time overall had a significant effect ($P < 0.001$) on frequency of rearing. In addition, the main effect of analgesia ($P = 0.045$) and the interaction of time and analgesia were also significant ($P = 0.015$), indicating that analgesia had a significant effect on rearing frequency at specific timepoints. The bup-ER mice showed a higher percentage of rearing than did saline controls at 24 h after surgery ($P = 0.013$). No significant differences were seen between the bup-HCl group and saline controls at any time point.

Cageside ethogram. Overall, time had a significant effect ($P < 0.001$) on the ethogram score. The main effect of analgesia ($P < 0.001$) and the interaction of time and analgesia were also significant ($P < 0.001$), indicating that analgesia had a significant effect on ethogram scores at specific timepoints. Scores of both bup-ER and bup-HCl groups were significantly lower than those of saline controls at 6 h ($P = 0.003$, < 0.001 respectively), 12 h ($P < 0.001$ for both groups), and 24 h after surgery ($P = 0.031$, < 0.001 respectively) (Figure 3C). Cageside ethogram scores did not significantly differ between buprenorphine groups at any time points.

Von Frey testing. Time had a significant effect on von Frey tests ($P < 0.001$) but analgesic treatment did not ($P = 0.07$). Both the bup-ER and bup-HCl groups differed significantly from controls at 6 h after surgery ($P = 0.006$, 0.011 respectively) (Figure 3D). However, Von Frey scores never differed significantly between the 2 buprenorphine groups. Saline controls had significantly lower thresholds for withdrawal than baseline at 6 h ($P = 0.012$), 12-h ($P = 0.022$), 48 h ($P = 0.021$), and 72 h after surgery ($P = 0.022$). No significant differences were detected between baseline and any postoperative time points in the buprenorphine groups.

Discussion

The results of this study demonstrate that Ethiqa XR, an extended-release formulation of buprenorphine, can achieve therapeutic levels in plasma by 30 min after administration, can be safely administered before surgery and in combination with common anesthetics, and reduces several behavioral correlates of pain after surgery as compared with saline controls. These results suggest that a single dose of bup-ER at the manufacturer recommended dosage of 3.25 mg/kg is a viable alternative to injecting bup-HCl every 8 h for mouse laparotomy.

In human pharmacokinetic studies of buprenorphine, a plasma concentration of 0.5 ng/mL or higher is the level that is generally deemed sufficient for pain management.[9] This concentration provided effective analgesia in at least 50% of trial populations, whereas levels above 1.0 ng/mL were effective in a majority of the clinical reports.[6,41] Experiments in animals, including rodents, have since relied on these human metrics to extrapolate that plasma levels above 1.0 ng/mL would also provide sufficient analgesia in a majority of animal populations.[17] However, this estimation may be overly conservative in mice, as demonstrated by reports that concentrations as low as 0.5 ng/mL also correlated with effective pain control in mice after surgery.[21] In the current study, the average plasma concentration of buprenorphine was above 0.5 ng/mL at all timepoints from 30 min to 48 h after administration. Previous studies have not measured plasma levels prior to 4 h after administration; thus, our data demonstrate rapid distribution in the blood after

