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Important Update:

In order to remain compliant with the most current regulatory guidelines, we have updated the labeling on our SR formulations from Buprenorphine and Meloxicam SR to Buprenorphine and Meloxicam in Polymer. **As of April 1, 2024, SR preparations mentioned in the attached study are now labeled as in Polymer**, with no changes to the formulation of the medication(s).

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Antinociceptive Effects of Sustained-Release Buprenorphine in a Model of Incisional Pain in Rats (*Rattus norvegicus*)

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Effective management of postoperative pain is an essential component of the care and welfare of laboratory animals. A sustained-release formulation of buprenorphine (Bup-SR) has recently been introduced to the veterinary market and has been reported to provide analgesia for as long as 72 h. Using evoked mechanical and thermal hypersensitivity tests, we here evaluated the antinociceptive effects of Bup-SR in a model of incisional pain in rats. Paw withdrawal responses were obtained before and 1 through 4 d after surgery. Rats are assigned to receive Bup-SR (0.3, 1.2, or 4.5 mg/kg SC once) or buprenorphine HCl (Bup HCl, 0.05 mg/kg SC twice daily for 3 d). Responses to mechanical and thermal stimuli in the 1.2 and 4.5 Bup-SR groups did not differ from those of rats in the Bup HCl group. Thermal latency on day 3 in rats that received 0.3 mg/kg Bup-SR was significantly different from baseline, indicating that this dose effectively decreased thermal hypersensitivity for at least 48 h. Marked sedation occurred in rats in the 4.5 Bup-SR group. Our findings indicate that Bup-SR at 0.3 or 1.2 mg/kg SC is effective in minimizing hypersensitivity with minimal sedation for at least 48 h (thermal hypersensitivity) and 72 h, respectively, in the incisional pain model in rats.

Abbreviations: Bup HCl, buprenorphine HCl; Bup-SR, sustained-release buprenorphine.

Effective management of postoperative pain management is an essential component of animal welfare that is emphasized in the 8th edition of *The Guide for the Care and Use of Laboratory Animals*.[17] Not only is controlling pain an ethical obligation, but uncontrolled pain can act as a stressor, leading to the deterioration of the animal and contamination of research results. Adequate treatment of postoperative pain is essential, because postoperative pain can alter cardiovascular function, prevent normal pulmonary function, and change hemodynamic values.[22]

Buprenorphine HCl (Bup HCl) is a standard of care for postoperative analgesia in rodents.[9] It is an opioid with both partial μ receptor agonistic and κ and δ receptor antagonistic activities.[23,30] It has a high therapeutic index[7,33] and is used ubiquitously in the laboratory environment for pain management.[20,29] Bup HCl has been shown to have analgesic properties both in acute and chronic rodent pain models and even shows promising results in the reduction of neuropathic pain.[4] Bup HCl is more effective in managing pain than are carprofen, ketoprofen, acetaminophen, tramadol, and tramadol–gabapentin.[25,26] Although Bup HCl provides effective analgesia, it also can have negative clinical side effects after administration, including decreased body weight gain,[1] pica,[5] respiratory depression,[10] and decreased water consumption.[16,18] When buprenorphine HCl is used acutely, it does not alter natural killer cell or macrophage activity[15,28]

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Important limitations of Bup HCl include the duration of action and method of administration. Administration of Bup HCl at 0.05 mg/kg has proven to be the standard of care, but doses must be administered at least every 12 h.[9,26] Handling, restraint, and readministration of the drug increases stress to the animal.[27] Recently introduced to the veterinary field, a sustained-release formulation of buprenorphine (Bup-SR) may eliminate (or at least greatly reduce) redosing requirements. A previous study[13] in rats found that buprenorphine-SR is adequate for providing analgesia at 1.2 mg/kg (calculated as 0.2 mg/kg every 12 h for 72 h) in a tibial defect model and is capable of attenuating thermal sensitivity of the hindpaw. In light of these results, the authors[13] concluded that Bup-SR may be an effective alternative for treating postsurgical pain in this model. In addition, Bup-SR has been tested in noninjured mice by using the hot-plate assay, and findings show that Bup-SR is effective for at least 12 h in male BalbC/J and SWR/J mice.[3]

