Journal of the American Association for Laboratory Animal Science
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Vol 00, No 00
Month 2023
Pages 1–7

Comparing Three Formulations of Buprenorphine in an Incisional Pain Model in Mice

Giselle Lopez-Echeverria,[1,*] Eden Alamaw,[1] Gregory Gorman,[3] Katechan Jampachaisri,[2] Monika K Huss,[1] and Cholawat Pacharinsak[1]

This study compared the therapeutic effects in mice of 3 different formulations of buprenorphine. These formulations were standard buprenorphine hydrochloride (Bup-HCL) and 2 different extended-release buprenorphine formulations (Bup-ER and Ethiqa-XR [Bup-XR]). Drugs were evaluated based on their ability to attenuate thermal hypersensitivity in a mouse plantar incisional pain model. We hypothesized that Bup-HCL would attenuate postoperative thermal hypersensitivity at 20 min after administration, and that Bup-ER and Bup-XR would attenuate thermal hypersensitivity at 40 min after administration. Male C57BL6/J mice were randomly assigned to 1 of 4 treatment groups: 1) saline, 5 mL/kg SC, once; 2) Bup-HCL, 0.1 mg/kg SC, once; 3) Bup-ER, 1 mg/kg, SC, once; and 4) Bup-XR, 3.25 mg/kg, SC, once. Thermal hypersensitivity was assessed on the day before surgery and again on the day of surgery at 20, 40, 60, 90, and 120 min after drug administration. Thermal hypersensitivity after surgery was not different among the Bup-HCL, Bup-ER and Bup-XR groups at any timepoint. In addition, all buprenorphine treatment groups showed significantly less thermal hypersensitivity after surgery than did the saline group. Subjective observations suggested that mice that received Bup-ER or Bup-XR became hyperactive after drug administration (83 and 75% of mice tested, respectively). Our results indicate that Bup-HCL, Bup-ER, or Bup-XR attenuate thermal hypersensitivity related to foot incision by 20 min after administration.

Abbreviations and Acronyms: Bup-ER, extended-release buprenorphine; Bup-HCL, buprenorphine hydrochloride; Bup-XR, extended-release buprenorphine (Ethiqa-XR)

DOI: 10.30802/AALAS-JAALAS-23-000011

Introduction

Providing effective and continuous analgesia is essential for preventing pain and distress in research animals. Uncontrolled pain poses ethical concerns and reduces animal welfare, which may alter scientific results.[14] Analgesics are required for animals undergoing painful surgical procedures, and effective management of postoperative pain is an essential component of animal welfare that is emphasized in *The Guide for the Care and Use of Laboratory Animals*.[2]

Buprenorphine-HCL (Bup-HCL) is an analgesic that is commonly used for the management of postoperative pain in rodents.[31] However, because of its short duration (3 to 12 h), this drug requires repeated dosing.[10,23,32,53,54] Two different extended-release injectable formulations of buprenorphine are commonly used in rodent research: Bup-ER (ZooPharm) and Ethiqa-XR (Bup-XR; Fidelis Animal Health). These formulations provide consistent analgesia for over 72 h, decreasing the need for frequent handling and injection.[10,17,29] Bup-ER and Bup-XR both use slow-release drug delivery technology[15,46] which gradually releases buprenorphine over time. Bup-ER uses a polymeric delivery system consisting of buprenorphine dissolved in a liquid polymer composed of lactide and caprolactone.[22] When Bup-ER is injected, buprenorphine is released slowly as the polymer biodegrades, leading to a drug delivery system that releases buprenorphine over several days.[33,46] For Bup-XR, buprenorphine is lipid-encapsulated and suspended in medium chain fatty acid triglyceride oil, which slowly release the drug over time.[45,46] Previous research has determined that Bup-ER[10,54] and Bup-XR[40,46] provide effective analgesia in rats and mice for up to 72 h. To the best of our knowledge, the onset of analgesia for Bup-ER and Bup-XR has not been previously reported.

