

# Evaluation of a Sustained-Release Formulation of Buprenorphine for Analgesia in Rats

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Preventing and minimizing pain in laboratory animals is a basic tenet of biomedical research and is warranted for ethical, legal, and scientific reasons. Postoperative analgesia is an important facet of pain management. A sustained-release formulation of buprenorphine was tested in rats for analgesic efficacy and plasma concentration over a 72-h time period. Rats were injected subcutaneously with either 1.2 mg/kg sustained-release formulation (Bup-SR), 0.2 mL/kg buprenorphine HCl (Bup-HCl), or an equivalent volume of sustained-release vehicle and tested in a thermal nociception model or a surgical postoperative pain model. In both models, Bup-SR showed evidence of providing analgesia for 2 to 3 d. Thermal latency response in rats that received the sustained-release formulation increased 28.4% and 15.6% compared with baseline values on days 1 and 2, respectively. Rats with a unicortical tibial defect and treated with Bup-SR showed similar willingness to bear weight on the hindlimbs as did negative-control animals (no surgery), demonstrated by counting vertical raises; rats treated with Bup-HCl had significantly fewer vertical raises than did control rats for 5 d after surgery. Plasma concentrations of buprenorphine remained over 1 ng/mL for 72 h after a single dose of Bup-SR. Taken together, the results indicate that this formulation of buprenorphine may be a viable option for treating postsurgical pain in laboratory rats.

**Abbreviations:** Bup-HCl, buprenorphine hydrochloride; Bup-SR, sustained-release formulation of buprenorphine.

Provision of postoperative analgesia is a necessary component of animal research and contributes to the principle of refinement by alleviating or minimizing pain and distress. Indeed, 3 of the 9 principles within the *US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training* specifically address pain and distress (Principles IV, V, and VI).<sup>31</sup> Buprenorphine is one of the most commonly used analgesics in rodents.<sup>5,6,49</sup> It is preferred for its activity as a partial agonist at the  $\mu$ -opioid receptor, ease of administration, and long duration of action and has been shown to be effective in a variety of pain models.<sup>9,27</sup> Another potential advantage of buprenorphine over full  $\mu$ -opioid receptor agonists such as fentanyl is buprenorphine's wider safety margin.<sup>55</sup> However, to maintain effective therapeutic levels, it must be administered at least 2 or 3 times daily. Frequent dosing requires more personnel effort and more handling of the animals being treated. Using the hot-plate and tail-flick assays, several authors demonstrated a duration of 6 to 8 h in rats (0.5 mg/kg) and 3 to 5 h in mice (2.0 mg/kg) for buprenorphine.<sup>22</sup> Despite many publications demonstrating buprenorphine's efficacy in both traditional pain assays and clinical assessments, its use remains controversial, and many studies cite unfavorable characteristics associated with its use such as loss of body weight, pica behavior, and lack of efficacy compared with other opioids.<sup>10,24,32,47,53</sup> In addition, the dose used varies widely, as shown in a review of the use of buprenorphine in animals, in which clinical doses in rats ranged from 0.01 to 0.5 mg/kg.<sup>48</sup>

Sustained-release drug formulations have been achieved for some analgesic drugs. Transdermal fentanyl patches have been used in sheep, dogs, and rabbits.<sup>1,17,21,28</sup> Liposomal formulations of oxymorphone and hydrocodone have been tested in mice and

rats.<sup>11,52</sup> However, no sustained-release analgesic that can be administered safely and effectively to rodents is yet available. A formulation that provides sustained release of buprenorphine for 72 h (buprenorphine HCl SR, ZooPharm, Fort Collins, CO) is now commercially available; this formulation can be administered subcutaneously and has been preliminarily evaluated in dogs and cats,<sup>57</sup> but heretofore has not been tested in rodents. The current study aimed to evaluate the use of buprenorphine HCl SR in rats by using a standard pain-assessment tool (thermal analgesiometry), measuring plasma concentrations over a 72-h period, and assessing the drug's ability to provide clinically relevant postoperative analgesia in a rat model of orthopedic surgical pain.

## Materials and Methods

**Animals.** Male Sprague-Dawley rats (275 to 300 g) were obtained from Charles River Laboratories (Wilmington, MA). The rats were free of the following agents: Sendai virus, pneumonia virus of mice, rat parvovirus, *Mycoplasma pulmonis*, sialodacryoadenitis virus, reovirus 3, lymphocytic choriomeningitis virus, Tyzzer disease virus, and cilia-associated respiratory bacillus. The rats were group-housed in polycarbonate caging with ad libitum access to food (Diet 7912, Harlan Laboratories, Indianapolis, IN) and tap water and a 12:12-h light:dark cycle. The University of Virginia Animal Care and Use Committee approved all animal experiments, and the program is AAALAC-accredited. Rats receiving surgery were euthanized by anesthetic overdose at the end of the study. Rats used for the noninvasive experiments (thermal sensitivity and plasma concentration determination) subsequently were used for training purposes.