The aim of the current study was to investigate the antinociceptive effects of Bup-SR in the plantar incisional pain model in rats.[2] This well-established model recapitulates postoperative pain due to injury or a minor procedure. Our group has extensive experience with this model, and we find that this model is reproducible, produces mechanical and thermal hypersensitivity, and leads to mild to moderate pain in rats. In previous studies using this model,[26] we found that rats showed signs of thermal hypersensitivity for as long as 4 d but that mechanical weight-bearing was decreased for only 1 d after surgery. We hypothesized that the antinociceptive effects of Bup-SR at all doses is comparable to those of twice-daily dosing of Bup HCl.

Materials and Methods

Animals. Adult male Sprague–Dawley rats (*Rattus norvegicus*; n = 21; weight, 330 to 375 g; Charles River, Wilmington, WA)

were used. Rats were free of rat coronavirus, rat Theiler virus, Kilham rat virus, rat parvovirus, Toolan H1 virus, rat minute virus, lymphocytic choriomeningitis virus, murine adenovirus types 1 and 2, reovirus type 3, Sendai virus, pneumonia virus of mice, *Mycoplasma pulmonis*, mites, lice, and pinworms. Rats were pair or singly housed in static microisolation cages on a 12:12-h dark:light cycle. They were fed a commercial diet (Teklad Global 18% Protein Rodent Diet 2018, Harlan Laboratories, Madison, WI) and were provided water filtered by reverse osmosis ad libitum. All experiments were approved by the Stanford Administration Panel for Laboratory Animal Care, and all rats were treated in compliance with *The Guide for the Care and Use of Laboratory Animals*.[17] Rats were weighed on the day prior to surgery and every day postoperatively until euthanasia. At the end of the study, rats were euthanized by carbon dioxide asphyxiation followed by physical methods.

Surgery. General anesthesia was induced with isoflurane inside an induction chamber. Rats then were maintained on a nonrebreathing anesthetic circuit mask by using isoflurane in 100% O_2. Cefazolin (20 mg/kg; GlaxoSmithKline, NC) and warm 0.9% NaCl (5 to 15 mL/kg) were administered once subcutaneously prior to incision. Sterile eye lubrication was applied after induction of anesthesia, and rats were kept on a circulating warm-water blanket. The plantar surface of the left (ipsilateral) hindpaw of each rat was prepared aseptically for surgery. The incisional pain model was created as previously described.[2] In brief, at approximately 0.5 cm distal to the tibiotarsal joint, a 1-cm longitudinal skin incision extending toward the digits was made on the plantar surface of the left (ipsilateral) hindpaw. The plantaris muscle was isolated, elevated slightly, and then incised longitudinally with care to avoid trauma to sites of muscle attachment. The incision was closed with 2 interrupted horizontal mattress sutures of 5-0 polyglactin 910. Triple-antibiotic ointment was applied to the wound. All rats were monitored closely until they recovered from anesthesia and then returned to their home cage. Rats recovered from surgery for 20 to 24 h prior to behavioral testing.

Study designs. Bup HCl (0.3 mg/mL; Hospira, Lake Forest, IL) and Bup-SR (1 mg/mL; Zoopharm, Fort Collins, CO) were used in this study. Rats were assigned randomly to 1 of 4 groups: Bup HCl (n = 3), in which the rats received Bup HCl at 0.05 mg/kg SC 15 min prior to skin incision followed by Bup HCl at 0.05 mg/kg BID for 3 d thereafter; 0.3 Bup-SR (n = 6), rats received Bup-SR at 0.3 mg/kg SC 15 min prior to surgery (equivalent to 6 doses of Bup HCl at 0.05 mg/kg); 1.2 Bup-SR (n = 6), in which rats received Bup-SR at 1.2 mg/kg SC 15 min prior to surgery (dosage based on a previous study[13]); and 4.5 Bup-SR (n = 6), in which rats received Bup-SR at 4.5 mg/kg SC (equivalent to 18 doses of Bup HCl at 0.5 mg/kg).

