Evaluation of an improved sustained-release buprenorphine formulation for use in mice

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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 ER. **As of July 1, 2022, SR preparations mentioned in the attached study are now labeled as ER,** with no changes to the formulation of the medication(s).

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Objective—To evaluate analgesic effects of an improved sustained-release buprenorphine (BUP-SR) formulation administered to mice.

Animals—36 male Swiss-Webster mice.

Procedures—Mice were assigned to each of 3 treatment groups (n = 12 mice/group). Treatments were administered SC (vehicle [control treatment], 1.5 mg of buprenorphine hydrochloride [BUP-HCl]/kg, and 1.5 mg of BUP-SR/kg). Mice were evaluated (total activity, gastrointestinal tract motility, respiratory rate, cataleptic behavior, and tail-flick and hot plate nociception tests) to determine behavioral and physiologic responses at 4, 24, and 48 hours after treatment administration. Body weight and respiratory rate were measured before and at each time point after treatment administration.

Results—SC administration of BUP-SR resulted in significant antinociception effects for 48 hours for the hot plate and tail-flick nociception tests without substantial adverse effects. Gastrointestinal tract motility and total activity were higher at 4 hours for mice receiving BUP-SR than for mice receiving the vehicle, but values were the same between these groups at 24 and 48 hours. The BUP-SR group had a lower respiratory rate than did the control group at all times after treatment administration. Mice treated with BUP-SR had no significant changes in body weight during the study, whereas mice treated with BUP-HCl had a significant decrease in body weight at 24 and 48 hours.

Conclusions and Clinical Relevance—BUP-SR administration resulted in antinociception effects for 48 hours. Results of this study indicated that the improved BUP-SR formulation could be safely administered SC and conferred superior analgesia, compared with that for BUP-HCl, in mice. (*Am J Vet Res* 2014;75:619–625)

Buprenorphine is a pharmacologically unique opioid narcotic used for the treatment of pain[1] and opioid dependency.[2] Buprenorphine is an oripavine derivative that has high affinity for the 3 classic opioid receptor subtypes (μ, δ, and κ) as well as the opioid receptor-like 1 subtype.[3] It traditionally has been classified as a partial agonist of μ-opioid receptors,[4] which is primarily responsible for its analgesic effects. Furthermore, buprenorphine is a partial agonist of the opioid receptor-like 1 subtype[5,6] and an antagonist of both δ-opioid[7] and κ-opioid[8] receptors.

Buprenorphine is reported to be 25 to 50 times as potent as morphine,[9] but there is a ceiling effect that can potentially reduce antinociceptive efficacy with progressively increasing doses. This has been commonly referred to as an inverted U-shaped or bell-shaped dose-response curve; this phenomenon has been observed with respect to antinociception, gastrointestinal tract motility, respiration, and physical dependence.[10,11] However, experiments with an array of antinociception pain models suggest that buprenorphine, when administered at a correct dosage, can cause profound analgesic effects.[12]

Buprenorphine is a popular analgesic agent for use in veterinary medicine, especially in research animals.[13] The pharmacokinetic profile for buprenorphine indicates slow association and dissociation kinetics for opioid receptors, thereby allowing long-lasting analgesic effects.[14] Because of the paradoxical reversal effect for buprenorphine at high doses, the likelihood of overdose in humans and other animals is lower, compared with that for many other potent μ-opioid receptor agonists used for the treatment of pain.[15] In addition, buprenorphine is highly lipophilic, which has facilitated the development of

Abbreviations

BUP-HCl	Buprenorphine hydrochloride
BUP-SR	Sustained-release buprenorphine
%MPE	Percentage of the maximum possible effect

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sustained-release transdermal patches in humans. These patches provide relief from pain for up to 96 hours and have been indicated for a variety of pain-related conditions, including severe chronic pain and pain associated with cancer.[16–18] Although buprenorphine transdermal patches are currently used on humans, they are of little use on rodents, including mice, because of concerns about patch displacement or consumption of patches and opioid-induced toxicosis. Furthermore, sites for patch application require special preparation, and various locations may yield different absorption profiles because of differences in skin thickness that may alter patch kinetic characteristics.[19,20]

Although the reported duration of action for buprenorphine depends on the specific testing methods used, its long duration of action (3 to 5 hours or longer in mice)[21] allows for longer intervals between doses, compared with the dosing interval for other commonly used opioid analgesics used for this purpose. Buprenorphine hydrochloride may be administered every 8 to 12 hours[22]; however, if doses or dosing intervals for BUP-HCl are not optimal, there is an increased likelihood of inadequate analgesia. In a recent study,[23] administration of a BUP-SR formulation in mice resulted in antinociception for only 12 hours. A major drawback of sustained-release formulations in mice used in previous studies[19,23] is that they cause skin ulcers at the site of injection. Sustained-release opioid formulations, if found to be safe, have the potential to provide more consistent analgesic relief and to result in labor savings and fewer instances of stressful animal handling.