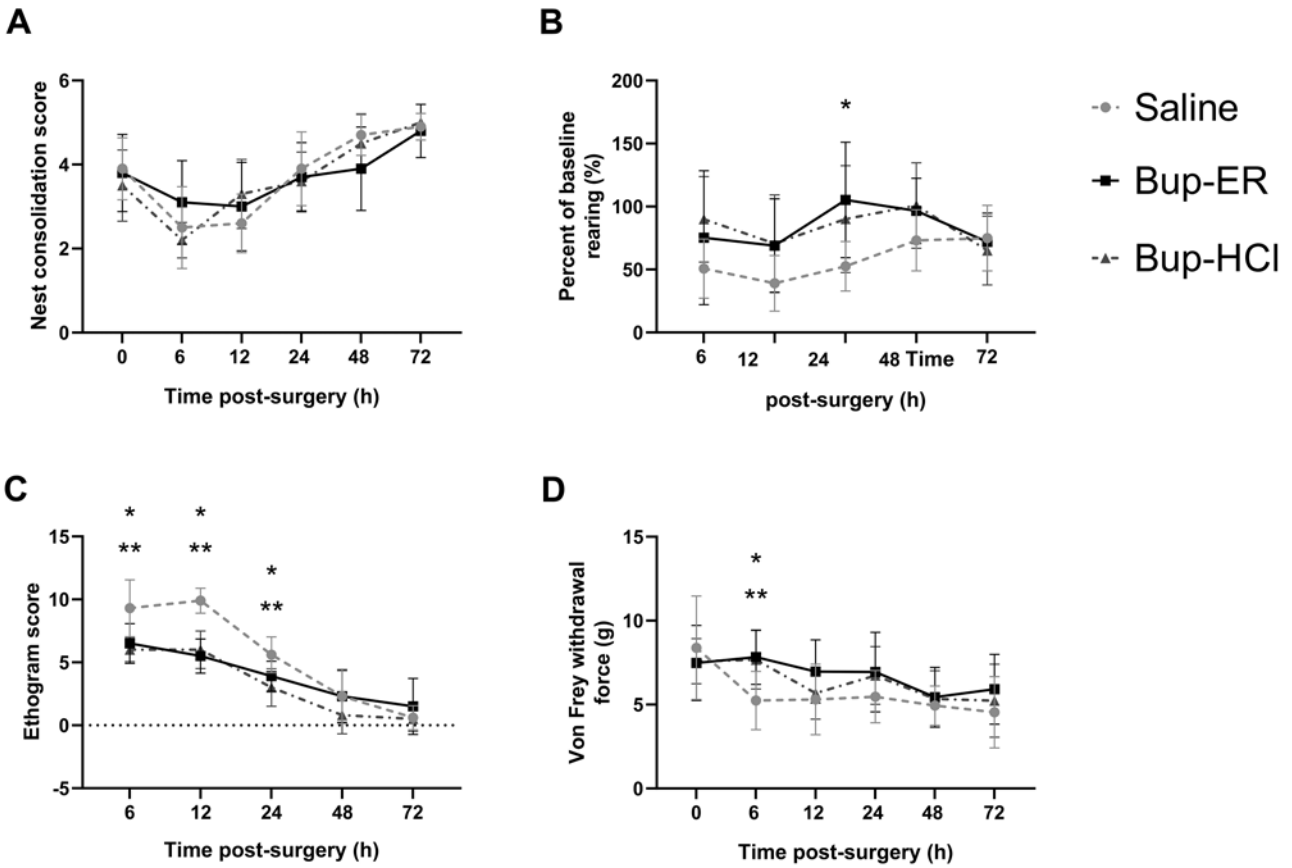

Figure 3. Behavioral assessments of postoperative pain in mice given extended-release buprenorphine (bup-ER), buprenorphine HCl (bup-HCl), or saline. (A) Nest consolidation scores, (B) rearing frequency as a percentage of baseline (presurgical rearing), (C) ethogram scores, and (D) von Frey withdrawal force are shown at 6, 12, 24, 48, and 72 h after surgery. Baseline scores for nest consolidation and von Frey tests were collected before surgery (t = 0 h). Values are shown as a mean ± SD, * indicates a significant difference ($P < 0.05$) between the bup-ER group and saline controls at a specific time point, ** indicates a significant difference ($P < 0.05$) between bup-HCl and saline controls at a specific time point.

subcutaneous injection. The maximum duration for which this therapeutic level is maintained varies among studies, ranging from 2 to 3 d after administration.[32,36] In our hands, average plasma levels fell below 1.0 ng/mL between 24 to 48 h, but remained above 0.5 ng/mL at 48 h after administration. Therefore, our pharmacokinetic data show that bup-ER will likely provide sufficient analgesia for a majority of mice up to 48 h after administration.

The concept of preemptive analgesia first evolved from the demonstration that changes in the central nervous system (CNS) which would normally occur after an injury could be suppressed by the administration of opioids prior to the injury.[24,42] The preemptive administration of opioids has subsequently been shown to reduce signs of pain in mice and other animals as compared with administration after the event.[16,25,35] Therefore, preemptive analgesia is widely encouraged, and sometimes mandated at the institutional level. However, preemptive administration of analgesics frequently coincides with the use of general anesthetics, which raises the concern of drug interactions. Respiratory depression, widely associated with the use of opioids, can be exacerbated by general anesthesia. In previous studies evaluating safety, buprenorphine or bup-ER was administered after surgery.[36,40] Thus, we sought to determine whether bup-ER could be safely administered prior to anesthesia. However, no clinically significant differences in rectal temperature, respiratory rate or recovery time were noted between bup-ER, bup-HCl, or saline when administered with either isoflurane or ketamine-xylazine anesthesia. Based on these results, safety does not appear to be a concern with preemptive administration of bup-ER. The current study evaluated physiologic effect of bup-ER after 10 to 20 min of anesthesia; future studies may evaluate safety of bup-ER up to and beyond 30 min to include the peak concentration periods seen in our pharmacokinetic study.