Behavioral assessment. Prior to behavioral studies, rats were allowed 15 to 30 min to acclimate after being moved to the behavioral testing room. Rats were tested between 0900 and 1100 at 1 d prior to surgery and then once daily for 4 consecutive days after surgery.

Withdrawal responses to mechanical stimuli. Rats were placed on top of an elevated wire mesh (1 cm^2 perforations) in a clear plastic chamber (23 × 13 × 13 cm) and were allowed to acclimate to the testing environment for 15 min. Von Frey monofilaments with calibrated bending forces were used to deliver punctate mechanical stimuli (force, 10 g) to both hindpaws over 10 consecutive trials. Each stimulus was applied for approximately 1 s with an interstimulus interval of approximately 5 s (Figure 1). Care was taken to stimulate random locations on the plantar surface. The pads, toes, and heels were avoided. Paw withdrawal responses were measured as the number of times a rat completely lifted its paw off the mesh during a total of 10 stimuli. Mechanical hypersensitivity was defined as a significant increase in paw withdrawal response frequency evoked by mechanical stimuli. The right hindpaw (contralateral) served as a control.

Withdrawal responses to thermal stimuli. Radiant heat was applied to the plantar surface of the hindpaw and withdrawal response latencies were determined. Rats were placed in a clear plastic chamber (23 × 13 × 13 cm) and allowed to acclimate for 15 min before testing. A 50-W light bulb was focused on the plantar surface of the hindpaw; a 33-s cutoff was set to prevent tissue damage (Figure 2). Each hindpaw was tested 4 times, alternating between hindpaws, and with at least 1 min between trials. The heat source was focused on the middle of the plantar surface of the hindpaw. Withdrawal latency was measured as the mean of the last 3 trials, to eliminate variability in the initial latency measurement. Thermal hypersensitivity was defined as a significant decrease in paw withdrawal latency evoked by heat stimuli. The right hindpaw (contralateral) served as a control.

Statistical analyses. Mean withdrawal responses were analyzed by using repeated-measures ANOVA with Bonferroni correction for multiple comparisons (SPSS, IBM, Somers, NY) to examine differences in withdrawal responses between groups and over time. Data were expressed as mean ± SEM. A P value of less than 0.05 was considered significant.

Results

The weight of rats in the Bup HCl, 0.3 Bup-SR, and 1.2 Bup-SR groups were similar before and after surgery throughout the study. However, the weight of rats in the 4.5 Bup-SR group was clinically (>10%) but not significantly reduced on days 3 (340.75 ± 12.15 g) and 4 (350.7 ± 8.3 g) compared with the baseline value (381.25 ± 11.65 g; Figure 3).

Mechanical hypersensitivity. Mechanical hypersensitivity on days 1 through 4 after surgery in rats that received Bup-SR was no different than that of those that received Bup HCl (Figure 4). Baseline values in the ipsilateral limb (range, 0.67 ± 0.33 to 1.00 ± 0.63 foot raises) did not differ between groups. In the ipsilateral limb, mechanical hypersensitivity in the Bup HCl group on days 1 (1.67 ± 1.67 foot raises), 2 (0.33 ± 0.33 foot raises), 3 (1 ± 1 foot raises), and 4 (1 ± 1 foot raises) did not differ from the baseline value (0.67 ± 0.33 foot raises). Similarly, mechanical hypersensitivity on days 1 through 4 did not differ from the baseline values for the 0.3 Bup-SR, 1.2 Bup-SR, and 4.5 Bup-SR groups, nor were there any differences in mechanical hypersensitivity in the ipsilateral limb between Bup-SR groups throughout the study. No significant differences were detected for the contralateral hindpaw between groups at any time point (Figure 4).