Both lactide and caprolactone have been used extensively to prepare biodegradable polymers and copolymers for inclusion in sutures, medical devices, and drug delivery systems.[24] Polylactide and polycaprolactone polymers and copolymers are hydrolyzed in the body to form lactic acid and hydroxycaproic acid, which are metabolized and eliminated through the Krebs cycle.[25,26] These polymers have been evaluated for tissue reactions and toxic effects and were found to be safe and biocompatible in humans and other animals.[27]

Formulations containing *N*-methyl-2-pyrrolidone yield insufficient blood concentrations 72 hours after administration.[19,23] Similarly, an improved BUP-SR created by use of a copolymer of lactide and caprolactone maintained plasma concentrations > 0.5 ng/mL at 72 hours, but the concentrations at that time point were inconsistent during analgesic testing conducted by one of the authors (JHW; unpublished data). The purpose of the study reported here was to evaluate that improved BUP-SR, which had been developed to provide sustained antinociceptive benefits for Ø 48 hours. Optimally, the adverse effects profile of any novel or improved opioid analgesic must be compared with that of the drug it is intended to replace. This is especially important for sustained-release products because they have the potential for a long duration of effect. In particular, respiratory and gastrointestinal tract effects of the improved BUP-SR needed to be evaluated and compared with those for the established BUP-HCl treatment. Finally, opioids, including buprenorphine, can cause both excitatory and sedative effects, and these results may differ considerably depending on the animal species and treatment dose.[22] Therefore, total animal activity and cataleptic behavior were tested to assess the behavioral effects attributable to the improved BUP-SR.

Materials and Methods

Animals—Male Swiss-Webster mice[a] were used for the study. Mice were 8 weeks old and had a body weight of 33 to 41 g. Mice were housed separately in individually ventilated polysulfone cages[b] with a light-to-dark cycle of 12 hours of light to 12 hours of darkness. Mice were provided with ad libitum access to food[c] and water. Mice were allowed to acclimatize to their cages for 1 week prior to the start of the study. In addition, mice were allowed to acclimatize to the testing room for 2 days prior to the start of the study. All procedures were approved by the Institutional Animal Care and Use Committee at West Virginia University.

Experimental procedures—Mice were randomly assigned to treatment groups (n = 12 mice/group) by use of a random number table. Group size was determined on the basis of results of a power analysis.[d] Mice in a respective treatment group received a single dose of vehicle (control treatment), BUP-HCl, or BUP-SR. All treatments were administered SC in the dorsal aspect of the neck by means of 1-mL Luer-lock syringes and 25-gauge needles.

The control treatment consisted of the BUP-SR vehicle at a volume equal to that of the BUP-SR treatment. Mice in the BUP-HCl group received 1.5 mg of BUP-HCl[e]/kg (volume, 0.17 to 0.21 mL), whereas mice in the BUP-SR group received 1.5 mg of BUP-SR[f]/kg (volume, 0.08 to 0.11 mL). The dose of buprenorphine was selected on the basis that it was within the range found to result in near maximal antinociceptive activity in mice for both the hot plate and tail-flick tests.[28] The BUP-SR formulation consisted of a biodegradable liquid polymer dissolved in a biocompatible solvent. The solvent used was a combination of *N*-methyl-2-pyrrolidone and triacetin; this mixture was used to solubilize the buprenorphine base so that it could be drawn into a sustained-release biodegradable matrix. In a preliminary unpublished study conducted by one of the authors (JHW) that involved 8-week-old male Swiss-Webster mice (n = 5/group), SC administration of 100 µL of *N*-methyl-2-pyrrolidone caused toxic skin reactions, but SC administration of 100 µL of triacetin did not, leading to its incorporation into the BUP-SR formulation used in the present study.