Limited data are available on the pharmacokinetics (PK) and clinical efficacy for extended-release buprenorphine formulations. A previous study evaluated the pharmacokinetics of buprenorphine in mice after an IV dose of 2.4 mg/kg beginning at 5 min after administration, at which time the plasma level was well above the accepted therapeutic concentration (1 ng/mL).[28,61] Another study[12] evaluated the plasma buprenorphine concentration after SQ administration of Bup-XR (3.25 m/kg) in mice; at 30 min after administration (the earliest timepoint measured), the buprenorphine concentration was 1.68 ± 0.84 ng/mL, which is higher than the therapeutic plasma buprenorphine concentration (1 ng/mL).[28] However, whether clinical analgesia occurs at these earlier timepoints is unknown, as there can be a difference between plasma drug concentration and effect.[50] The earliest timepoints that have been evaluated in terms of clinical analgesic efficacy are 1 to 2 h for Bup-ER in mice (hypersensitivity or activity models),[10,36] and 24 h in rats (hypersensitivity model).[13] The earliest timepoints that have evaluated clinical analgesic efficacy for Bup-XR are 4 h for mice[46] and rats[4] (hypersensitivity model). These time lapses between dosing and testing create concern and uncertainty regarding the time of onset of analgesic efficacy for Bup-ER and Bup-XR, especially given their extended-release formulation

Submitted: 02 Feb 2023. Revision requested: 08 Mar 2023. Accepted: 10 Apr 2023.
[1]Department of Comparative Medicine, Stanford University, Stanford, California; [2]Department of Mathematics, Naresuan University, Phitsanulok, Thailand; and [3]Pharmaceutical Sciences Research Institute, McWhorter School of Pharmacy, Samford University, Birmingham, Alabama
**Corresponding author. Email: glopezec@stanford.edu*

technology which may cause delay in the time to attenuate hypersensitivity.

Preemptive analgesia can reduce the possibility of intraoperative nociception and decrease the duration and severity of postoperative pain.[37,39] Rodent surgical procedures commonly use isoflurane as an anesthetic agent; isoflurane provides a rapid rate of induction and recovery from anesthesia,[3] but does not provide analgesia.[11,25] Frequently, rodents receive analgesics during preparation for a surgical procedure[10,13,14,17,23,25,29,35,37,39,54]; however, surgical preparation time may require only a few minutes, and the procedures themselves may also be of short duration (5 to 15 min).[41,51] Because the time of onset of an analgesic effect is unknown, it is also not known when Bup-ER or Bup-XR must be administered in relation to surgery and recovery to provide clinically effective analgesia.

The aim of this study is to examine if the onset of the analgesic effect of Bup-HCL, Bup-ER, and Bup-XR was within 20 min using the incisional pain model, hot plate assay, and plasma buprenorphine concentration in mice. We hypothesized that Bup-HCL would provide effective analgesia in a mouse incisional pain model within 20 min after administration, but that Bup-ER and Bup-XR would not provide effective analgesia until 40 min.

Materials and Methods

Mice. Adult male C57BL/6 mice (*Mus musculus*; *n* = 80; weights of 25 to 30 g; The Jackson Laboratory, Bar Harbor, ME) were used for this study. Mice were healthy and free of minute virus of mice, mouse hepatitis virus, mouse rotavirus, Theiler murine encephalomyelitis virus, Sendai virus, murine adenovirus 1 and 2, mouse parvovirus, mouse norovirus, ectromelia virus, lymphocytic choriomeningitis virus, pneumonia virus of mice, respiratory enteric virus 3 (Reovirus 3), *Mycoplasma pulmonis*, endo- and ectoparasites, and pinworms. Mice were group housed in individually ventilated cages (Innovive, San Diego, CA) on ALPHA-dri bedding with Enviro-dri (Lab Supply, Fort Worth, TX) for bedding and enrichment. Mice had unrestricted access to a commercial diet (Teklad Global 18% Protein Rodent Diet 2018; Harlan Laboratories, Madison, WI) and to acidified water in bottles (Innovive, San Diego, CA) ad libitum. Rooms were maintained under climate-controlled conditions, on a 12:12-h light:dark cycle at a temperature of 20 to 26 °C and 30% to 70% relative humidity. All experiments were conducted with approval by the Administrative Panel for Laboratory Animal Care at Stanford University (APLAC). All mice were treated in accordance with the *Guide for the Care and Use of Laboratory Animals*.

Mice were acclimated in the facility for a minimum of 3 d prior to baseline testing. All mice were weighed at baseline and again on days 1 and 2 after surgery. At the end of the study, mice were euthanized by carbon dioxide asphyxiation followed by cervical dislocation. The mice used for plasma collection were euthanized with an isoflurane overdose and exsanguination followed by cervical dislocation.