Thermal hypersensitivity. On days 1 through 4 after surgery, thermal hypersensitivity in the 1.2 and 4.5 Bup-SR groups did not differ from that of rats given Bup HCl (Figure 5). For Bup HCl, differences in mean thermal hypersensitivity in the ipsilateral limb on days 1 (8.37 ± 2.60 s), 2 (11.11 ± 1.59 s), 3 (7.93 ± 1.00 s), and 4 (9.72 ± 2.16 s) did not differ from the baseline value (10.46 ± 0.61 s). Incision of the plantar aspect of the hindpaw did not significantly reduce withdrawal latencies in response to thermal stimulation in rats in the 1.2 or 4.5 Bup-SR groups. There was a significant ($P < 0.05$) difference in thermal latency for the 0.3 Bup-SR group on day 3 (7.94 ± 0.87 s) as compared with baseline values (12.98 ± 1.80 s). However, there was no significant difference in thermal hypersensitivity on day 1 (8.37 ±2.60 s) or 2 (11.11 ±1.60 s) compared with the baseline value

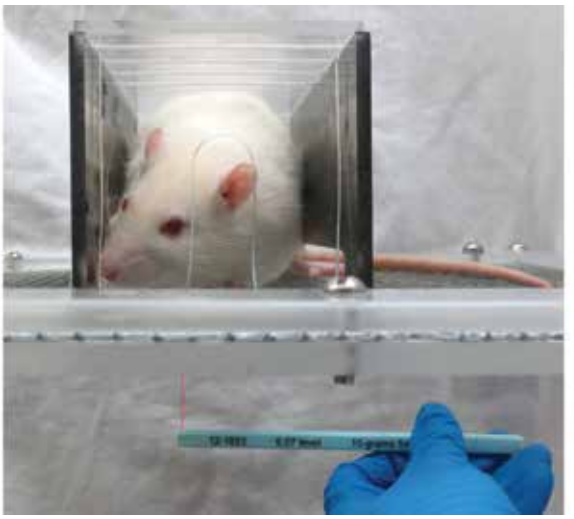

Figure 1. Experimental set-up for assessing mechanical hypersensitivity. The rat is placed on wire mesh, and the von Frey device is applied to each hindpaw 10 times.

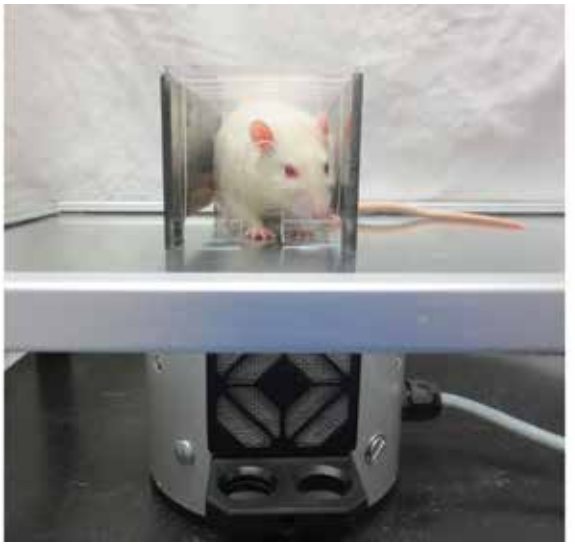

Figure 2. Experimental set-up for assessing thermal hypersensitivity. A radiant heat source is applied to the plantar aspect of the rat's hindpaws.

in the 0.3 Bup-SR group. These results indicate that rats in the 0.3 Bup-SR group failed to return to baseline thermal latency between 48 and 72 h. There were no significant differences between time points in the Bup HCl group for either the ipsilateral or contralateral paw throughout the study. Withdrawal latency in the contralateral paw differed ($P < 0.05$) between time points in the 1.2 Bup-SR and 4.5 Bup-SR groups (Figure 5), which also showed significant differences in the contralateral paw between the baseline value and day 1. This result is likely due to sedation, which was detected clinically in both groups. Sedation was severe in rats that received 4.5 mg/kg Bup-SR and mild to moderate in some rats given 1.2 mg/kg Bup-SR. Clinical signs of sedation including sleeping in the testing apparatus, lethargy, and decreased appetite.