Evaluation of effects—On the basis of prior preliminary pharmacokinetic profiling for samples obtained at 0, 3, 6, 12, 24, 48, 72, and 96 hours after buprenorphine administration (JHW; unpublished data), it was determined that blood concentrations would remain consistently high for at least 48 hours in all mice. Therefore, only time points that provided the best comparison between BUP-SR and BUP-HCl were selected for further evaluation. Thus, mice were evaluated before (time 0) and 4, 24, and 48 hours after administration of the vehicle, BUP-HCl, or BUP-SR. The 4-hour time point was chosen as the approximate point of peak

effect, as determined on the basis of previous pharmacokinetic analyses of buprenorphine-treated mice.[21]

Body weight of each mouse was assessed before each evaluation period. After body weight was measured, all mice were allowed to acclimatize to the testing room for 60 minutes. Each mouse was then placed inside a testing chamber (a clear plastic box without bedding) and allowed to acclimatize for an additional 30 minutes. Respiratory rate for each mouse was determined after the 30-minute acclimatization period in this testing chamber. An investigator who was not aware of the treatment administered to each mouse counted each full inhalation and exhalation cycle for 10 seconds; each count was multiplied by 6. Total activity was then measured by use of an automated computer-counting activity monitoring system,[g] which consisted of two 16 × 16-photon beam arrays separated in height by 5 cm. Total activity was quantified for a 30-minute period. Ambulatory movements, fine movements (eg, grooming), and rearing movements (eg, standing upright on hind limbs) were each counted; the number of movements was then combined to yield a total activity score. Testing chambers were cleaned between mice. Gastrointestinal tract motility was determined as the number of fecal pellets produced by each mouse during the 60-minute period within the testing chamber.[29]

Hot plate nociception testing was then performed. Mice were placed in a plastic cylinder[h] atop a uniformly heated black anodized aluminum plate.[i] Time until the first sign of excessive shaking, lifting, or licking of the hind paws was determined and recorded as the response latency. A maximum of 30 seconds was used as a cutoff for response latency to avoid tissue damage. Two response latencies were recorded before drug administration (baseline) and used to ensure nociception reflexes were clinically normal; a mean baseline latency of 8 to 10 seconds was required before testing was allowed to proceed. Response latency was recorded at each of the respective time points after treatment. Data obtained were reported as the %MPE, which was indicative of antinociception activity and was calculated by use of the following equation:

$$\%MPE = ([TL - BL]/[CL - BL]) \times 100$$

where TL is the response latency at a given time point, BL is the baseline response latency, and CL is the cutoff response latency.

Cataleptic behavior was assessed next. The forepaws of each mouse were draped over a horizontal metal rod located 3 cm above the bench surface, and the interval until the mouse disengaged both forepaws was recorded. The investigator who performed the measurements was not aware of the treatment administered to each mouse.

Finally, tail-flick antinociception testing was performed. Mice were positioned so that tails were in the beam of an overhead halogen light source.[j] Time until the first sign of a rapid tail flick was determined and recorded as the response latency. A maximum of 10 seconds was used as a cutoff for response latency to avoid tissue damage. Two values were recorded before treatment administration (baseline), and a mean baseline latency of 2 to 4 seconds was required before testing was allowed to proceed. Response latency was recorded at each of the respective time points after treatment. Data obtained were reported as the %MPE.

Data analysis—All data analyses including normality testing were performed with a statistical software package.[k] Data were evaluated by means of repeated-measures ANOVAs to examine time- and treatment-dependent effects. Tukey and Bonferroni post hoc tests were used for pairwise comparisons. For all analyses, α was set at 0.05 and any value of $P < 0.05$ was considered significant.

Results

Animals—Mice tolerated the injections well. No skin reactions were detected at the site of injection for any mice during the study period.

Body weight—Repeated-measures ANOVA revealed a significant ($P < 0.001$) difference in body weight among time points. However, Bonferroni post hoc analysis revealed that body weight for the BUP-SR group did not differ significantly from body weight for the control group at 4, 24, and 48 hours. Bonferroni post hoc analysis also revealed that body weight for the BUP-HCl group did not differ significantly from body weight of the control group at 4, 24, and 48 hours and that body weight did not differ significantly between the BUP-SR and BUP-HCl groups at 4, 24, and 48 hours.