**Experimental groups and drugs.** Three different experiments were conducted: a thermal nociception response study (12 rats), a tibial defect study (40 rats), and a plasma concentration study (9 rats). Rats were distributed randomly into treatment groups, and the end observer was blinded to treatment group

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allocation. Randomization included housing rats within treatment groups among different cages and mixing of treatment groups within cages. Rats were housed 2 or 3 per cage. Rats received either sustained-release buprenorphine HCl (Bup-SR; ZooPharm, Fort Collins, CO), buprenorphine HCl (Bup-HCl; Hospira, Lake Forest, IL), or the sustained-release polymer as vehicle control, with dosages provided as described later. According to information obtained from ZooPharm, Bup-SR consists of a biodegradable polymer delivery system and the drug buprenorphine hydrochloride. The biodegradable polymer delivery system is a 50:50 (w/w) solution of a biodegradable polymer dissolved in a biocompatible organic solvent. The biodegradable polymer is a copolymer with a 50:50 molar ratio of DL-lactide to  $\epsilon$ -caprolactone. The copolymer has an average molecular weight of approximately 5500 Da.<sup>39</sup>

**Sensitivity to a thermal stimulus.** After several days of acclimation to handling followed by 2 consecutive days of acquisition of baseline thermal latency data and body weights (see below), rats were given either 1.2 mg/kg Bup-SR ( $n = 6$ ) or 0.2 mg/kg Bup-HCl ( $n = 6$ ). Both drugs were administered subcutaneously on the rat's dorsum. Rats were tested once daily on days 1, 2, and 3 (24, 48, and 72 h after dosing) by a blinded observer. Thermal latency was evaluated by using a thermal analgesiometer (Plantar Analgesia Meter Model 390G, IITC, Woodland Hills, CA). Rats were placed on an acrylic glass platform maintained at 28 °C and acclimated to the chamber for 10 to 15 min prior to testing. A focused thermal heat stimulus was delivered from a fixed distance to the plantar surface of the hindpaw, and the time until the paw was lifted in response to the heat stimulus was defined as the latency interval. Thermal heat intensity was adjusted initially to develop a baseline latency interval in nonmedicated rats of 9 to 10 s. During establishment of baseline latency, if mean values for a rat's right and left hind paws differed by more than 1 s, the rat was excluded from the study. Thereafter, only the right paw response was determined. The device was set to cut off automatically at 20 s to avoid any potential for thermal injury to the rat's paw. Each rat was tested 3 to 4 times per session, and mean latency response was calculated. The mean and SEM were calculated for each group for each day of testing.

**Tibial defect model.** Rats were randomized into 4 experimental groups each containing 10 rats. The experimental groups were: tibial defect treated with Bup-SR at 1.2 mg/kg SC; tibial defect treated with buprenorphine HCl at 0.2 mg/kg SC; anesthesia only plus Bup-SR at 1.2 mg/kg SC; anesthesia only plus control sustained-release vehicle (volume, 0.2 mL). A sham surgical group was not performed because of ethical considerations associated with having an additional group of rats subjected to a surgical procedure without analgesia, therefore groups receiving just anesthesia were included. A no-surgery group given Bup-SR was included to determine whether administration of Bup-SR had any adverse effects of its own that might be masked in the surgery group. The group receiving buprenorphine HCl received a single rather than multiple doses, to differentiate analgesic duration from the Bup-SR group. Baseline ethograms and body weights were established prior to surgery. Rats were anesthetized with ketamine (45 to 50 mg/kg IP) and medetomidine (0.5 mg/kg IP). The left leg was shaved to remove fur and prepped for sterile surgery. A skin incision was made over the lateral aspect of the proximal tibia. Soft tissue was retracted gently from the bone and the periosteum removed. By using a compressed air powered drill, a unicortical defect (2 × 4 mm) was created in the proximal tibia. The surgical site was kept moist and cool with sterile saline. The

skin incision was closed by using 6-0 polypropylene suture in a simple interrupted pattern. Rats were identified by ear notch and given a single subcutaneous dose of either Bup-SR or Bup-HCl. Anesthesia only groups received either a single dose of Bup-SR or the sustained-release vehicle only. Atipamezole was administered subcutaneously (0.1 mL) as an anesthetic reversal agent. Rats were provided supplemental heat during and after surgery or anesthesia until ambulatory again.

**Ethogram.** Observers were blinded to treatment group, although surgical and anesthesia groups were easily distinguishable visually. During each daily evaluation period, rats were assessed for body weight, food and water consumption, general activity, use of affected leg, and number of vertical raises (standing on hindlegs only). Food intake was determined by subtracting the weight of any uneaten pellets from the known starting weight. Water consumption was determined similarly by subtracting the remaining volume from the known starting volume. Both food and water were always available in excess of consumption. General activity in the rat's home cage while observed for 1 min was scored as follows: 0, no activity; 1, not as active as expected but some movement and exploration around cage; 2, normal activity level with exploration of all 4 corners. Total number of vertical raises when placed in novel empty cage were counted over a 2-min period.<sup>35</sup> A full vertical raise was defined as standing on both hindlimbs, with both hindlimbs supporting the entire body weight, torso fully extended, with front paws in the air or against the side of the cage. A partial vertical raise was defined as standing on both hindlimbs but without full extension of the torso; a partial raise was given one half the value of a full stand. Pain at the surgical site was evaluated by palpating the leg at the surgical area. Vocalization during palpation of the affected leg or when the rat was picked up was considered a sign of pain. Likewise, any guarding of the leg or decreased use of the leg was noted. Presence of porphyrin at the nares or eyes was noted also. Rats were evaluated before surgery (baseline) and subsequently on days 1, 2, 3, 5, and 8 after surgery. Figure 1 summarizes the assessment paradigm.