Discussion

This present study demonstrates that, in a rat model of plantar incisional pain, mechanical and thermal postoperative hypersensitivity after twice-daily dosing with Bup HCl (0.5 mg/kg)

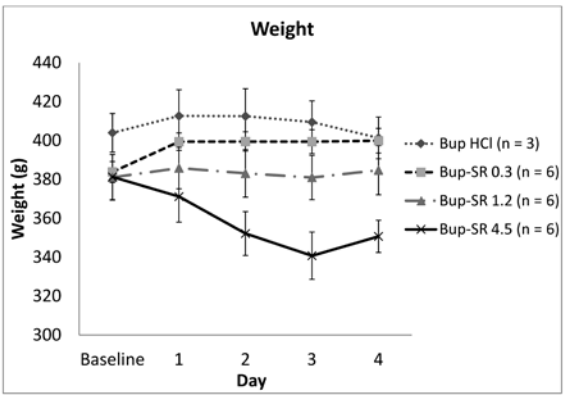

Figure 3. Weights of rats that received Bup HCl or Bup-SR at various dosages (mg/kg). *, Value is significantly ($P < 0.05$) different from baseline for group.

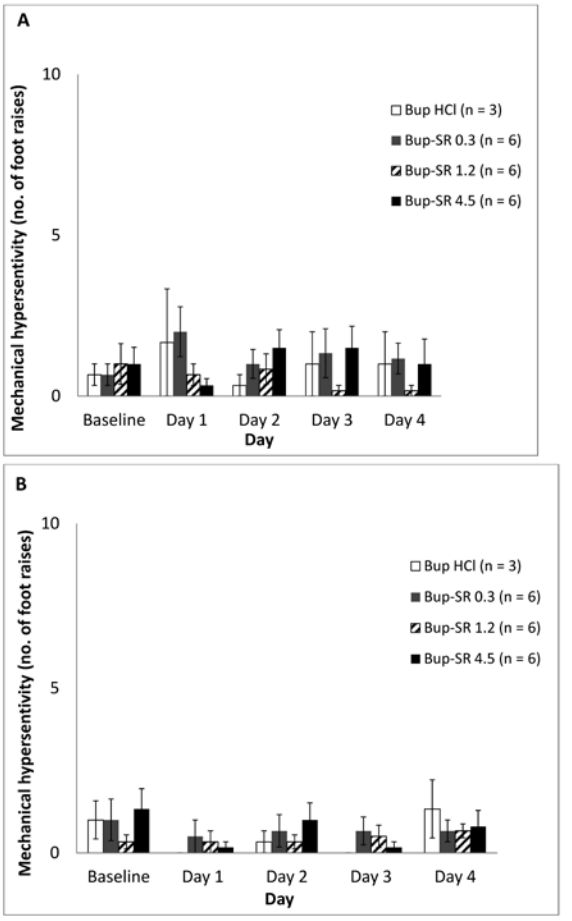

Figure 4. Effects of Bup HCl or Bup-SR at various dosages (mg/kg) on mechanical hypersensitivity (number of foot raises, mean ± SEM) of (A) ipsilateral and (B) contralateral paws. *, Value is significantly ($P < 0.05$) different from baseline for group; #, value is significantly ($P < 0.05$) different from that for Bup HCl at the same time point.