A 1-way repeated-measures ANOVA revealed a significant ($P < 0.001$) difference in body weight for the BUP-HCl group among time points. Tukey post hoc analysis revealed a significant ($P = 0.01$) increase in body weight (2.4% increase) for the BUP-HCl group at 4 hours, compared with body weight at 0 hours (**Figure 1**). Additionally, there was a significant ($P < 0.001$) decrease in body weight for the BUP-HCl group between 4 and 24 hours (4.9% decrease) as well as between 4 and 48 hours (4.1% decrease). Similarly, there was a significant ($P = 0.01$) decrease in body weight from 0 to 24 hours (2.7% decrease) and 0 to 48 hours (1.8% decrease).

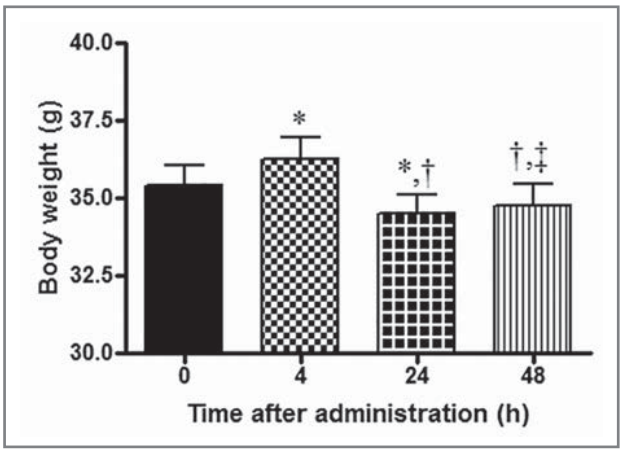

Figure 1—Mean ± SEM body weight in mice (n = 12) before (time 0) and after treatment with BUP-HCl (1.5 mg/kg, SC). *Value differs significantly ($P = 0.01$) from the value at time 0. †Value differs significantly ($P < 0.001$) from the value at 4 hours. ‡Value differs significantly ($P < 0.05$) from the value at time 0.

Respiratory rate—Repeated-measures ANOVA revealed a significant ($P < 0.001$) difference in respiratory rate among treatment groups and among time points. Bonferroni post hoc analysis revealed that administration of BUP-SR resulted in a significant decrease in respiratory rate, compared with that for the control group, at 4 ($P < 0.001$), 24 ($P < 0.001$), and 48 ($P < 0.05$) hours **(Figure 2)**. Bonferroni post hoc analysis also revealed that the BUP-HCl group had a significant ($P = 0.01$) decrease in respiratory rate, compared with the respiratory rate for the control group, at 4 hours; however, there was not a significant difference in respiratory rates between these groups at 24 and 48 hours.

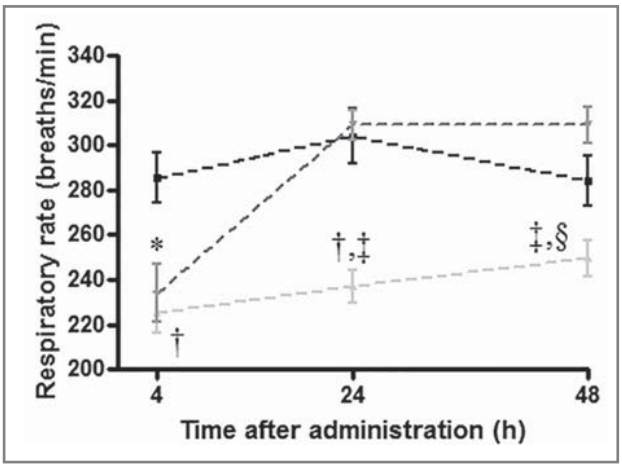

Figure 2—Mean ± SEM respiratory rate in mice (n = 12/group) 4, 24, and 48 hours after treatment with a vehicle (control treatment [black squares]), BUP-HCl (1.5 mg/kg, SC [dark gray inverted triangles]), or BUP-SR (1.5 mg/kg, SC [light gray triangles]). *,†Within a time point, value differs significantly (*$P = 0.01$; †$P < 0.001$) from the value for the control group. ‡Within a time point, value differs significantly ($P < 0.001$) from the value for the BUP-HCl group. §Within a time point, value differs significantly ($P < 0.05$) from the value for the control group.