Group size determinations. A power analysis for group size determinations was determined for the thermal hypersensitivity testing. The 12 animals per timepoint used gained 82.6% power analysis, 0.2 effect size, and 0.45 correlation among repeated measures. For the pharmacokinetic study, the main purpose for this part was to show plasma concentration levels to confirm the limit quantification and not to statistically compare them, therefore two mice per timepoint were used and a statistical comparison for the plasma samples was not performed.

Figure 1 shows the study design for the incisional pain model and pharmacokinetic studies described below.

Surgery: Incisional pain model. Anesthesia was induced in mice (*n* = 48) by administering 5% isoflurane in 100% O_2

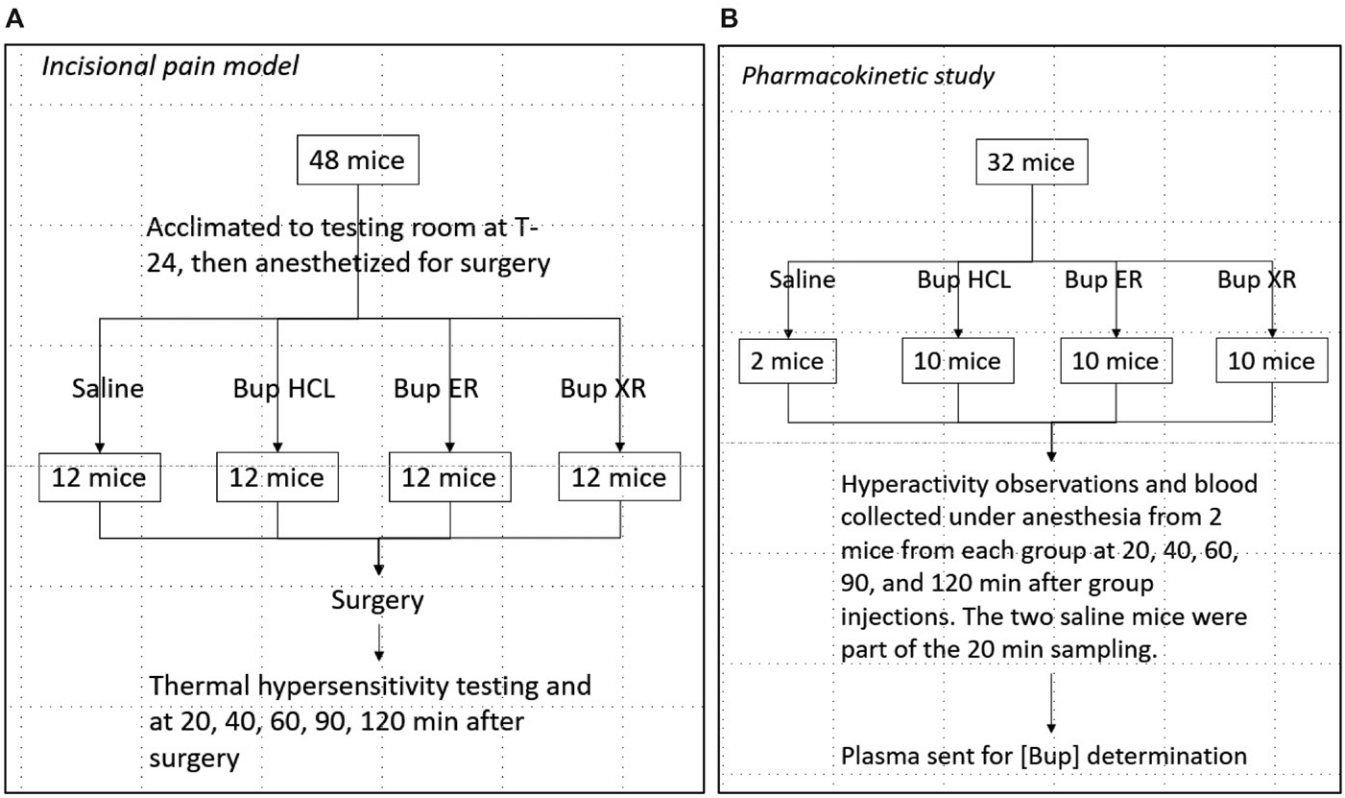

Figure 1. Study design for the incisional pain (A) and pharmacokinetic studies (B).