were similar to those at the preoperative baseline; single doses of Bup-SR (0.3, 1.2, and 4.5 mg/kg) are no different than twice-daily Bup HCl the control of postoperative mechanical and thermal hypersensitivity, although the duration of effect differed among doses; and, although efficacious in ameliorating postsurgical mechanical and thermal hypersensitivity, Bup-SR (4.5 mg/kg) led to weight loss and sedation. Therefore in light of these data, we recommend the use of Bup-SR at 0.3 or 1.2 mg/kg—but not 4.5 mg/kg—for the management of

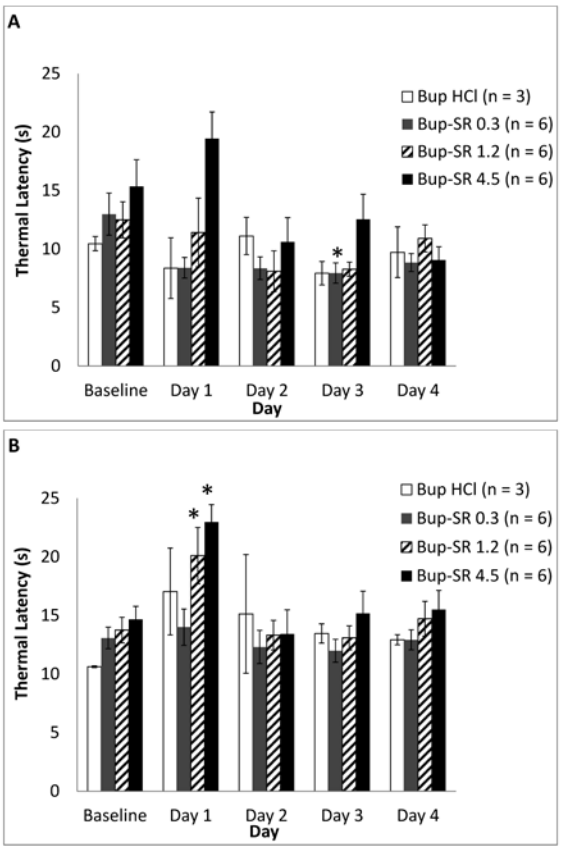

Figure 5. Effects of Bup HCl or Bup-SR at various dosages (mg/kg) on thermal latency (s; mean ± SEM) of (A) ipsilateral and (B) contralateral paws. *, Value is significantly ($P < 0.05$) different from baseline for group; #, value is significantly ($P < 0.05$) different from that for Bup HCl at the same time point.

postoperative incisional pain in male adult Sprague–Dawley rats. Actual dosing requirements may vary, depending on the weight, strain, or sex of the animals used or the level of pain associated with different experimental situations. Our results support the hypothesis that the antinociceptive effect of Bup-SR is comparable to that of twice-daily dosing with Bup HCl. Mechanical and thermal hypersensitivity in rats that received a single dose of Bup-SR at 1.2 or 4.5 mg/kg were no different for at least 72 h than those associated with twice-daily dosing of Bup HCl. Lack of a significant decrease in thermal latency through day 2 provides evidence that 0.3 mg/kg Bup-SR SC was effective in diminishing thermal hypersensitivity to a point similar to baseline for at least 48 h. Thermal latency of animals that received a single dose of 0.3 mg/kg Bup-SR was no different for at least 48 h than that of twice-daily dosing of Bup HCl. Mechanical latency in rats given a single dose of 0.3 mg/kg Bup-SR was no different for at least 72 h than that associated with twice-daily dosing of Bup HCl.