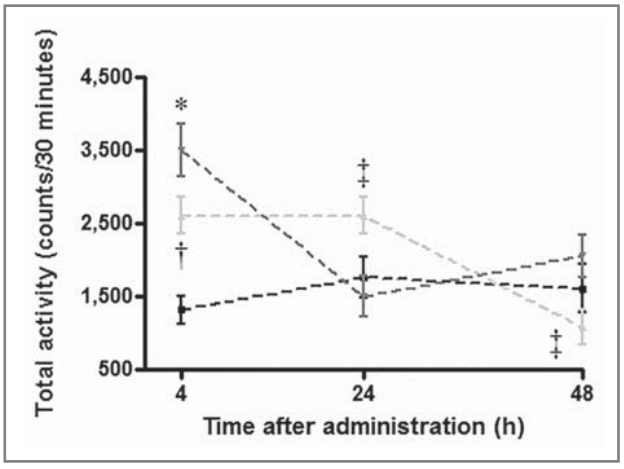

Figure 3—Total activity in mice (n = 12 mice/group) 4, 24, and 48 hours after treatment with a vehicle (control treatment), BUP-HCl, or BUP-SR. At each time point, the numbers of ambulatory movements, fine movements (eg, grooming), and rearing movements (eg, standing upright on hind limbs) were counted; the numbers of movements were then combined to yield a total activity score. Results reported are the mean ± SEM. *,†Within a time point, value differs significantly (*$P < 0.001$; †$P = 0.01$) from the value for the control group. ‡Within a time point, value differs significantly ($P < 0.05$) from the value for the BUP-HCl group. *See* Figure 2 for remainder of key.

Bonferroni post hoc analysis revealed no significant difference in respiratory rate between the BUP-SR and BUP-HCl groups at 4 hours, but the respiratory rate for the BUP-SR group was significantly ($P < 0.001$) lower than the respiratory rate for the BUP-HCl group at 24 and 48 hours.

Total activity—Repeated-measures ANOVA revealed a significant difference in total activity among treatment groups ($P < 0.05$) and among time points ($P < 0.001$). Bonferroni post hoc analysis revealed that the BUP-SR group had a significantly ($P = 0.01$) higher total activity, compared with total activity for the control group, at 4 hours **(Figure 3)**; however, total activity did not differ significantly between these groups at 24 and 48 hours. Bonferroni post hoc analysis also revealed that the BUP-HCl group had a significantly ($P < 0.001$) higher total activity, compared with total activity for the control group, at 4 hours, but total activity did not differ significantly between these groups at 24 and 48 hours. Total activity did not differ significantly between the BUP-SR and BUP-HCl groups at 4 hours but did differ significantly between these groups at 24 and 48 hours.

Gastrointestinal tract motility—Repeated-measures ANOVA revealed a significant difference in gastrointestinal tract motility among treatment groups ($P = 0.01$) and among time points ($P < 0.001$). Bonferroni post hoc analysis revealed that the BUP-SR group had significantly ($P < 0.001$) less gastrointestinal tract motility, compared with gastrointestinal tract motility for the control group, at 4 hours **(Figure 4)**; however, gastrointestinal tract motility did not differ significantly between these groups at 24 and 48 hours. Bonferroni post hoc analysis also revealed that the BUP-HCl group had significantly ($P < 0.001$) less gastrointestinal tract motility, compared with gastrointestinal tract motility for the control group, at 4 hours; however, gastrointestinal tract motility did not differ significantly between these groups at 24 and 48 hours. There was no