(1 to 2 L/min) in an induction chamber. Anesthesia was maintained using 1 to 3% isoflurane administered by nose cone. The mice were placed in sternal recumbency with the left (ipsilateral) hind paw dorsal surface down [the right (contralateral) hindpaw was served as control (no surgery on contralateral hindpaw)]. Mice were then placed on a circulating warm-water blanket, and the eyes were treated with sterile ophthalmic ointment. The plantar surface was aseptically prepared with betadine scrub and alcohol. A single dose of cefazolin (20 mg/kg SC; GlaxoSmithKline, Research Triangle Park, NC) and warmed 0.9% saline (5 mL/kg) were injected SC between the shoulder blades 1 to 2 min before making the incision in order to replicate other previous studies.[6,46] Sufficient anesthetic depth was confirmed before incision by testing for an absence of a withdrawal movement by a toe pinch. A 0.5-cm longitudinal skin incision was made using #15 blade. The underlying plantaris muscle was then elevated using iris tissue forceps and transected longitudinally with the point of a #15 blade without disturbing the attachment. Fine tipped curved forceps were then inserted into the incision and were used to elevate the muscle for 5 s. Saline (0.9% NaCl; Hospira, Lake Forest, IL) was applied to the surgical site and blotted with a sterile cotton swab to remove excess fluid. Gentle pressure was applied with sterile gauze until hemostasis is achieved. The incision was then immediately closed using one horizontal mattress suture of 5-0 silk suture. After surgery, mice were placed in a recovery cage placed over a warm-water blanket and were monitored until recovery. Mice were returned to their home cage once fully recovered.

Study design for incisional pain model. C57BL6/J mice were randomly assigned to one of 4 treatment groups (n = 12 mice per group). Mice received either 1) Saline (5 mL/kg SC; 0.9% NaCl, Hospira;, Lake Forest, IL, 0.13 cc); 2) buprenorphine hydrochloride (0.1 mg/kg SC; Bup-HCL, 0.3 mg/mL, Hospira; Lake Forest, IL, 0.01 cc); 3) extended-release buprenorphine (1 mg/kg SC; Bup-ER, 0.5 mg/mL, ZooPharm; Fort Collins, CO, 0.05 cc); or 4) extended-release buprenorphine (3.25 mg/kg, SC; Bup-XR, 1.3 mg/mL; Fidelis, North Brunswick, NJ, 0.06 cc). All injections were administered between the scapulae 1 to 2 min before the skin incision. Bup-HCL, Bup-ER, and Bup-XR were administered using a 1-ml Luer lock syringe and 22-gauge needle. To prevent leakage, digital pressure was applied at the injection site for 5 s.

Thermal hypersensitivity testing To determine baseline values, mice were acclimated in the testing room for 15 to 30 min before testing on the day before surgery. Variability in thermal hypersensitivity testing and time from D-1 and time of surgery was not significant. Thermal hypersensitivity testing was then performed between 0800 and 1200 at 20, 40, 60, 90, and 120 min after surgery by observers who were blinded to the treatment group.

An aluminum hot-plate apparatus (IITC Life Science) was used to assess thermal hypersensitivity. The temperature of the hot-plate was set and calibrated at a constant 52 °C. A clear acrylic open-top barrier surrounded the surface of the hot-plate. A tripod holding a recording device was directed at the mouse, hot plate, timer, and mirror. The mirror was placed directly behind the hot-plate apparatus to allow observers to clearly see the video recordings. An unrestrained mouse was placed on the metal surface of the hot plate and was recorded on video to detect latency for nocifensive behaviors. Nocifensive behaviors on either on the incised hind paw or the contralateral hind paw included licking a hind paw, jumping, lifting a hind paw (entire paw or toes only, without taking a step), or fluttering a hind paw (shaking the paw while standing or stepping). The mouse was removed from the hot plate after showing nocifensive behaviors or a predetermined cut-off time of 30 s to avoid tissue injury. The 30-s cutoff is based on the authors' experiences with the hotplate testing modality.[23]

Video recordings were evaluated by 2 observers who were unaware of the treatment group. Thermal latency was defined as the time taken to observe nocifensive behaviors (or time to removal from the plate. Thermal hypersensitivity was defined as the significant decrease in thermal latency.

