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Efficacy of Sustained-Release Buprenorphine in an Experimental Laparotomy Model in Female Mice

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Mice purportedly require dosing with the opioid buprenorphine (Bup-HCl) at least every 8 to 12 h to maintain an adequate plane of analgesia. Here we used an experimental laparotomy model to determine the clinical efficacy of sustained-release formulations of buprenorphine (Bup-SR) after surgery in mice. Female CD1 mice underwent laparotomy and received either Bup-SR (0.6 mg/kg), Bup-HCl (0.1 mg/kg every 12 h), or saline (every 12 h). Pain was assessed at 1, 3, 6, 12, 24, 48, and 72 h according to the frequency of several behaviors (general activity, wheel-running activity, rearing, grooming, wound licking, orbital tightening, and percentage of integrated nest material) and daily body weight. Over time, wheel running was increased and wound licking was decreased in Bup-SR–treated mice compared with Bup-HCl– and saline-treated mice. Compared with Bup-HCl– and saline-treated mice, Bup-SR–treated mice had increased general activity and percentage of integrated nest material and decreased orbital tightening for 1 to 6 h after surgery. The Bup-HCl– and saline-treated mice had similar general activity, orbital tightening scores, and wheel running activity. Rearing activity and body weight did not differ throughout the study, and none of the observed behaviors differed between groups at 24, 48, and 72 h after surgery. These results suggest that Bup-SR at 0.6 mg/kg provides adequate analgesia after laparotomy in mice and can be used as an alternative analgesic in this context. Furthermore, Bup-HCl at 0.1 mg/kg every 12 h may be inadequate in providing analgesia for abdominal procedures in mice.

Abbreviations: Bup-HCl, buprenorphine hydrochloride; Bup-SR, sustained-release buprenorphine; TINT, time-to-integrate-to-nest test.

Postoperative or postprocedural analgesia is imperative to eliminate undue pain or distress in murine models. Opioids are common analgesics used to treat postoperative pain in laboratory mice. The μ and κ opioid receptors are the most frequently targeted for analgesic activity, and opioid compounds are classified as either agonists, partial agonists, or agonist–antagonists. Buprenorphine (Bup-HCl) is a partial μ agonist commonly used to reduce pain in laboratory mice in a variety of models.[3,10,15,23] The most common dosing regimens for Bup-HCl are at least every 8 to 12 h.[9] This frequency requires additional handling, and according to previous efficacy studies[11] and our most recent pharmacokinetic studies,[22] Bup-HCl may not retain therapeutic concentrations during the entire dosing interval. Sustained-release formulations of buprenorphine (Bup-SR) reduce the amount of animal handling and were shown to retain therapeutic concentrations for as long as 48 h.[22]

Bup-SR was first shown to provide sustained plasma concentrations for 72 h in rats and demonstrated efficacious analgesia in a rat tibial defect model for 72 h[10] and a rat incisional pain model.[4] We performed a pharmacokinetic analysis of Bup-SR in female CD1 mice and found the formulation, when given at 0.6 mg/kg, resulted in plasma concentrations that exceeded the reported therapeutic concentration for 24 to 48 h.[22] A similar pharmacokinetic analysis was performed in male C57BL/6 mice and demonstrated that Bup-SR at 1.2 mg/kg resulted in therapeutic concentrations that lasted as long as 12 h.[6] Several studies recently have evaluated the clinical efficacy of Bup-SR in mouse models, and 3 studies demonstrated varying clinical efficacy in mice by using a thermal nociception model. Specifically, one study demonstrated the efficacy of Bup-SR for 12 h in male BALB/cJ and SWR/J mice when given at 1.0 mg/kg,[2] whereas another demonstrated antinociceptive effects in male Swiss–Webster mice for as long as 48 h when given at 1.5 mg/kg.[17] In addition, Bup-SR at 2.2 mg/kg was effective against thermal nociception in female C57BL/6 mice for 24 h; clinical efficacy was further assessed in an experimental embryo transfer model and persisted for at least 24 h postoperatively.[19]

The current study sought to determine the clinical efficacy of Bup-SR at 0.6 mg/kg for 72 h after experimental laparotomy in female CD1 mice. We found that Bup-SR provided adequate analgesia, greater than that in Bup-HCl– or saline-treated mice, for the first 12 h after surgery, whereas there was no clinically discernable difference in the analgesic response after the 12-h time point. These results suggest Bup-SR is a suitable alternative to Bup-HCl for abdominal surgical procedures in mice.