**Pharmacokinetics of buprenorphine.** Blood samples were collected into heparinized collection tubes from rats given either Bup-SR at 0.9 mg/kg, Bup-SR at 1.2 mg/kg, or Bup-HCl at 0.1 mg/kg ( $n = 3$  for each drug group; total of 9 rats). Rats given Bup-SR 0.9 mg/kg had blood samples collected at 4, 8, 24, 48, and 72 h after administration. For the immediate-release drug group (Bup-HCl), blood was collected only at the 4-, 8-, and 24-h time points because we anticipated that no detectable levels would be present after 24 h. Subsequently, blood samples were collected at 8, 24, and 48 h from a group of rats treated with 1.2 mg/kg Bup-SR to determine whether an increased dose provided increases in measured plasma levels. Due to financial constraints, only limited time points were collected for this group. Samples were centrifuged and plasma collected and stored at -80 °C until analyzed. Samples were processed by using HPLC followed by double mass spectroscopy (Azopharma, Maryland Heights, MO) for buprenorphine concentration, with a lower limit of detection of 0.05 ng/mL.

**Statistical analysis.** Data were analyzed by using GraphPad Prism (version 5.02, GraphPad Software, San Diego, CA). ANOVA was used to analyze body weights and food and water consumption. Two-way ANOVA was used to analyze thermal latency and vertical raises, and Bonferroni posttests were used to determine differences between treatment groups at each time point. One-way ANOVA was determined to evaluate differences between tibial defect treatment groups. Data are expressed as

Parameter	Assessment
Body weight	Measured once daily
Food and water consumption	Measured once daily
No. of vertical raises in 2-min period	Full raises given 1 point; partial raises given 1/2 point
General activity score	0: quiet, little activity; 1: moderate activity; 2: normal, very active
Use of affected leg score, local pain	1: slight use, vocalizes when leg palpated; 2: moderate use, some guarding of leg; 3: favoring leg slightly; 4: normal

Figure 1. Clinical assessment and ethogram.

mean  $\pm$  SEM, and the threshold for significance was established at a  $P$  value of less than 0.05.

## Results

**Thermal sensitivity of hindpaw.** Paw withdrawal latency in rats was measured in response to a thermal stimulus before and after subcutaneous administration of either Bup-SR or Bup-HCl. Thermal intensity level was set to provide a baseline latency interval of 9 to 10 s (actual baseline latency,  $9.7 \pm 0.9$  s), and the right paw was assessed in all rats, for consistency. Mean latency over the 3-d period was 11.2 s for Bup-SR rats (peaked on day 1 at 12.3 s), and 9.3 s for Bup-HCl rats. Thermal latency (% change from baseline) increased through day 2 in rats given Bup-SR, with maximal latency on day 1 of 28.4% over baseline, 15.6% on day 2, and a return to near baseline on day 3 (Figure 2). The 3-day difference between the 2 treatment groups was statistically significant ( $P < 0.05$ ; 2-way ANOVA), and the difference between the treatment groups on individual days was statistically significant ( $P < 0.005$ ; unpaired  $t$  test) on day 2. Body weight in both groups did not change over the course of the 3 d, and there was no difference in body weights between the 2 groups at any time point. No adverse effects on behavior were observed with either treatment. However, in some rats, in which a small portion of the Bup-SR dose administered seemed to seep back out of the injection site immediately upon withdrawal of the needle, skin irritation including erythema and scabbing of the skin around the injection site developed over the next 24 h. This reaction occurred primarily in the first few rats injected, and the injection technique was modified subsequent to this observation. Thereafter, the skin was tented up during both drug injection and needle withdrawal and pinched at the needle site for approximately 15 s after needle withdrawal, and the needle was withdrawn more slowly. With this method, occurrence of skin irritation was reduced dramatically.

**Tibial defect model.** The ethogram, which included numeric scoring of general activity and use of affected leg (Figure 1), showed no statistical significance between treatment groups. Activity score was lowest on day 2 after surgery for both surgery groups. Similarly, body weight and food and water consumption did not vary significantly over the time period evaluated between the 4 experimental groups. When evaluated for the number of vertical raises exhibited over a 2-min period, rats that received Bup-SR after surgery performed more vertical raises than did those that received Bup-HCl after surgery ( $P < 0.05$ ; one-way ANOVA), but significantly fewer than those given Bup-SR after anesthesia alone ( $P < 0.01$ ); the treated no-surgery group was not significantly different than the control group that received vehicle only. In addition, rats treated after surgery with Bup-HCl demonstrated fewer vertical raises than did all 3 other experimental groups, and this decrease in willingness