Pharmacokinetic study. The mice used in this study were observed behaviorally for general activity levels throughout the testing by observers who were blind to the treatment group. Hyperactivity was defined as a visual perception of an increase in locomotor activity consisting of repetitive rotations and increased exploratory activity at 20, 40, 60, 90, and 120 min. The activity level of each mouse was scored as increased or normal.

To analyze plasma drug concentration, a total of 32 mice were randomly assigned to the following treatment groups: 1) saline; 5 mL/kg SC (n = 2); 2) buprenorphine hydrochloride 0.1 mg/kg SC (n = 10); 3) extended-release buprenorphine (ZooPharm) 1 mg/kg SC (n = 10); or 4) extended-release buprenorphine (Fidelis Animal Health) 3.25 mg/kg, SC (n = 10). These mice were solely used for plasma collection and did not receive an incision or thermal hypersensitivity testing. For drug administration, mice were induced with 5% isoflurane in 100% O_2 (2 L/min) using an induction chamber. Two mice were used for each timepoint (20, 40, 60, 90, and 120 min) and treatment. For blood collection, the mice were anesthetized as described for drug administration. After a surgical plane of anesthesia was confirmed by toe pinch, whole blood was collected by retroorbital collection followed by cervical dislocation. A minimum of 0.8 mL of whole blood was collected in 1-mL lithium heparin microtainers. The blood samples were spun in a microcentrifuge at 2,500 rpm for 20 min. A minimum plasma volume of 0.25 mL was collected and transferred into 1-mL cryogenic tubes. Samples were stored in a −80 °C freezer before shipment for analysis.

Plasma samples were sent to McWhorter School of Pharmacy Pharmaceutical Sciences Research Institute (Samford University, Birmingham, AL) for analysis. Liquid chromatography-tandem mass spectrometry was used to measure plasma buprenorphine concentration. Buprenorphine standard solutions were prepared by spiking 50:50 DI water:acetonitrile to provide concentrations ranging from 0.2 to 200 ng/mL. Buprenorphine plasma samples and standards (100 µL) were fortified with internal standard (50 ng/mL terfenadine). Acetonitrile (1 mL) was added to precipitate the plasma proteins, was added, and the mixture was vortexed and centrifuged. The organic layer was moved to a clean test tube and evaporated to dryness under nitrogen in a 50 °C water bath set. Samples were reconstituted in diluted solvent and analyzed by HPLC MS/MS. Matrix matched standards and QC samples were prepared using blank control plasma. A noncompartmental pharmacokinetic analysis was performed for plasma buprenorphine concentration by using PKSolver 2.0 software.

Statistical analysis. The measurement for the time until thermal hypersensitivity was taken as continuous parametric methods of analysis were used. To assess significance of differences in thermal hypersensitivity responses by group and over time, 2-way repeated measures ANOVA with Bonferroni correction for multiple comparisons (R Development Core Team, Vienna, Austria) was performed (pharmacokinetic study was not statistically analyzed). Data are expressed as mean ± SEM. A P value of less than 0.05 was considered significant.

Results

Thermal hypersensitivity. Thermal hypersensitivity was not different between treatment groups at baseline (D-1). Mice in the saline (control) group had significantly ($P < 0.05$) lower thermal latency at all postoperative timepoints (20, 40, 60, 90, and 120 min) as compared with baseline (Figure 2). Thermal latency in the Bup-HCL, Bup-ER, and Bup-XR groups did not significantly differ from baseline, or from one another, at any of the timepoints tested on day 1 after surgery (20, 40, 60, 90, and 120 min). Thermal latency of the saline group was significantly lower ($P < 0.05$) than that of Bup-HCL, Bup-ER, and Bup-XR groups for all postoperative timepoints (20, 40, 60, 90, and 120 min).

Behavioral observations in the thermal hypersensitivity group. After drug administration, locomotor activity was significantly increased in Bup-ER (83%) and Bup-XR (75%) treated groups after surgery based on subjective observations. The hyperactivity began as early as 20 min after injection and continued throughout the study (120 min). Hyperactivity was not observed in the saline or Bup-HCl groups. No other abnormal behaviors were noted.