Materials and Methods

Mice. Female Crl:CD1(ICR) mice (weight, 20 to 30 g; age, 8 to 10 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. Mice were housed individually with unrestricted access to Teklad Irradiated Diet 2918 (Harlan Laboratories, Madison, WI) and filter-sterilized

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water during the 3 to 7 d before initiating the studies and after surgery. Mice were maintained on a 14:10-h light:dark cycle at a temperature of 21 to 24 °C. All experimental procedures were approved by the IACUC.

Experimental laparotomy model. To determine whether Bup-SR provided pain relief comparable to that of Bup-HCl or saline, a behavioral pain scoring system was used in a postlaparotomy model. Mice were randomly distributed into 3 groups: saline (n = 7), Bup-HCl treatment (n = 8), and Bup-SR (n = 8). Approximately 20 min prior to surgery performed at 0900, mice were treated with Bup-SR (0.6 mg/kg SC; Zoopharm, Windsor, CO), Bup-HCl (0.1 mg/kg SC; Ricket Benckiser Healthcare, London, United Kingdom), or saline (0.3 mL). Anesthesia was induced and maintained in the mice using isoflurane (Fluriso, VetOne, MWI Veterinary Supply, Boise, ID), and their abdomens were shaved and prepared aseptically for surgery by using a chlorhexadine 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. The small intestines were exposed and manipulated with the surgical instruments for 2 min. Light pressure was applied to a section of midjejunum for 60 s by using hemostats that clamped until the locking mechanism touched. The intestines were replaced in the abdomen, the abdominal wall incision was closed with 5-0 absorbable suture (Ethicon, Johnson and Johnson, New Brunswick, NJ), and the skin was closed with surgical staples (Becton Dickinson, Franklin Lakes, NJ). The mice were returned to their cages and monitored until they had recovered. Bup-HCl was given every 12 h subcutaneously for 60 h, and the Bup-SR and saline-treated (control) groups received subcutaneous saline injections every 12 h for 60 h.

Analgesic controls. To determine the effects of the analgesics on mouse behavior without surgery, mice were randomly distributed into 2 groups: Bup-HCl (n = 7) and Bup-SR (n = 7). Mice were anesthetized and treated with either Bup-HCl or Bup-SR as described earlier, and behaviors assessed.

Behavioral assessments. Baseline assessments were performed prior to surgery or treatment (time point 0), and indicators of pain were assessed at 1, 3, 6, 12, 24, 48 and 72 h afterward. Observers were blinded to the treatment groups. At each observation time point, 2 observers scored each mouse in the laparotomy model over a 5-min period, and the average scores of the observers were recorded. Each mouse in the nonsurgery group was scored for a 5-min period at each observation time point by an observer blinded to the treatment groups. Behaviors scored as indicators of pain or distress included general activity, wheel running, rearing, grooming, wound licking, orbital tightening, and the percentage of integrated nest material. General activity was scored on a scale of 0 to 2 (0: quiet, little activity; 1: moderate activity; 2: normal, very active). Velometers (Bontrager Trip 1, Trek Bicycle, Waterloo, WI) were placed on the running wheels to count the revolutions per minute at each time point. The frequency of rearing, grooming, and wound licking was tallied during the 5-min observation period. The orbital-tightening measure is a modification of the facial grimace scale,[1,24] in which the level of pain experienced was correlated with a score on a scale of 0 to 2 (0, no orbital tightening; 1, moderate orbital tightening; 2, severe orbital tightening); the cumulative observations over the 5-min period were summed to provide the score. The percentage of integrated nesting material was determined by placing 25% of the nesting material (Bed-r' Nest, The Andersons, Maumee, OH) on the opposite side of the cage during anesthesia recovery and assessing the amount that had been integrated into the nest at each time point. This measure is a modification of a previously described procedure[27] and uses a large volume of material given one time rather than small amounts given at different time intervals. In addition, body weights were recorded every 24 h, with the baseline (0 time point) recorded 24 h before the procedure. After completion of the study, mice were euthanized and necropsied to evaluate the surgical site.

Statistical analysis. Statistical analysis was done by using SAS (SAS Institute, Cary, NC) Proc Mixed for each response variable (general activity, grooming, orbital tightening, rearing, no. of revolutions per minute, percentage of integrated nesting material, wound licking). Fixed effects included treatment (Bup-HCl, Bup-SR, saline), time (baseline, 1, 3, 6, 12, 24, 48, 72 h), and treatment×time interaction. Mouse (nested within treatment) was included as a random effect to account for repeated measures. To help satisfy model assumptions, responses (except for general activity, percent of integrated nesting material and weight) were square-root–transformed. For all analyses, posthoc pairwise comparisons were performed. P values less than 0.05 were considered statistically significant. The scoring between the 2 observers blinded to the treatment groups was assessed for agreement. The κ statistic was calculated for general activity. The paired t test was used to evaluate the agreement between the 2 observers for the other variable.