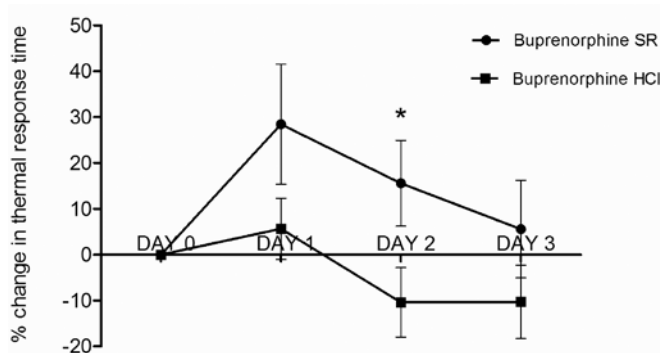


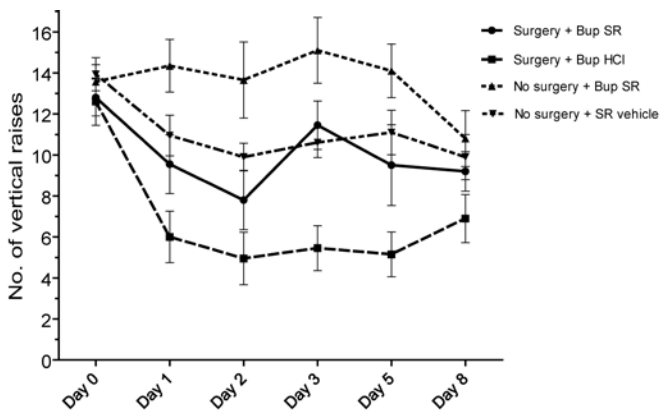
Figure 2. Paw withdrawal response (% change from baseline; mean  $\pm$  SEM) to a thermal stimulus in rats treated with either sustained-release buprenorphine (Bup-SR) or buprenorphine HCl (Bup-HCl). Thermal intensity level was set to provide a baseline latency interval of 9 to 10 s. Thermal latency increased in Bup-SR rats through day 3. The difference between the 2 treatment groups over the 3-d period was statistically different ( $P < 0.05$ ; 2-way ANOVA). \* There was an additional significant ( $P < 0.005$ ; unpaired  $t$  test) treatment effect on day 2.

to support full weight on both hindlegs persisted throughout the 8-d testing period, ranging from 45% to 61% of presurgical levels ( $P < 0.05$ ; Figure 3). Among the rats in the tibial-defect study, skin irritation (mild) was seen in only 1 rat given Bup-SR.

**Plasma concentration of buprenorphine.** Plasma concentration of buprenorphine at the observed time points was highest at 4 h for both Bup-HCl (0.1 mg/kg) and Bup-SR (0.9 mg/kg) at similar levels (2.8 ng/mL and 2.7 ng/mL, respectively), although there was a large variability at this early time point for both drugs. In addition, levels at 8 h were similar for both formulations, including a higher dose group for Bup-SR (1.2 mg/kg). At 24, 38, and 72 h, only Bup-SR achieved levels close to or above 1 ng/mL. Although the differences in plasma profile were not statistically different, group sizes were small ( $n = 3$ ), and early time point variability likely contributed to lack of statistical significance. Figure 4 A shows plasma concentrations over the entire time period tested. Figure 4 B shows a dose-dependent effect at the 24-h time point.

## Discussion

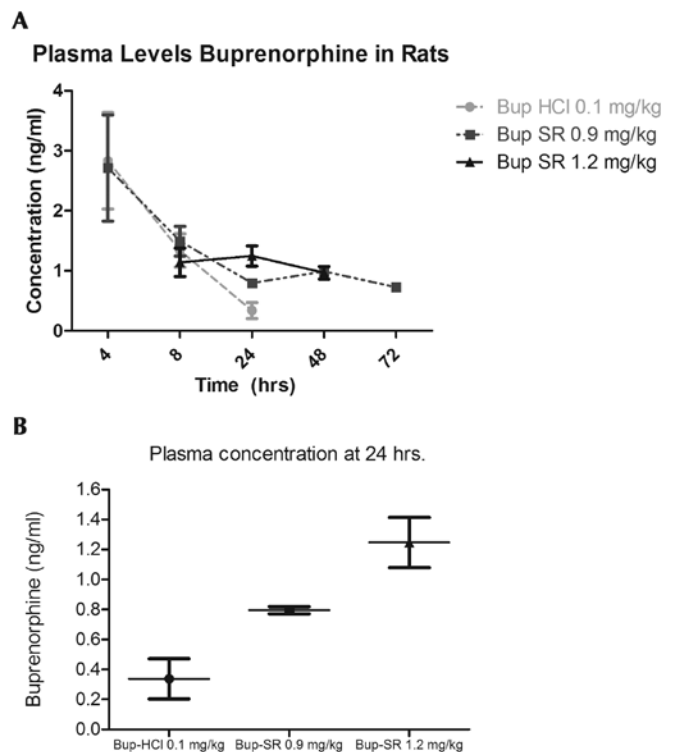
Buprenorphine has been shown to possess strong binding affinity to  $\mu$ -opioid receptors and an antinociceptive potency 25 to 40 times higher than that of morphine.<sup>13,34</sup> It is generally accepted that buprenorphine exerts primarily  $\mu$ -opioid receptor agonist,  $\kappa$ -opioid receptor antagonist, and  $\delta$ -receptor agonist effects.<sup>34,45</sup> Buprenorphine-induced amelioration of postsurgical pain has been substantiated in a variety of species,<sup>49</sup> and buprenorphine has been used clinically in humans for 30 y.<sup>34</sup>



**Figure 3.** Hindlimb weight-bearing (no. of vertical raises in 2 min; mean  $\pm$  SEM) in rats with a unicortical tibial defect. Control rats received control sustained-release polymer under anesthesia. Among those that underwent surgery, rats treated with sustained-release buprenorphine (Bup-SR) performed more vertical raises than did rats treated with buprenorphine HCl (Bup-HCl) ( $P < 0.05$ ), but significantly fewer than those that had anesthesia alone (no surgery) with Bup-SR ( $P < 0.01$ ). Number of vertical raises did not differ between the 2 no-surgery groups. Rats treated after surgery with buprenorphine HCl demonstrated fewer vertical raises than did all 3 other experimental groups; this decrease in willingness to support full weight on both hindlegs persisted throughout the 8-d testing period and ranged from 45% to 61% of presurgical levels ( $P < 0.05$ ).