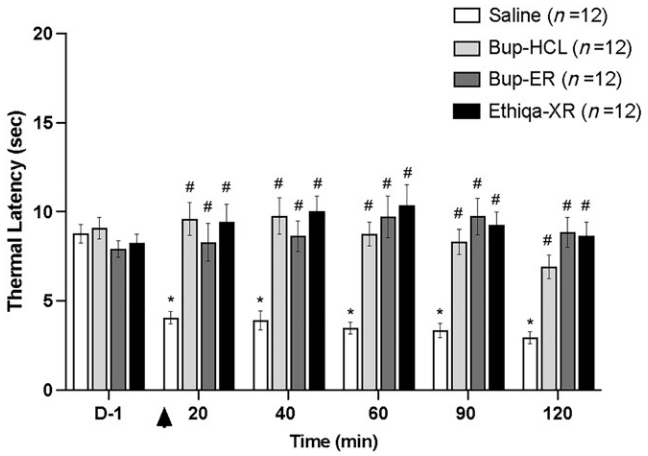

Figure 2. Thermal hypersensitivity (mean ± SEM) of ipsilateral and contralateral paws. Arrow indicates the time of treatment. *Significantly different ($P < 0.05$) from baseline within the treatment group. #Significantly different ($P < 0.05$) from saline at the specified timepoint.

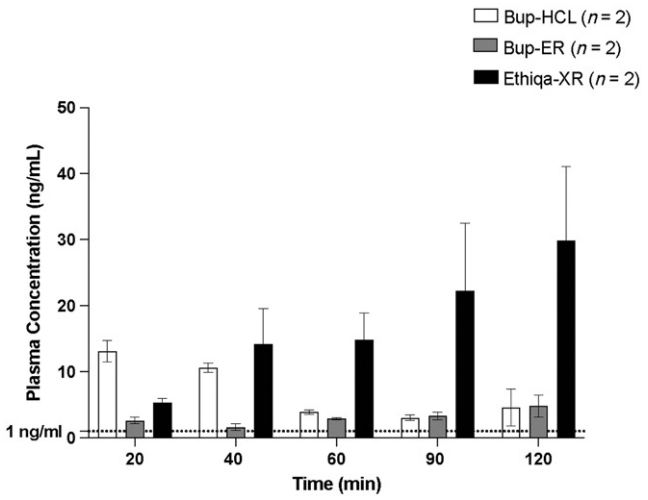

Figure 3. Plasma drug concentrations (ng/mL, mean ± SEM) in mice (n = 2/group/timepoint) treated with either Bup-HCL, Bup-ER, or Bup-XR. Samples were analyzed at 20, 40, 60, 90, and 120 min after drug administration. (n = number of mice at each timepoint).

Plasma buprenorphine pharmacokinetic study. Plasma buprenorphine concentrations as a function of time are shown for each dose in Figure 3. The measured plasma concentrations were above the generally accepted therapeutic range (1 ng/mL) for all treatment groups at all measured timepoints. In the Bup-HCL group, the highest plasma buprenorphine concentration was detected at 20 min after drug administration and subsequently fell until the final timepoint at 120 min (Figure 3). At 20 min, plasma concentrations of Bup-HCL were higher than the concentrations of Bup-SR and Ethiqa-XR. In contrast to the Bup-HCL group, plasma buprenorphine concentration in Bup-ER and Bup-XR groups gradually increased over time and peaked at 120 min. Due to the short duration of the experiment, the terminal elimination phases for the Bup-ER and Bup-XR groups were not reached and so PK data was not calculated for these groups. Saline injected mice were tested at the 20 min timepoint and were negative (plasma buprenorphine level was not detected).

Discussion

This study demonstrates that all 3 buprenorphine formulations (Bup-HCL, Bup-ER, and Bup-XR) effectively attenuate postoperative thermal hypersensitivity as soon as 20 min after administration in a mouse model of incisional pain. No significant differences were detected in thermal hypersensitivity attenuation among Bup-HCL, Bup-ER, or Bup-XR at any study timepoints (20, 40, 60, 90, and 120 min). The plasma buprenorphine concentration was at or above the therapeutic concentration (1 ng/mL[28]) for all 3 treatment groups at all timepoints (20, 40, 60, 90, and 120 min).