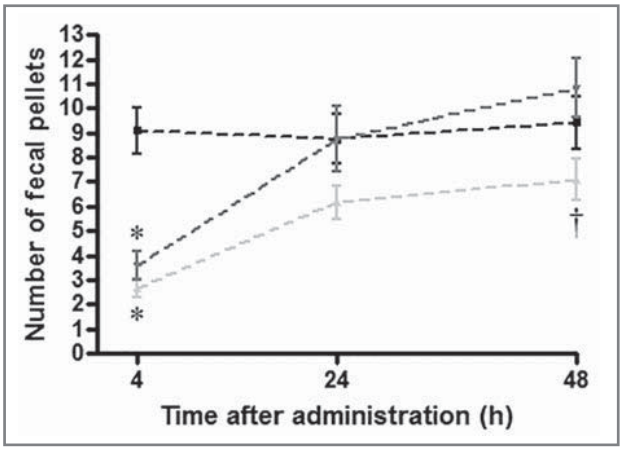

Figure 4—Gastrointestinal tract motility in mice (n = 12/group) 4, 24, and 48 hours after treatment with a vehicle (control treatment), BUP-HCl, or BUP-SR. Results reported are the mean ± SEM number of fecal pellets for each mouse during a 60-minute period within a testing chamber. *Within a time point, value differs significantly ($P < 0.001$) from the value for the control group. †Within a time point, value differs significantly ($P < 0.05$) from the value for the BUP-HCl group. *See* Figure 2 for remainder of key.

significant difference in gastrointestinal tract motility between the BUP-SR and BUP-HCl groups at 4 and 24 hours; however, the BUP-SR group had significantly less gastrointestinal tract motility, compared with that of the BUP-HCl group, at 48 hours.

Hot plate nociception testing—Peak antinociception effects were evident at 4 hours for both the BUP-SR and BUP-HCl groups. Repeated-measures ANOVA revealed a significant ($P < 0.001$) difference in latency for hot plate nociception testing among treatments groups and among time points. Bonferroni post hoc analysis revealed that the BUP-SR group had significantly greater antinociception, compared with antinociception for the control group, at 4 ($P < 0.001$), 24 ($P < 0.001$), and 48 ($P = 0.01$) hours **(Figure 5)**. Bonferroni post hoc analysis also revealed that the BUP-HCl group had significantly ($P < 0.001$) greater antinociception, compared with antinociception for the control group, at 4 hours, but antinociception did not differ significantly between these groups at 24 and 48 hours. There was no significant difference in antinociception between the BUP-SR and BUP-HCl groups at 4 hours, but the BUP-SR group had significantly ($P < 0.001$) greater antinociception, compared with antinociception for the BUP-HCl group, at 24 and 48 hours.

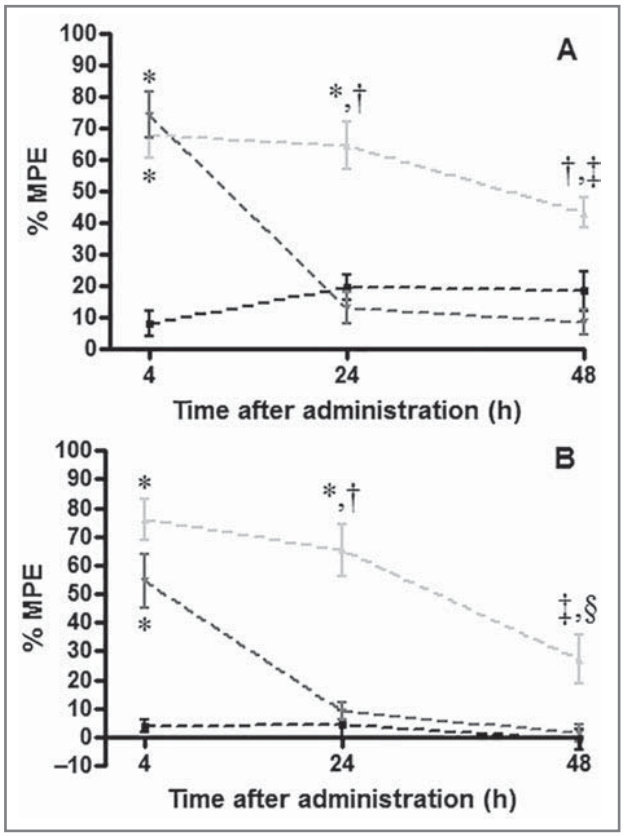

Figure 5—Mean ± SEM response latency during hot plate (A) and tail-flick (B) nociception tests in mice (n = 12/group) 4, 24, and 48 hours after treatment with a vehicle (control treatment), BUP-HCl, or BUP-SR. The %MPE was calculated by use of the following equation: %MPE = ([TL – BL]/[CL – BL]) × 100, where TL is the response latency at a given time point, BL is the baseline response latency, and CL is the cutoff response latency. The BL represents the mean of 2 response latencies determined before treatment administration. The CL was 30 and 10 seconds for the hot plate and tail-flick nociception tests, respectively. *Within a time point, value differs significantly ($P < 0.001$) from the value for the control group. †Within a time point, value differs significantly ($P < 0.001$) from the value for the BUP-HCl group. ‡Within a time point, value differs significantly ($P = 0.01$) from the value for the control group. §Within a time point, value differs significantly ($P < 0.05$) from the value for the BUP-HCl group. *See* Figure 2 for remainder of key.