Due to its long duration of action, buprenorphine is one of the most widely used opioid analgesics in veterinary clinical practices.<sup>8,16,54</sup>

As would be expected, the analgesic efficacy of buprenorphine in rats has been established in many studies. For example, several studies demonstrated significant differences in analgesic and behavioral responses to buprenorphine in August Copenhagen Irish (ACI) and Brown Norway (BN) rats.<sup>3,4</sup> Similarly, others found buprenorphine to be an effective analgesic in a variety of acute and chronic pain models using both mice and rats with an  $ED_{50}$  ranging from 0.0024 to 0.16 mg/kg when given intravenously.<sup>9</sup> Doses determined to provide effective postoperative pain in rats range from 0.05 mg/kg<sup>15</sup> to 0.5 mg/kg.<sup>22,41</sup> However, other studies have not found significant analgesic action with buprenorphine in rats and mice at doses tested<sup>23,24,43</sup> or have seen adverse effects on food consumption and body weight.<sup>32,33</sup> The optimal dose seems to remain an open question and is affected further by strain variation. Oxymorphone was found to be more effective than buprenorphine in alleviating pain-related behaviors after intestinal resection;<sup>24</sup> however, rats in the cited study received a much higher dose (0.5 mg/kg every 6 h) than those given in other studies. The increased dose used in the present study (1.2 mg/kg Bup-SR) was based on an equivalent dosing schedule of either 0.2 mg/kg every 12 h or 0.13 mg/kg every 8 h for 3 d. The basis for the chosen dose in this study was to select a dose sufficiently high to detect a measurable clinical response if present, even though that dose was higher than some reported in the literature. The lower dose of 0.9 mg/kg Bup-SR was based on equivalent dosing with buprenorphine hydrochloride at 0.1 mg/kg every 8 h for 3 d. At these doses, increased thermal latency was seen on days 1, 2, and 3, compared with baseline levels and those associated with Bup-HCl. Thermal latency response is a common measure of nociceptive pain and is often used to assess hyperalgesia in models of neuropathic pain.<sup>46</sup> Future studies with the Bup-SR formulation will test whether lower doses comparable to single doses of 0.05 mg/kg are efficacious. However, it is notable that



**Figure 4.** (A) Plasma concentration of buprenorphine in rats given either buprenorphine HCl (Bup-HCl; 0.1 mg/kg) or sustained-release buprenorphine (Bup-SR; 2 different doses) at the time points shown ( $n = 3$ ). Bup-HCl and Bup-SR had similar peak plasma levels at 4 h (2.8 and 2.7 ng/mL, respectively), which declined to 1.4 and 1.5 ng/mL by 8 h. Although Bup-HCl continued to decline at 24 h, Bup-SR maintained a steady-state level averaging 1.1 ng/mL through the 72-h time point. Bup-SR at 1.2 mg/kg measured at 8, 24, and 48 h showed plasma levels close to those obtained with 0.9 mg/kg, remaining at or above 1 ng/mL. (B) Dose-dependent effect of Bup-HCl, 0.9 mg/kg Bup-SR, and 1.2 mg/kg Bup-SR at 24 h after administration ( $n = 3$ ).

even with the 1.2-mg/kg dose, plasma buprenorphine concentrations remained less than 1.5 ng/mL after the initial peak.

Although the thermal nociception assay successfully identified an analgesic effect, pain threshold measurement may not be entirely predictive of postsurgical pain. Therefore, paw withdrawal latency to a thermal stimulus was combined with other assessments often used for determining postoperative status of animals including food consumption, body weight, and a behavioral ethogram.<sup>19,29,44</sup> Neither treatment group nor time after surgery elicited a significant change in body weight or food or water consumption. Furthermore, no pica behavior was observed, as had been reported in other rat studies.<sup>10</sup> The current findings differ from other studies, which demonstrated decreased food intake or body weight associated with buprenorphine administration.<sup>3,5,7,12,25,32,51</sup> In several studies, rats lost weight during the first few postoperative days despite same or increased food consumption.<sup>6,32</sup> Although the adverse effect of opioid analgesics in rodents seems to vary in incidence and severity, another plausible explanation for the lack of negative clinical effects seen with Bup-SR is that this sustained-release formulation results in more steady-state plasma levels, as substantiated by the plasma concentration measurements. Perhaps the intermittent high peak levels associated with periodic dosing result in some of the observed detrimental clinical effects. Indeed, a similar discrepancy was seen with repeated injections of oxymorphone compared with liposome-encapsulated oxy-

morphone administered to rats after intestinal resection.<sup>37</sup> Rats given liposome-encapsulated oxymorphone had significantly higher body weight gain compared with that of rats given nonencapsulated oxymorphone.