The aim of this study was to determine whether postoperative thermal hypersensitivity would be attenuated within 20 min after treatment with 1 of 3 different formulations of buprenorphine in a model of incisional pain. This is a well-established model that produces mild to moderate thermal hypersensitivity for 2 to 7 d in mice.[4,7,8,18,46,49] In this study, saline-treated mice showed thermal hypersensitivity that was present at 20 min after surgery and lasted for the 120 min duration of the study.

The hot plate assay was used to assess thermal hypersensitivity.[38] Thermal latency was defined as the time taken for a mouse to exhibit nocifensive behavior involving ipsilateral or contralateral hindlimbs after placement on the hot plate.[10] Although nocifensive behaviors can involve both fore and hind limbs, we recorded only those involving the hind limbs. Hind limb nocifensive behaviors are reportedly a more reliable indicator of thermal hypersensitivity than as forelimbs, which are frequently used for grooming and exploration and have inconsistent contact with the metal surface.[20] The nocifensive behaviors we used consisted of licking a hind paw, lifting a hind paw independent of taking a step, jumping, or fluttering a hind paw while standing or stepping.[10] Exploratory behaviors (walking, sniffing, urinating, defecating, rearing, and lifting a front paw) were excluded.[10] If the mouse did not display nocifensive behaviors within the predetermined cut-off time (30 s), the mouse was removed from the hot plate to prevent tissue damage.[10,44,56,58]

Preemptive analgesia is often recommended before any surgical or painful procedure to prevent peripheral or central sensitization. Therefore, knowledge of the onset of the analgesic effect is vital to ensure that the correct timing of analgesic administration in relation to surgery. Thermal hypersensitivity for Bup-HCL, Bup-ER, and Bup-XR did not differ at any study timepoint (20, 40, 60, 90, or 120 min) as compared with their preoperative baseline values. All buprenorphine treated groups

had significantly longer thermal latency times as compared with the saline treated group at all study timepoints. This indicates that all 3 buprenorphine formulations (Bup-HCL, Bup-ER, and Bup-XR) attenuated hypersensitivity as compared with saline at all study timepoints (20, 40, 60, 90, and 120 min). These current results differed from those in another study[36] in which different testing modalities were used (general activity/running wheels/nesting (non-stimulus evoked testing) vs thermal hypersensitivity (stimulus evoked hotplate testing from this current study). Other factors for these different outcomes include test conditions, time of testing, environment/facilities, different personnel, age, sex [male C57BL/6 compared with female Crl:CD1(ICR)], and models used (incisional pain compared with laparotomy models), vendors (Jackson compared with Charles River Laboratories)].[9,20,34,52,55,63] In addition, all buprenorphine groups achieved therapeutic plasma buprenorphine concentrations (1 ng/mL)[25] throughout the study period (20, 40, 60, 90, and 120 min).

Bup-HCl, Bup-ER, and Bup-XR produced different plasma buprenorphine concentrations during the study. For the Bup-HCL group, the plasma buprenorphine concentration was highest at the 20 min and decreased thereafter at each timepoint. In contrast to Bup-HCL, plasma buprenorphine concentration in Bup-ER and Bup-XR groups gradually increased over time and peaked at 120 min. This was presumably due to carrier vehicles that release buprenorphine over an extended period. Our previous studies in C57BL6/J and NSG immunodeficient mice showed that plasma buprenorphine concentration peaked within 4 to 8 h after drug injection followed by a gradual decline over the course of the study.[6,46] In the present study, the elimination phase of the drugs had not begun at the 120-min timepoint and a decline in plasma concentration was not observed. Therefore, we could not calculate the half-life, Cl, and Vd. Buprenorphine plasma concentrations appeared to be higher in the Bup-XR group than in the other buprenorphine groups. This could be due to the differences in carrier vehicles and doses (Bup-XR, 3.25 mg/kg; Bup-ER, 1 mg/kg; and Bup-HCL, 0.1 mg/kg). The highest plasma concentration timepoint for the Bup-XR group was not associated with a significant effect on attenuation of thermal hypersensitivity as compared with the other treatment groups at that timepoint. Because only 5 to 10% of mu opioid receptors are required to provide buprenorphine-induced analgesia, it is possible that at this plasma concentration, all mu receptors were all saturated with buprenorphine.[5,59,26,27,42,62] Therefore, bell-shaped curve effects of buprenorphine (with a plateau of hypersensitivity response) occur despite higher drug concentration.[21,42]