Results

One mouse in the Bup-SR and one mouse in the Bup-HCl group died immediately after surgery and were not included in the analysis. In addition, another mouse in the Bup-SR group died prior to the 72-h time point and is included in the analysis up to that time point. Necropsy revealed severe intestinal necrosis and obstruction at the point of injury. The clamped intestinal area of the remaining mice was evident during necropsy but lacked signs of obstruction or intestinal tears. There were no injection site lesions noted in any mice during the course of the study.

Baseline behavioral assessments were performed prior to surgery and analgesic treatment and are represented in the tables as time point 0. The activity and behavior of the mice prior to surgery and at 1, 3, 6, 12, 24, 48, and 72 h afterward are summarized in Table 1. Baseline values did not differ between groups, and there was a significant ($P < 0.05$) time effect for all variables tested. The Bup-SR mice were more active than the Bup-HCl mice at 1 and 3 h postoperatively and more active than the saline-treated group at 1, 3 and 6 h after surgery ($P < 0.01$). General activity after 12 h postoperatively did not differ between groups. Treatment had a significant ($P < 0.01$) effect on the number of revolutions per minute over time. Wheel-running activity was greater ($P < 0.03$) in the Bup-SR–treated mice at 3 and 6 h compared with both the Bup-HCl– and saline-treated groups. Rearing behavior did not differ among groups. Grooming behavior was increased at 3 h and decreased at 24 h postoperatively in the Bup-HCl-treated group compared with the Bup-SR treated mice ($P < 0.03$). Treatment had a significant ($P < 0.01$) effect on wound licking over time. Wound licking in the saline–treated group was increased compared with baseline for the first 6 h after surgery; was increased compared with Bup-SR at 1, 3, and 6 h; and was increased compared with Bup-HCl at 1 h ($P < 0.01$ for all comparisons). There was also a significant decrease in wound licking between the Bup-SR and Bup-HCl treated groups at 3, 6, and 12 h. There were no intergroup differences in wound-licking behavior at 24 h and beyond. Although orbital tightening seemed to be increased in the saline and Bup-HCl groups, the difference was significant

Table 1. Postoperative behavioral scores (mean ± 1 SD) in mice treated with saline, Bup-HCl, or Bup-SR after surgery