Assessment of pain using an orthopedic surgical model proved to be difficult. The tibial defect model of bone pain is expected to elicit mechanical hyperalgesia and allodynia, as well as limb guarding and lifting.<sup>30</sup> The rats in the current study did not show obvious signs of favoring the surgically altered leg as expected, even 1 d after surgery. Movement around both the home cages and the testing cage was remarkably normal. Use of the vertical raise scoring system was adapted from a study of skeletal pain in a mouse fracture model<sup>35</sup> and seemed to be an effective measurement of willingness to bear weight on hindlegs after surgery. Rats treated with Bup-HCl (single dose) had fewer vertical raises on days 1, 2, 3, and 5 than did control rats ( $P < 0.05$ ; 2-way ANOVA with Bonferroni posttests). In contrast, rats treated with Bup-SR had similar scores to control rats at all time points. Interestingly, rats treated with Bup-SR that underwent anesthesia but no surgical intervention showed significantly more vertical raises than did Bup-SR treated rats with surgery on days 1 and 2. In other words, the buprenorphine-treated group of no-surgery rats seemed to show an increased activity level unrelated to presence or absence of pain. However, the 2 nonsurgical groups given either Bup-SR or vehicle only did not differ. One possible explanation for this observation is the nonspecific increase in general activity level due to buprenorphine, which has been noted to occur in both rats<sup>14,20,48</sup> and mice.<sup>14,38</sup> Other tools such as static or dynamic weight-bearing assessments or activity monitors (for example, HomeCageScan System, Clever Sys, Reston, VA) as used elsewhere<sup>50</sup> might provide more objective assessments and are under consideration for future studies.

The skin irritation seen in some of the rats is clearly a consideration in the use of Bup-SR. Although administration techniques may avoid complications in most animals, and although the degree of inflammatory response and necrosis were not severe in any of the animals in the current study, the potential for adverse effects, especially if used in smaller animals such as mice, is a concern. Histopathology of the affected area was considered; however, the likelihood of obtaining useful information beyond confirming a local inflammatory response seemed minimal. Reformulation with a different solvent may resolve this drawback.

Plasma concentrations were determined for 2 different dosages of Bup-SR as well as a single dose of Bup-HCl. Surprisingly a small yet detectable level of buprenorphine was found at 24 h ( $0.34 \pm 0.13$  ng/mL) in the Bup-HCl group; however, this concentration is unlikely to provide analgesia. The levels measured for doses of the sustained-release formulations over the 72-h period (close to or above 1 ng/mL throughout) were consistent with measurements found in other species. For example, unpublished data from a study with Bup-SR in 3 dogs given 0.27 mg/kg SC showed an average plasma concentration remaining over 1 ng/mL from 1 to more than 72 h after injection.<sup>57</sup> Pharmacokinetic values determined from those dogs included a maximal concentration of 2.14 ng/mL at time of 1 h; that result parallels the findings in this study in rats of a maximal concentration of 2.7 ng/mL at 4 h (earliest time point measured) at a dose of 0.9 mg/kg. The number of samples obtained for determining plasma concentrations in the current study was small due to the expense of the assay. However, the data indicate that plasma levels within a range considered to provide analgesia are achievable with the Bup-SR formulation. Other pharmacokinetics studies in animals typically have followed distribution after intravenous

administration.<sup>26,36,56</sup> However, a study examining pharmacokinetics of transdermal buprenorphine in dogs found that use of a 70- $\mu$ g/h patch in dogs resulted in a sustained plasma concentration of 0.7 to 1.0 ng/mL after the initial 36-h period after application.<sup>2</sup> In humans, the minimal effective analgesic concentration is considered to be 0.1 ng/mL, and the target plasma concentration is 0.5 to 0.7 ng/mL.<sup>18</sup>

Although transdermal formulations of analgesic drugs such as fentanyl and buprenorphine have not yet been practical in rodents due to their small body size and the likelihood of ingesting a dermally applied product, a variety of other sustained-release formulations have been explored. Liposome-encapsulated hydromorphone was effective in a rat model of neuropathic pain,<sup>52</sup> and similarly formulated oxymorphone was effective in ameliorating splenectomy-induced pain in mice.<sup>11</sup> Novel depot formulations of buprenorphine prodrugs were evaluated in rats and found to prolong nociceptive activity for as long as 70 h, as compared with a single dose of buprenorphine HCl (5 h).<sup>42</sup> A recent study examined the efficacy of slow-release morphine and hydromorphone preparations in a rat model of thermal nociception,<sup>40</sup> similar to the methodology used in the current study (paw withdrawal latency to a thermal stimulus). Both of the formulations used are oral formulations used in humans, and single doses of these 2 drugs in rats prolonged thermal latency as long as 3 h and 7 h compared with baseline values, respectively.<sup>40</sup>

Despite ongoing and important research in this area of investigation, none of these cited studies appears to offer a viable available alternative to current dosing regimens for analgesia. Using both a thermal nociceptive model and a model of orthopedic surgical pain, the current study suggests the efficacy of a commercially available product (sustained-release buprenorphine) in providing analgesic activity in rats. Although concerns remain regarding the potential for skin irritation with this product and further investigation will be necessary to better determine optimal dosing and efficacy in treating postsurgical pain, sustained-release buprenorphine may provide a valuable option for treating postoperative pain in laboratory animals. The use of a sustained-release analgesic with efficacy over a 48- to 72-h period would provide increased ease of administration, potentially better compliance with analgesic dosing, less handling (and therefore less stress) of animals, and more consistent and even plasma and tissue drug concentrations. Taken together, these factors represent a significant refinement in animal welfare.