While buprenorphine formulations are considered to have a wide safety margin,[4,23,60] reported side effects are respiratory depression,[19] cardiovascular depression,[16] sedation,[14] pica,[1,4,23] nausea,[57] constipation,[57] disturbed circadian rhythm,[31] and body weight changes.[4,8,23] Hyperactivity is another side effect that has been noted with extended-release formulations of buprenorphine.[46] Hyperactivity can occur after buprenorphine administration as a result of activation of μ opioid receptors.[43] In this study, we also observed hyperactivity in the Bup-ER and Bup-XR treatment groups. Although this hyperactivity may affect thermal latency observation, both blind observers were focused only on the endpoint behaviors previously described. In addition, thermal latency readings from both observers were similar throughout the study. In a previous study with C57BL6/J male mice, hyperactivity was observed at 4 and 24 h after administration of mice treated with Bup-ER (83 and 17%, respectively) and Bup-XR (83 and 67%, respectively).[46] Another study evaluating Bup-ER (0.6 to 1.5 mg/kg SC) reported hyperactivity at 4 h in Swiss-Webster mice.[30] In our current study, the only abnormal clinical observation noted was hyperactivity, which was seen in Bup-ER (83%) and Bup-XR (75%) at 20 min and lasted throughout the study (120 min).

This study has some limitations: 1) Because each timepoint included only 2 mice, any true differences between the groups at any of the studied timepoints may have been missed due to insufficient power; 2) the hot plate test can result in learned behavioral responses possibly leading to faster reaction times after multiple exposures to the hot plate.[20,24,48] Our study did not test the mice over multiple days and we saw no apparent learned behavior, but with thermal hypersensitivity studies that last multiple days, this could be a confounding factor[20]; 3) during the hot plate assay, all 4 paws and the tail were exposed to the heat stimulus. According to the literature, this is generally not an issue when systematically testing the effects of compounds, but it could increase the pain or discomfort felt by the mouse[20]; 4) we only evaluated evoked thermal hypersensitivity. We did not use other experimental methods of pain evaluation such as mechanical hypersensitivity or spontaneous behavior evaluation because of the many timepoints we wanted to evaluate during the study, which left time only for testing thermal hypersensitivity; 5) a challenge in this study was assessing some of the more subtle nocifensive behaviors, such as a fluttering hind paw. These subtle behaviors required subjective assessment of the video recording by the observers. To minimize these variables, 2 observers confirmed behavior evaluation during the observation of video recordings and the recorded thermal latency was averaged; 6) the earliest timepoint used to evaluate the onset of analgesic effect was 20 min after administration. The onset of analgesia could have occurred much earlier than 20 min. We chose 20 min as the earliest timepoint to evaluate for practical reasons. The time needed for mouse animal preparation, surgery, and recovery was approximately 7 to 12 min. Furthermore, we wanted to avoid any confounding factors associated with the residual effects of isoflurane anesthesia and allow sufficient time for the mice to fully recover; 7) we only used one pain model (an incisional pain model). We used this model because it is a well-established way to produce mild to moderate thermal hypersensitivity[4,7,8,18,46,49]; and 8) we only used male mice for this study, even though female mice show more hypersensitivity after injury.[47] We used only males to reduce the number of mice we needed to evaluate to determine hypersensitivity efficacy between timepoints. Finally, we evaluated only one dosage of each formulation of buprenorphine. We used the manufacture's recommended dosages[1,10] and dosages that we have previously used in the incisional pain model.[46] The use of lower or higher doses of each buprenorphine formulation could change study results.

The primary goal of this current study was to compare the analgesic efficacy of 3 commonly used buprenorphine formulations using thermal hypersensitivity testing. Our study used thermal hypersensitivity attenuation to show that Bup-HCL (0.1 mg/kg, SC), Bup-ER (1 mg/kg, SC), and Bup-XR (3.25 mg/kg, SC) were effective at 20 min after administration mice with incisional pain. Based on these results, we recommend that all 3 buprenorphine formulations (Bup-HCL, Bup-ER, and Bup-XR) be administered 20 min before surgery or a painful procedure.

Acknowledgments

We would like to thank Janis Atuk-Jones, Benjamin Franco, and Dr Gregory Gorman at Samford University (plasma analysis). The Stanford Department of Comparative Medicine supported this study.

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