	Time (h) after laparotomy	Treatment group		
		Saline	Bup-HCl	Bup-SR
General activity	0	1.9 ± 0.2	2.0 ± 0	1.9 ± 0.2
	1	0.9 ± 0.6	0.8 ± 0.5	1.4 ± 0.6[a]
	3	1.1 ± 0.5	1.1 ± 0.4	1.8 ± 0.4[a]
	6	1.0 ± 0.7	1.25 ± 0.5	1.7 ± 0.3[b]
	12	1.6 ± 0.7	1.4 ± 0.7	1.6 ± 0.5
	24	1.6 ± 0.4	1.3 ± 0.7	1.5 ± 0.6
	48	1.5 ± 0.6	1.5 ± 0.6	1.7 ± 0.6
	72	1.9 ± 0.2	1.6 ± 0.6	1.6 ± 0.4
Wheel running	0	26.8 ± 14.2	29.6 ± 26.3	25.7 ± 23.7
	1	10.5 ± 23.0	3.7 ± 8.9	11.5 ± 16.5
	3	11.2 ± 19.2	4.9 ± 8.1	37.2 ± 43.9[a]
	6	5.2 ± 10.4	5.7 ± 7.6	31.55 ± 25.9[a]
	12	15.6 ± 19.8	9.1 ± 9.9	29.2 ± 35.3
	24	22.8 ± 12.9	7.3 ± 10.1	20.1 ± 7.6
	48	43.2 ± 33.2	21.1 ± 26.1	9.5 ± 16.1[b]
	72	45.5 ± 27.8	37.5 ± 38.1	35.1 ± 16.5
Rearing	0	26.7 ± 12.1	24.6 ± 13.5	20.6 ± 10.7
	1	2.4 ± 1.5	3.8 ± 4.8	4.6 ± 6.5
	3	6.6 ± 9.6	7.8 ± 10.1	13.0 ± 18.2
	6	9.2 ± 14.5	10.4 ± 11.6	14.5 ± 11.5
	12	17.1 ± 26.4	10.0 ± 12.0	10.1 ± 1.9
	24	18.8 ± 21.2	22.7 ± 35.6	18.6 ± 21.2
	48	17.7 ± 12.1	23.2 ± 25.5	24.1 ± 28.1
	72	34.6 ± 14.9	24.6 ± 33.8[b]	31.8 ± 38.6
Grooming	0	3.6 ± 3.4	3.3 ± 5.2	9.8 ± 22.8
	1	6.6 ± 8.0	5.5 ± 11.1	3.1± 2.1
	3	9.4 ± 11.5	30.7 ± 41.3[d]	5.2 ± 5.9
	6	4.8 ± 6.2	8.3 ± 10.4	4.5 ± 10.9
	12	6.3 ± 11.7	18.5 ± 25.1	6.5 ± 10.3
	24	9.8 ± 9.5	3.5 ± 3.6[d]	16.8 ± 17.5
	48	9.8 ± 7.3	4.6 ± 4.2	12.8 ± 23.7
	72	4.6 ± 4.6	7.9 ± 2.9	6.2 ± 10.2
Wound licking	0	0 ± 0	0 ± 0	0 ± 0
	1	52.3 ± 28.7	32.3 ± 42.9[b]	6.87 ± 11.1[a]
	3	56.8 ± 53.5	47.9 ± 54.9	13.3 ± 22.8[a]
	6	33.7 ± 21.3	26.6 ± 31.4	1.1 ± 1.7[a]
	12	19.2 ± 30.2	46.3 ± 45.9	11.5 ± 18.1[e]
	24	12.9 ± 16.8	7.4 ± 11.6	16.4 ± 18.9
	48	7.4 ± 13.1	7.1 ± 10.7	2.0 ± 3.3
	72	2.9 ± 6.9	3.6 ± 5.4	9.7 ± 14.8
Orbital tightening	0	0 ± 0	0 ± 0	0 ± 0
	1	19.7 ± 11.4	22.9 ± 10.4	8.9 ± 6.7[a]
	3	18.1 ± 19.0	8.6 ± 7.3	6.25 ± 8.3[b]
	6	11.5 ± 11.5	6.3 ± 6.4	1.3 ± 1.2[b]
	12	10.2 ± 21.2	8.9 ± 12.7	3.1 ± 7.8
	24	4.1 ± 6.5	9.3 ± 15.1	4.8 ± 6.9
	48	1.1 ± 1.5	10.4 ± 16.2	2.9 ± 6.9
	72	0.3 ± 0.6	13.7 ± 23.5[c]	6.3 ± 10.8

Table 1. Continued.

	Time (h) after laparotomy	Treatment group		
		Saline	Bup-HCl	Bup-SR
Percentage (%) of integrated nesting material	0	NA	NA	NA
	1	18.5 ± 32.8	9.2 ± 18.1	6.3 ± 6.8
	3	27.1 ± 22.1	35.7 ± 31.7	48.1 ± 23.63
	6	52.1 ± 49.3	54.6 ± 40.4	66.3 ± 34.2
	12	62.8 ± 46.8	69.3 ± 41.5	94.1 ± 8.6[b]
	24	100 ± 0	98.3 ± 4.1	95 ± 11.1
	48	100 ± 0	100 ± 0	100 ± 0
	72	100 ± 0	100 ± 0	100 ± 0

NA, not applicable
Each value represents 7 mice except for Bup-SR at 72 h (n = 6)
[a]Value significantly ($P < 0.05$) different from those for the Bup-HCl and saline groups.
[b]Value significantly ($P < 0.05$) different from that for the saline-treated mice.
[c]Value significantly ($P < 0.05$) different from those for Bup-SR and saline groups.
[d]Value significantly ($P < 0.05$) different from those for the Bup-SR–treated mice.
[e]Value significantly ($P < 0.05$) different from those for the Bup-HC1–treated mice.

from that of the Bup-SR group only at 1 h for the Bup-HCl–treated mice ($P < 0.05$) and at 3 and 6 h for the saline-treated mice ($P < 0.05$). The Bup-HCl–treated mice had increased ($P < 0.03$) orbital tightening at 72 h compared with that of the saline and Bup-SR treated groups. There were no differences in the TINT except at 12 h, when the Bup-SR group was higher ($P < 0.03$) than the saline-treated mice.