Cataleptic behavior—Repeated-measures ANOVA revealed a significant ($P = 0.01$) difference in cataleptic behavior among time points. Bonferroni post hoc analysis revealed that cataleptic behavior for the BUP-SR group did not differ significantly, compared with cataleptic behavior for the control group, at 4, 24, and 48 hours. Bonferroni post hoc analysis revealed that cataleptic behavior for the BUP-HCl group also did not differ significantly, compared with cataleptic behavior for the control group, at 4, 24, and 48 hours. Cataleptic behavior did not differ significantly between the BUP-SR and BUP-HCl groups at 4, 24, or 48 hours.

Tail-flick nociception testing—Peak antinociception effects were evident at 4 hours for both the BUP-SR and BUP-HCl groups (Figure 5). Repeated-measures ANOVA revealed a significant ($P < 0.001$) difference in latency for tail-flick nociception testing among treatment groups and among time points. Bonferroni post hoc analysis revealed that the BUP-SR group had significantly greater antinociception, compared with antinociception for the control group, at 4 ($P < 0.001$), 24 ($P < 0.001$), and 48 ($P = 0.01$) hours (Figure 5). Bonferroni post hoc analysis also revealed that the BUP-HCl group had greater antinociception, compared with antinociception for the control group, at 4 hours ($P < 0.001$), but antinociception did not differ significantly between these groups at 24 and 48 hours. Antinociception did not differ significantly between the BUP-SR and BUP-HCl groups at 4 hours; however, the BUP-SR group had significantly greater antinociception, compared with antinociception for the BUP-HCl group, at 24 ($P < 0.001$) and 48 ($P < 0.05$) hours.

Discussion

The study reported here was conducted to determine the antinociceptive effects of an improved BUP-SR formulation with reduced opioid-related adverse effects. A BUP-SR that can be administered SC is advantageous for a number of reasons. Clinical pain assessment in rodents is challenging, even with refined assessment tools such as the mouse grimace scale.[30,31] Thus, continuous analgesia is more desirable than a fixed intermittent dosing regimen, which leaves open the possibility of wide swings in drug concentrations, periods of inadequate pain relief, and inadvertent instances of noncompliance with treatment administration. Furthermore, repeated administration of BUP-HCl has the potential to cause adverse effects, such as respiratory depression, gastrointestinal tract stasis, and reduced food consumption and body weight loss, at the time of peak concentrations without the benefit of continuous analgesic relief.[32] Loss of body weight has been reported in buprenorphine-treated animals.[33] Results for the present study are consistent with those of previous studies because a loss in body weight was detected

at 24 and 48 hours after administration in mice treated with BUP-HCl. Surprisingly, mice treated with BUP-SR did not have significant weight loss up to 48 hours after administration. Although this result should be confirmed, it is possible it reflected smaller variations in buprenorphine blood concentrations and, conceivably, fewer adverse effects when BUP-SR was used.

The behavioral effects after administration of commercially available BUP-SR formulations have been reported.[19,23] In those studies,[19,23] skin lesions were detected as early as the first day after administration. To our knowledge, particularly for Swiss-Webster mice, the BUP-SR formulation used in the study reported here is the first that diminishes the risk for skin lesions at the site of injection.

Antinociception effects can range from 12 to 72 hours after administration of BUP-SR formulations; disparities in these reported findings may be explained by differences in experimental methods, blood concentration, or degrees of binding to species' opioid receptors or the development of hyperalgesia related to higher peak concentrations.[34] In addition, differences in antinociceptive effects and development of hyperalgesia may reflect differences among rodent species or mouse strains. The potential for hyperphagia in rodents (especially rats) may not be diminished after administration of BUP-SR, compared with the potential for hyperphagia in rodents administered BUP-HCl.[19] To optimize benefits for the use of BUP-SR formulations in mice, whereby they would undergo long periods without observation during routine use, it is important to screen for potential adverse effects, including effects on respiration, gastrointestinal tract motility, body weight, total activity, and cataleptic behavior. There are multiple clinically relevant adverse effects associated with opioid narcotic use, with respiratory depression being one of the most dangerous.[35] In the present study, there was a significant decrease in respiratory rate for mice administered BUP-SR, compared with that in mice administered the control treatment, for the duration of the study (48 hours). However, tidal volume was not measured. Clinical studies[36,37] indicate that there is a ceiling effect for buprenorphine with regard to respiratory depression, which thereby minimizes the risk when compared with the risk for respiratory depression with conventional μ-opioid receptor agonists. Interestingly, it has been suggested in 1 study[38] that analgesic efficacy may not be constrained by this ceiling effect.