Bup HCl has been used as a standard of care for analgesia in a variety of laboratory animal species and provides effective control of mild to moderate pain,[29] multiple routes of administration, and minimal respiratory depression.[11] The use of Bup HCl at doses exceeding the maximal effective dose may have less analgesic efficacy.[32] Although Bup HCl is a controlled drug and, unlike full opiate agonists, is ineffectively antagonized by naloxone, it provides a wide margin of safety.[8] Generally, twice-daily administration of Bup HCl is recommended. In the present study, we investigated preemptive single dosing with Bup-SR at 3 different doses (0.3, 1.2, and 4.5 mg/kg) compared with a standard of care, Bup HCl at 0.05 mg/kg BID. Although minor and moderate surgical pain likely require different doses of buprenorphine HCl, a previous study[26] established that buprenorphine HCl at 0.05 mg/kg every 12 h provides adequate attenuation of hyperalgesia in the incisional pain model in rats.[26]

Although Bup HCl has been known to be generally safe, it has some side effects; dose-dependent cardiovascular depression[24] and sedation[31] and interference with gastrointestinal motility[6] and dose-dependent have all been reported in a wide range of species. In our study, 0.3 mg/kg Bup-SR did not result in any observable clinical effects. Rats in the 1.2 Bup-SR group showed only signs of mild sedation, but those in the 4.5 Bup-SR group had severe sedation. In addition, none of the rats in the Bup HCl or 0.3 and 1.2 Bup-SR groups lost more than 10% of body weight, unlike those in the 4.5 Bup-SR group, whose weight loss manifested on days 3 and 4 after administration. Despite the lack of statistically significant weight lost in the 4.5 Bup-SR rats, we recommend using lower doses Bup-SR (for example, 0.3 or 1.2 mg/kg) to avoid clinically evident weight loss and sedation.

In future studies, we plan to measure plasma drug levels after the administration of Bup-SR at 0.3 and 1.2 mg/kg, to confirm the maintenance of adequate concentrations for at least 48 h. A previous study[13] demonstrated that Bup-SR administered subcutaneously at 1.2 mg/kg maintained plasma levels greater than 1 ng/mL for more than 72 h. Plasma levels of Bup HCl given at 0.1 mg/kg peaked at 2.8 ng/mL and declined to 1.4 ng/mL at 8 h and continued to decrease at the 24-h time point.[13] Bup-SR administered at 0.9 mg/kg results in similar plasma concentrations to those of the 1.2-mg/kg dosage, and plasma levels of 0.1 to 0.5 ng/mL buprenorphine are necessary to maintain analgesia in humans.[12] In mice, plasma concentrations of 1 to 10 ng/mL buprenorphine have been associated with analgesia.[34]

Previous studies using Bup-SR have reported skin lesions, ulcerations, self-mutilation and scabbing at the site of administration.[3,13] In our current study, we noted no erythema, ulcerations, or irritation of the skin at the administration site for Bup-SR during the 7-d period drug delivery. The development of new techniques to administer analgesics postoperatively to rodents is becoming a common focus in laboratory animal medicine. Injectable Bup-SR is a substantial refinement in analgesia because it produces a minimal handling stress. In addition, studies using subcutaneous cholesterol–triglyceride–buprenorphine pellets show promising results,[14] as does Bup HCl in drinking water and food gels. Oral Bup HCl administered in drinking water after an initial postsurgical subcutaneous injection of the drug may also prove to be an effective alternative to additional injections of Bup HCl.[18] Bup HCl administered orally via gelatin or other food stuffs increases thermal antinociceptive threshold for only 1 h.[21] However, another study[19] finds that higher oral doses produce adequate serum levels of buprenorphine for longer time periods.

Finally, the cost of a single 1.2 mg/kg dose of Bup-SR (ZooPharm) for a 350-g rat is $1.47, whereas its standard-of-care equivalent (6 doses of Bup HCl) would cost $5.10. This difference in cost does not include labor charges, which would further increase the difference between total expenses. Therefore, using a sustained-release form of buprenorphine is a good option financially.

A sustained-release form of buprenorphine that provides analgesia over a course of 72 h is a considerable refinement in postoperative care in veterinary medicine. Our study suggests that Bup-SR at 0.3 or 1.2 mg/kg provides effective antinociception

in the incisional pain model in rats for 48 to 72 h without noteworthy side effects. Additional studies measuring plasma concentration levels related to behavioral antinociception and possible synergistic effects Bup-SR are warranted.

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