There was good agreement between the observers when evaluating general activity (κ 0.72, $P < 0.0001$), the frequency of grooming (average difference 1.08, $P = 0.6$), rearing (average difference 0.09, $P = 0.8$), and wound licking (average difference 2.11, $P = 0.05$). However, the agreement between the observers' evaluation of the more subjective measurements of orbital tightening (average difference 1.39, $P = 0.003$) and the percentage of integrated nesting material (average difference 3.32%, $P = 0.013$) was only moderate at each time point.

The mice that did not have surgery and that only received analgesics after anesthesia frequently were resting during the observation periods and as a result had low general activity levels and grooming behavior (Table 2). Behavior did not differ between the mice that received Bup-HCl without surgery compared with Bup-SR without surgery with the exception of 3 individual time points: general activity was increased at 12 h, grooming was decreased at 24 h, and percentage of integrated nesting material was decreased in the Bup-SR group without surgery compared with the Bup-HCl group without surgery. Both groups of analgesic-treated mice had decreased running-wheel activity at 1, 3, 6, and 12 h compared to baseline values ($P < 0.05$) after anesthesia and analgesic administration; this difference disappeared at 24 h. In addition, according to observations, the analgesia-only mice integrated more nesting material at earlier time points than did the groups that had surgery. Because these studies were conducted separately from the laparotomy model and used different observers, the behavioral parameters were not compared statistically between the analgesia-only and surgical group.

Body weight was decreased after surgery in all mice in laparotomy groups and remained below baseline throughout the 72-h observation period (Table 3). There was no significant difference in the daily body weights between groups. The body weight of mice that received analgesics without surgery had a nonsignificant decrease in body weight compared to their initial weight during the first 24 h after anesthesia and analgesic administration. This decrease was less pronounced than that in the laparotomy groups and disappeared by 48 h. Daily body weight did not differ between groups that did not undergo laparotomy.

Discussion

We based the loading dose of Bup-SR (0.6 mg/kg SC) in the current study on the cumulative dose of Bup-HCl of 0.1 mg/kg every 12 h. In a previous study, we demonstrated that this dose provides a plasma concentration of 0.5 to 1 ng/mL for 48 h,[22] supporting the hypothesis that Bup-SR use provides analgesia in a surgical model of pain for at least 48 h. Although the therapeutic plasma concentration has not been determined for mice, it is within the therapeutic concentration of Bup in humans and rats, which is commonly referenced for other rodents including mice[14,19] and is the targeted plasma concentration of the commercially available Bup-SR formulations. The current study demonstrated that the clinical efficacy of Bup-SR is superior to that of Bup-HCl according to results from our ethogram to assess postoperative pain in mice. Furthermore we demonstrated that giving Bup-HCl every 12 h does not provide adequate analgesia for pain due to abdominal surgery in mice.

The evaluation of pain in mice can be difficult because they are a prey species that typically masks signs of pain.[26,30] Several parameters are available to assess the degree of pain in mice, including behavioral assessments, physiologic changes such as body weight and food and water intake, nesting behavior, and facial grimacing. We used a compilation of several previous published mouse ethograms to assess analgesic efficacy[1,20,28,32] as well as recently recognized parameters, including modifications of the mouse grimace scale[24,25] and the TINT.[27]

Many of the parameters we used to assess pain demonstrated the difficulties in assessing pain behavior during a 5-min observation period, because significant differences were not discernable until after statistical analysis was performed. For example, the activity level of the saline-treated group was subtly different from that of the mice that received analgesics. This difficulty regarding pain assessment is further complicated by an individual observer's interpretation of pain behavior in mice. The more objective measures—frequency of rearing, wound licking, grooming, and general activity—had better interobserver agreement than did those that were more subjective—orbital

Table 2. Postoperative behavioral scores (mean ± 1 SD; $n = 7$) in mice treated with Bup-HCl or Bup-SR in the absence of surgery