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## References

1. Ahern BJ, Soma LR, Boston RC, Schaer TP. 2009. Comparison of the analgesic properties of transdermally administered fentanyl and intramuscularly administered buprenorphine during and following experimental orthopedic surgery in sheep. *Am J Vet Res* 70:418–422.
2. Andaluz A, Moll X, Ventura R, Abellan R, Fresno L, Garcia F. 2009. Plasma buprenorphine concentrations after the application of a 70- $\mu$ g/h transdermal patch in dogs. Preliminary report. *J Vet Pharmacol Ther* 32:503–505.
3. Avsaroglu H, Sommer R, Hellebrekers LJ, van Zutphen LFM, van Lith HA. 2008. The effects of buprenorphine on behaviour in the ACI and BN rat inbred strains. *Lab Anim* 42:171–184.

4. **Avsaroglu H, van der Sar AS, van Lith HA, van Zutphen LFM, Hellebrekers LJ.** 2007. Differences in response to anaesthetics and analgesics between inbred rat strains. *Lab Anim* **41**:337–344.
5. **Blaha MD, Leon LR.** 2008. Effects of indomethacin and buprenorphine analgesia on the postoperative recovery of mice. *J Am Assoc Lab Anim Sci* **47**:8–19.
6. **Bomzon A.** 2006. Are repeated doses of buprenorphine detrimental to postoperative recovery after laparotomy in rats? *Comp Med* **56**:114–118.
7. **Brennan MP, Sinusas AJ, Horvath TL, Collins JG, Harding MJ.** 2009. Correlation between body weight changes and postoperative pain in rats treated with meloxicam or buprenorphine. *Lab Anim (NY)* **38**:87–93.
8. **Capner CA, Lascelles BDX, Waterman-Pearson AE.** 1999. Current British veterinary attitudes to perioperative analgesia for dogs. *Vet Rec* **145**:95–99.
9. **Christoph T, Kogel B, Schiene K, Meen M, De Vry J, Friderichs E.** 2005. Broad analgesic profile of buprenorphine in rodent models of acute and chronic pain. *Eur J Pharmacol* **507**:87–98.
10. **Clark JA, Myers PH, Goelz MF, Thigpen JE, Forsythe DB.** 1997. Pica behavior associated with buprenorphine administration in the rat. *Lab Anim Sci* **47**:300–303.
11. **Clark MD, Krugner-Higby L, Smith LJ, Heath TD, Clark KL, Olson D.** 2004. Evaluation of liposome-encapsulated oxymorphone hydrochloride in mice after splenectomy. *Comp Med* **54**:558–563.
12. **Cooper DM, Hoffman W, Wheat N, Lee HY.** 2005. Duration of effects on clinical parameters and referred hyperalgesia in rats after abdominal surgery and multiple doses of analgesic. *Comp Med* **55**:344–353.
13. **Cowan A.** 2003. Buprenorphine: new pharmacological aspects. *Int J Clin Pract Suppl* **133**:3–8.
14. **Cowan A, Doxey JC, Harry EJR.** 1977. The animal pharmacology of buprenorphine, an oripavine analgesic agent. *Br J Pharmacol* **60**:547–554.
15. **Curtin LI, Grakowsky JA, Suarez M, Thompson AC, DiPirro JM, Martin LBE, Kristal MB.** 2009. Evaluation of buprenorphine in a postoperative pain model in rats. *Comp Med* **59**:60–71.
16. **Dohoo SE, Dohoo IR.** 1996. Factors influencing the postoperative use of analgesics in dogs and cats by Canadian veterinarians. *Can Vet J* **37**:552–556.
17. **Egger CM, Glerum L, Michelle Haag K, Rohrbach BW.** 2007. Efficacy and cost-effectiveness of transdermal fentanyl patches for the relief of postoperative pain in dogs after anterior cruciate ligament and pelvic limb repair. *Vet Anaesth Analg* **34**:200–208.
18. **Evans HC, Easthope SE.** 2003. Transdermal buprenorphine. *Drugs* **63**:1999–2010.
19. **Firth AM, Haldane SL.** 1999. Development of a scale to evaluate postoperative pain in dogs. *J Am Vet Med Assoc* **214**:651–659.
20. **Flecknell PA, Liles JH.** 1992. Evaluation of locomotor activity and food and water consumption as a method of assessing postoperative pain in rodents, p 482–488. In: Short CE, Van Poznak A, editors. *Animal pain*. New York (NY): Churchill Livingstone.
21. **Foley PL, Henderson AL, Bissonette EA, Wimer GR, Feldman SH.** 2001. Evaluation of fentanyl transdermal patches in rabbits: blood concentrations and physiologic response. *Comp Med* **51**:239–244.
22. **Gades NM, Danneman PJ, Wixson SK, Tolley EA.** 2000. The magnitude and duration of the analgesic effect of morphine, butorphanol, and buprenorphine in rats and mice. *Contemp Top Lab Anim Sci* **39**:8–13.
23. **Gades NM, Wixson SK, Danneman PJ, Tolley EA.** 2001. Inefficacy of buprenorphine in rats. *Contemp Top Lab Anim Sci* **40**:7.