The Animal Welfare Act and Regulations[1,2] classify laparotomies, along with any procedures that enter a body cavity, as major operative procedures. In rats, the most acutely painful period after a laparotomy occurs between 270 to 390 min after surgery.[38] Similarly, in mice undergoing a vasectomy, significantly elevated corticosterone levels occurred in the immediate postoperative period,[43] indicating that pain and discomfort were most severe in the first 6 h. A study examining the efficacy of a long-lasting, highly concentrated formulation of buprenorphine also found that differences between treated mice and saline controls were most significant between 6 to 12 h postoperatively.[23] Our study showed that bup-ER and bup-HCl could improve recovery and control pain as compared with saline levels in the immediate postoperative period. The 2 buprenorphine groups had significantly lower ethogram scores (indicating less pain) at 6 and 12 h after surgery. Furthermore, at 6 h after surgery, bup-HCl and bup-ER treated mice were significantly more tolerant of noxious mechanical stimuli on the foot pad than saline controls, likely indicating that secondary hyperalgesia and mechanical allodynia were most severely

triggered in control mice. Trends in nesting consolidation scores also demonstrated the motivation to build a nest was recovered more quickly in buprenorphine-treated mice than control animals. Therefore, as compared with the control group, both bup-ER and bup-HCl were able to adequately control pain in the immediate postoperative period, which is arguably the most crucial period for intervention.

The current study also suggests that sufficient pain management was present at 24 h after surgery and that bup-ER dampened secondary hyperalgesia and allodynia for the entire monitoring period. Ethogram scores were significantly lower in the bup-ER group as compared with the controls at 24 h after surgery. Rearing frequency showed an overall treatment effect across the entire postoperative period, with mice given bup-ER showing more rearing by 24 h after surgery. In contrast, the rearing frequency of saline controls did not return to baseline until 48 h after surgery. In addition, both of the buprenorphine groups had von Frey withdrawal thresholds that were similar to baseline throughout the experimental period, whereas control mice showed hyperalgesia and allodynia at several timepoints, including at 72 h after surgery. These differences indicate that buprenorphine can prevent central sensitization and mitigate persistent postoperative pain.

Pharmacokinetic and behavioral data combined indicate that plasma levels of bup-ER above 1.0 ng/mL produced a robust analgesic effect during the first 24 after laparotomy in mice. Although it is debated whether plasma levels of 0.5 ng/mL provide adequate analgesia,[17,21,22] our data suggest that this level was adequate for controlling pain after the first 24 h after laparotomy. We saw no behavioral evidence of a loss of efficacy between 24 and 48 h in any of the parameters measured in the bup-ER group. This is likely due to the fact that mice were experiencing less pain after the first 24 h and thus no longer required the same level of analgesic to relieve pain. In more painful models, a reduction in plasma levels from 1.0 to 0.5 ng/mL could be more clinically apparent.

Anecdotal and published reports of dermal masses, abscesses, and ulcerations have been associated with the use of sustained-release buprenorphine in people and animals.[8,34] The company that produces compounded sustained-release buprenorphine attributed these adverse reactions to intradermal rather than subcutaneous administration.[44,45] Gross skin lesions or visible adverse reactions were not noted by animal care staff or by researchers during behavioral assessments of mice that had received a subcutaneous injection of bup-ER.

The main limitation of our pharmacokinetic study is that only male mice were used. The limitations of the efficacy study center on the use of a single strain of mouse and specific type of surgery for testing bup-ER. Therefore, our results may not represent the ability of this product to provide analgesia in other species, surgical models, or painful procedures. Based on convergence with buprenorphine-treated groups, the period of acute pain in saline controls for this laparotomy model appeared to end somewhere between 24 to 48 h after surgery. Therefore, future studies could investigate the efficacy of bup-ER after surgeries that cause longer periods of acute pain. In addition, behavioral and mechanical measurements of pain continue to be challenging in mice due to the subjectivity of available methods and the tendency of prey species to hide their pain.[13,19,33] We addressed any potential inconsistencies by using a single, blind observer for all assessments of pain and by including several physical and behavioral methods to assess pain.

The viscosity of bup-ER presented some difficulties when drawing up the medication for administration. Manufacturer instructions recommend using a 20- or 23-gauge needle,[11] whereas another study recommended insulin syringes to minimize loss.[18] For this study, a 23-gauge needle was used for ease of withdrawal. However, a marked amount of hub loss was noted with this method when reviewing our controlled substance records. Although the manufacturer estimates that a 3-mL vial of bup-ER can provide 60 full doses, we suspect that hub loss would prevent use of the full volume provided. This anticipated loss may also influence its use when considering the price of bup-ER compared with bup-HCl.

In conclusion, this study demonstrates that bup-ER is a comparable alternative to bup-HCl for postoperative pain management after mouse laparotomy. This medication can be safely administered before anesthesia and surgery, and a single subcutaneous dose can provide adequate analgesia for up to 48 h, based on pharmacokinetic and clinical data. Bup-ER offers a welfare advantage for laboratory rodents as it requires only one injection and thus translates into less frequent handling and less stress for animals as compared with bup-HCl.

Acknowledgments

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