	Time (h) after treatment	Treatment group	
		Bup-HCl	Bup-SR
General activity	0	1.4 ± 0.5	1.4 ± 0.8
	1	1.4 ± 0.5	1.4 ± 0.5
	3	1.6 ± 0.5	1.6 ± 0.5
	6	1.0 ± 0.8	1.0 ± 0.8
	12	0.8 ± 0.9	1.7 ± 0.8[a]
	24	1.3 ± 0.9[b]	0.7 ± 0.8
	48	0.7 ± 0.8	0.9 ± 0.7
	72	1.1 ± 0.7	1.0 ± 0.8
Wheel running	0	18.7 ± 9.9	18.0 ± 9.9
	1	3.6 ± 5.5[b]	3.8 ± 5.1[b]
	3	8.1 ± 10.4[b]	3.2 ± 10.7[b]
	6	4.3 ± 12.2[b]	0.8 ± 1.3[b]
	12	4.3 ± 4.9[b]	1.0 ± 1.3[b]
	24	18.2 ± 13.3	9.8 ± 8.6[b]
	48	13.1 ± 7.4	11.5 ± 7.8
	72	14.6 ± 8.8	12.5 ± 7.4
Rearing	0	7.4 ± 9.2	9.5 ± 7.6
	1	5.0 ± 8.5	8.9 ± 8.3
	3	6.1 ± 6.6	3.0 ± 6.2
	6	4.3 ± 7.3	2.6 ± 3.4[b]
	12	4.4 ± 7.1	9.6 ± 8.3
	24	4.0 ± 5.7	2.0 ± 4.4[b]
	48	1.3 ± 1.9[b]	2.1 ± 2.9[b]
	72	2.3 ± 3.1	1.9 ± 2.4[b]
Grooming	0	2 ± 1.6	1.3 ± 0.9
	1	1.4 ± 1.5	1.1 ± 1.1
	3	1.9 ± 1.7	1.3 ± 1.4
	6	1.7 ± 2.1	0.6 ± 0.8
	12	1.7 ± 2.1	0.6 ± 0.8
	24	0.6 ± 1.5[b]	1.7 ± 1.4[a]
	48	2.1 ± 2.7	1.0 ± 0.8
	72	1.8 ± 2.5	1.3 ± 1.6
Wound licking	0	0 ± 0	0 ± 0
	1	0 ± 0	0 ± 0
	3	0 ± 0	0 ± 0
	6	0 ± 0	0 ± 0
	12	0 ± 0	0 ± 0
	24	0 ± 0	0 ± 0
	48	0 ± 0	0 ± 0
	72	0 ± 0	0 ± 0
Orbital tightening	0	0 ± 0	0 ± 0
	1	0 ± 0	0 ± 0
	3	0 ± 0	0 ± 0
	6	0 ± 0	0 ± 0
	12	0 ± 0	0 ± 0
	24	0 ± 0	0 ± 0
	48	0 ± 0	0 ± 0
	72	0 ± 0	0 ± 0
Percentage (%) of integrated nesting material	0	NA	NA
	1	39.3 ± 33.7	27.9 ± 11.3
	3	58.6 ± 40.2	32.1 ± 36.9[a]
	6	62.3 ± 36.9	81.5 ± 32.9
	12	85.0 ± 30.1	75.0 ± 25.0
	24	100.0 ± 0.0	96.4 ± 9.4
	48	100.0 ± 0.0	100.0 ± 0.0
	72	100.0 ± 0.0	100.0 ± 0.0

NA, not applicable
[a]Value significantly ($P < 0.05$) different from that for the Bup-HCl group.
[b]Value significantly ($P < 0.05$) different from the baseline value.

tightening and the amount of nesting material integrated to the nest. After statistical analysis, 4 parameters were most informative regarding whether the pain from the procedure was mitigated (or not): wound licking and wheel-running activity, both of which showed a significant effect of treatment over time, and general activity and orbital tightening, which had a significant treatment effect at specific time points, particularly early in the study.

Wound licking was highest, suggesting increased pain, in the saline-treated group. The increased wound licking in the Bup-HCl–treated mice compared with the Bup-SR–treated mice for the first 12 h might reflect the waxing and waning plasma levels of buprenorphine associated with Bup-HCl. The kinetics of Bup-HCl in mice after various routes of administration revealed that the serum concentration of Bup rapidly declined after a subcutaneous bolus.[21] Our previous pharmacokinetic evaluation of Bup-HCl demonstrated that plasma concentrations were undetectable as early as 4 h after administration.[22] This pattern is consistent with previous evaluations of Bup-HCl[11] and more recent analgesic assessments, which found that 8-h intervals were too long to adequately provide analgesia from Bup-HCl after an embryo transfer procedure.[19]

General activity is a reasonable objective measure of mouse pain after a painful procedure, and its assessment can be conducted relatively quickly. Comparisons should be made with baseline activity of mice prior to surgery. Wheel running provides a subjective measurement of activity and has been used to assess pain in previous studies. For example, wheel-running activity was used to evaluate the effectiveness of analgesics in mice immunized with complete Freund adjuvant,[23] and in male ICR mice undergoing a splenectomy.[5] In both cases, wheel-running activity was increased in groups that received analgesics. In our study, wheel running tended to be higher in Bup-SR–treated mice than the Bup-HCl and saline treated groups suggesting the analgesia was effective. In addition, orbital tightening has been previously used to assess pain in mice[1] and is a component of the mouse grimace scale.[24,25] We chose to simplify the mouse grimace scale to orbital tightening as it appears to be one of the more readily visible signs demonstrated in the mouse grimace scale and easy to assess and train personnel to recognize. Although the difference was not statistically significant, the cumulative score for orbital tightening was increased in the saline- and Bup-HCl–treated mice, suggesting that analgesia was inadequate in those groups.