Similar to traditional μ-opioid receptor agonists, buprenorphine can cause a decrease in gastrointestinal tract motility,[10] which leads to concerns about constipation. The BUP-SR evaluated in the present study caused an initial decrease in gastrointestinal tract motility, as was evident at 4 hours after administration. However, a rebound effect was detected because gastrointestinal tract motility responses at 24 and 48 hours mirrored responses for the control treatment. In future studies, pure μ-opioid receptor agonists could be evaluated and their effects compared with those for BUP-SR to evaluate respiration, gastrointestinal tract motility, and efficacy for a sustained-release formulation in situations where buprenorphine's analgesic properties may be insufficient.[39,40]

An increase in total activity has been reported in rodents treated with buprenorphine.[10,41–44] Those results are consistent with results for both the BUP-SR and BUP-HCl groups at 4 hours after administration in the present study. Activation of μ-opioid receptors is crucial for buprenorphine-induced hyperactivity.[44] It has been suggested[45] that increases in opioid-induced activity may correlate with the tendency toward human drug reuse. In the present study, hyperactivity was observed in mice receiving BUP-SR only at 4 hours, after which activity returned to a level comparable to that of the control group, whereas substantial antinociceptive effects were sustained for at least 48 hours after administration.

Future studies with the BUP-SR formulation used in the present study should be conducted to determine variations in antinociception that may exist between animals with acute and chronic pain as well as variations in efficacy for various types of pain. Buprenorphine can be an effective analgesic agent with respect to multiple types of pain that reflect a complex assembly of specific pain modalities.[12] Side-by-side comparisons that use methods to induce long-lasting pain, in addition to the methods used in the present study to induce short-term pain, will further advance the development of optimized use of BUP-SR and other opioid formulations.

A BUP-SR could be beneficial for postsurgical treatment of mice because it could provide more continuous analgesia, more stable opioid binding of receptors, and more stable blood concentrations as well as reducing handling stress. The novel BUP-SR in the present study sustained significant antinociceptive activity in mice for up to 48 hours as assessed by the use of thermal nociception testing.

Results for the present study were obtained from mice that had not undergone surgical intervention. Thus, there is the potential that these results may differ from those of mice undergoing surgery and receiving the same BUP-SR. Additional studies are needed to optimize the dosing regimen and efficacy. It is anticipated that improved alleviation of pain and distress will directly improve animal well-being and reduce experimental variation and may potentially reduce the number of mice needed in future studies. Overall, the data reported here support the potential use of this improved BUP-SR for painful procedures in mice to better alleviate pain and distress for an extended period of at least 24 hours without the need for administration of additional doses.

a. Taconic, Germantown, NY.
b. Sealsafe Next IVC Blue Line, Tecniplast USA Inc, Philadelphia, Pa.
c. 2018 Teklad global 18% protein rodent diet, Harlan Laboratories Inc, Indianapolis, Ind.
d. GraphPad Instat 3.0, GraphPad Software Inc, San Diego, Calif.
e. Buprenex, 0.3 mg/mL, Reckitt Benckiser Pharmaceuticals Inc, Richmond, Va.
f. 0.6 mg/mL, provided by WildPharm, Windsor, Colo.
g. San Diego Instruments, San Diego, Calif.
h. 10.2 cm ID × 15.2 cm length, IITC Life Science Inc, Woodland Hills, Calif.
i. 27.5 × 26.3 × 1.5 cm, 53°C, IITC Life Science Inc, Woodland Hills, Calif.
j. IITC Life Science Inc, Woodland Hills, Calif.
k. GraphPad Prism 4.0, GraphPad Software Inc, San Diego, Calif.

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