Table 3. Body weight (g) of individual mice

		Laparotomy groups			Analgesic-only groups	
Time (h)	Mouse no.	Saline	Bup-HCl	Bup-SR	Bup-HCl	Bup-SR
0	1	29.3	28.6	22.6	26.2	29.0
	2	30.2	27.0	25.5	25.9	24.6
	3	27.5	25.5	22.8	22.9	24.8
	4	29.0	29.0	28.5	26.3	26.1
	5	30.7	29.8	31.4	29.9	26.0
	6	30.4	27.4	31.3	31.6	24.2
	7	28.5	27.1	29.4	28.5	27.4
	mean ± 1 SD	29.4 ± 1.2	27.8 ± 1.5	27.8 ± 3.6	27.3 ± 2.9	26.0 ± 1.7
24	1	27.5	26.4	21.3	26.2	23.8
	2	27.2	23.4	23.4	25.3	24.4
	3	26.6	25.5	22.0	23.6	24.0
	4	27.3	27.5	27.2	25.6	25.4
	5	28.4	29.2	28.6	29.1	25.1
	6	29.6	29.2	25.4	29.6	ND
	7	27.1	22.3	25.6	27.2	27.1
	mean ± 1 SD	27.7 ± 1.0	26.2 ± 2.7	24.8 ± 2.7	26.7 ± 2.1	24.9 ± 1.2
48	1	26.5	26.8	21.7	26.9	28.4
	2	25.9	22.4	21.5	25.2	24.8
	3	27.4	24.8	20.5	23.8	25.0
	4	28.3	26.0	26.6	25.5	25.5
	5	29.5	28.8	29.2	29.3	25.5
	6	29.1	26.2	29.1	30.7	23.9
	7	28.0	26.1	28.3	27.7	28.9
	mean ± 1 SD	27.8 ± 1.3	25.9 ± 1.9	25.2 ± 3.9	27.0 ± 2.4	26.0 ± 1.9
72	1	26.9	27.3	22.2	27.3	28.9
	2	25.7	22.1	20.6	25.2	25.3
	3	27.3	23.8	ND	23.9	26.9
	4	28.4	25.1	26.7	26.4	26.0
	5	29.1	28.5	30.3	29.7	26.1
	6	28.7	27.3	29.4	31.6	24.3
	7	28.3	24.8	ND[a]	27.2	27.2
	mean ± 1 SD	27.7 ± 1.2	25.6 ± 3.9	25.8 ± 4.3	27.3 ± 2.6	26.4 ± 1.5

ND, not done
Body weight did not change significantly in any group.
[a]Mouse died

Nest building has become a useful tool to assess the welfare of laboratory mice and has been used as a means to evaluate pain.[12,27] The TINT provides a subjective measurement to assess analgesic efficacy. One study using the TINT[27] found that mice undergoing a carotid artery injury are likely to fail the TINT for as long as 2 d after surgery. In the current study, all mice began to integrate the nesting material by 1 h after surgery, but the Bup-SR treated group appeared to integrate more of the nesting material in less time than did the Bup-HCl– and saline-treated mice; however, these differences were not statistically significant. Perhaps the results would have been more revealing had we conducted the TINT daily. For example, in the original study,[27] a small amount of nesting material was placed across from the main nest daily, mice were observed for 10 min, and the times to first interaction and total integration were recorded; a failed TINT was considered to be no interaction during the 10-min observation time. Others have used the complexity of nest building as an indicator of pain.[18] We did not specifically record the complexity of the nesting material but did note that all mice, regardless of treatment, built nests.

Mice that underwent surgery typically had a reduced body weight for 72 h postoperatively, and mice that did not have surgery had a reduced body weight for 24 h after the anesthetic event and analgesic administration. The decreased body weight in our mice, which did not return to baseline with analgesic administration, is similar to other studies in which mice were unable to gain weight postoperatively even with analgesics.[1,13,15,29,31] These findings suggest that weight changes may not be an effective means for evaluating analgesic efficacy. Alternatively, even though the Bup-HCl we provided is a commonly used dose and despite Bup-SR maintaining better plasma concentrations than does Bup-HCl,[22] the dosage we used may have been insufficient to overcome postoperative anorexia.

We attributed the deaths of 2 mice that did not recover from anesthesia to anesthesia and not the surgical procedure. In addition, one mouse in the Bup-SR–treated group that died due to intestinal necrosis and obstruction. Every effort was taken to ensure consistent clamping pressure and duration, and some of the variability between individual mice in each group might be attributed to the degree of pain induced with the model.

The mice that received saline after surgery were included to provide appropriate comparisons among treatment groups. Although many of the parameters evaluated did not demonstrate a difference between groups, several suggested the Bup-HCl treatment was ineffective at providing analgesia. Mice that experience pain or distress are known to have reduced wheel running activity.[5,7,23,31] The wheel-running activity of the saline-treated group was similar to that of the Bup-HCl–treated group, as was the general activity level. Unlike previous studies in which mice have increased activity after buprenorphine administration,[8,16] the mice that received analgesics without surgery in our study did not have increased activity and frequently were observed resting during the observation periods. In addition, neither the Bup-HCl– or Bup-SR–treated mice that underwent surgery had increased general activity or wheel-running activity compared with their baseline data. These data suggest that the activity of the surgical mice was not influenced by buprenorphine administration. Orbital tightening and wound licking were both increased in the saline- and Bup-HCl–treated mice compared with the Bup-SR treated mice. These findings suggest the Bup-HCl dosing regimen used in this study did not ameliorate the pain associated with the surgical procedure. Similar to previous studies, our clinical observations were subtle and most noticeable during the first 12 h after surgery.[25] In retrospect, both the saline- and Bup-HCl–treated mice would have benefitted from receiving rescue analgesia.

The efficacy of Bup-SR in the current study was comparable to that in a previous study, in which mice that received Bup-SR experienced as long as 24 h of analgesia, on the basis of their ethogram in a laparotomy for embryo transfer.[19] Similar to our study, the surgical procedure was relatively brief (less than 5 min). Unlike our study, the amount of trauma induced was less severe, the dose of Bup-SR was greater (2.2 mg/kg compared with 0.6 mg/kg), and the duration of observations were shorter (24 h compared with 72 h) in the previous study.[19] In addition, the efficacy in the current study is similar to that in 2 rat studies. Rats treated with Bup-SR at 0.9 mg/kg after a tibial defect model had adequate analgesia.[10] Although the surgical model was different in the cited study,[10] there was no difference in activity or body weight between the Bup-SR– and the Bup-HCl–treated groups, but the increase in vertical raises suggested that Bup-SR provided greater analgesia.[10] In addition, rats in an incisional pain model that were treated with 0.3 and 1.2 mg/kg Bup-SR demonstrated analgesic efficacy for as long as 72 h.[4]

One notable finding in our current study is how poorly Bup-HCl controlled pain even when given every 12 h. Although numerous references support the use of Bup-HCl as twice-daily treatments,[9] several pharmacokinetic studies have found that Bup-HCl does not maintain plasma concentrations for more than 4 h.[6,22] Furthermore, using a thermal nociception assay (hot plate and tail flick), one author showed 3 to 5 h of analgesic effect in mice given 2 mg/kg Bup-HCl.[11] In another study, Bup-HCl given every 8 h did not provide adequate analgesia in a laparotomy model.[19] This body of evidence suggests that mice given Bup-HCl at an interval greater than every 8 h might not receive adequate analgesia, suggesting that dosing more frequently, such as every 4 to 6 h, may be necessary to achieve sustained analgesia.

In the current study, we found that Bup-SR was satisfactory in providing analgesia to mice in an experimental laparotomy model. Since this study was done, we have used this formulation to provide analgesia in several mouse surgical models including ovariectomies, hindlimb amputations, and other laparotomy procedures without complications. Given our current results and our continued experience with Bup-SR, we find it to be an acceptable alternative analgesic for murine studies requiring prolonged, sustained analgesia. In addition, we found that wound licking, activity levels, and orbital tightening were the most reliable indicators of pain that could be assessed in a timely manner. Using these parameters, we conclude that Bup-HCl provided inconsistent analgesia when given every 12 h and thus urge investigators to dose mice with Bup-HCl more frequently, especially during the first 24 h after surgery, to achieve appropriate analgesia.

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