24. **Gillingham MB, Clark MD, Dahly EM, Krugner-Higby LA, Ney DM.** 2001. A comparison of two opioid analgesics for relief of visceral pain induced by intestinal resection in rats. *Contemp Top Lab Anim Sci* **40**:21–26.
25. **Goecke JC, Awad H, Lawson JC, Boivin GP.** 2005. Evaluating postoperative analgesics in mice using telemetry. *Comp Med* **55**:37–44.
26. **Gopal S, Tzeng T-B, Cowan A.** 2002. Characterization of the pharmacokinetics of buprenorphine and norbuprenorphine in rats after intravenous bolus administration of buprenorphine. *Eur J Pharm Sci* **15**:287–293.
27. **Hayes JH, Flecknell PA.** 1999. A comparison of pre- and postsurgical administration of bupivacaine or buprenorphine following laparotomy in the rat. *Lab Anim* **33**:16–23.
28. **Hofmeister EH, Egger CM.** 2004. Transdermal fentanyl patches in small animals. *J Am Anim Hosp Assoc* **40**:468–478.
29. **Holton L, Reid J, Scott EM, Pawson P, Nolan A.** 2001. Development of a behaviour-based scale to measure acute pain in dogs. *Vet Rec* **148**:525–531.
30. **Houghton AK, Hewitt E, Westlund KN.** 1997. Enhanced withdrawal responses to mechanical and thermal stimuli after bone injury. *Pain* **73**:325–337.
31. **Interagency Research Animal Committee.** 1985. US government principles for the utilization and care of vertebrate animals used in teaching, research and training. Federal Register, vol 50, no. 97. Washington (DC): Office of Science and Technology Policy.
32. **Jablonski P, Howden BO, Baxter K.** 2001. Influence of buprenorphine analgesia on postoperative recovery in 2 strains of rats. *Lab Anim* **35**:213–222.
33. **Jacobson C.** 2000. Adverse effects on growth rates in rats caused by buprenorphine administration. *Lab Anim* **34**:202–206.
34. **Johnson RE, Fudala PJ, Payne R.** 2005. Buprenorphine: considerations for pain management. *J Pain Symptom Manage* **29**:297–326.
35. **Koewler NJ, Freeman KT, Buus RJ, Herrera MB, Jimenez-Andrade JM, Ghilardi JR, Peters CM, Sullivan LJ, Kuskowski MA, Lewis JL, Mantyh PW.** 2007. Effects of a monoclonal antibody raised against nerve growth factor on skeletal pain and bone healing after fracture of the C57BL/6J mouse femur. *J Bone Miner Res* **22**:1732–1742.
36. **Krotscheck U, Boothe DM, Little AA.** 2008. Pharmacokinetics of buprenorphine following intravenous administration in dogs. *Am J Vet Res* **69**:722–727.
37. **Krugner-Higby L, Smith L, Clark M, Heath TD, Dahly E, Schiffman B, Hubbard-VanStelle S, Ney D, Wendland A.** 2003. Liposome-encapsulated oxymorphone hydrochloride provides prolonged relief of postsurgical visceral pain in rats. *Comp Med* **53**:270–279.
38. **Kuribara H, Uchihashi Y.** 1994. Interactions of opioids with caffeine: evaluation by ambulatory activity in mice. *J Pharm Pharmacol* **46**:141–144.
39. **Lance W.** 2010. Personal communication. Information on formulation of SR buprenorphine. Wildlife Pharmaceuticals, Fort Collins, CO.
40. **Leach MC, Bailey HE, Dickinson AL, Roughan JV, Flecknell PA.** 2010. A preliminary investigation into the practicality of use and duration of action of slow-release preparations of morphine and hydromorphone in laboratory rats. *Lab Anim* **44**:59–65.
41. **Leese T, Husken PA, Morton DB.** 1988. Buprenorphine analgesia in a rat model of acute pancreatitis. *Surg Res Commun* **3**:53–60.
42. **Liu KS, Tzeng JI, Chen YW, Huang KL, Kuei CH, Wang JJ.** 2006. Novel depots of buprenorphine prodrugs have a long-acting antinociceptive effect. *Anesth Analg* **102**:1445–1451.
43. **Martin LB, Thompson AC, Martin T, Kristal MB.** 2001. Analgesic efficacy of orally administered buprenorphine in rats. *Comp Med* **51**:43–48.
44. **Molony V, Kent JE.** 1997. Assessment of acute pain in farm animals using behavioral and physiological measurements. *J Anim Sci* **75**:266–272.
45. **Pick CG, Peter Y, Schreiber S, Weizman R.** 1997. Pharmacological characterization of buprenorphine, a mixed agonist–antagonist with  $\kappa_3$  analgesia. *Brain Res* **744**:41–46.
46. **Ren K, Dulner R.** 1999. Inflammatory models of pain and hyperalgesia. *ILAR J* **40**: 111–118.
47. **Richardson CA, Flecknell PA.** 2005. Anaesthesia and postoperative analgesia following experimental surgery in laboratory rodents: are we making progress? *Altern Lab Anim* **33**:119–127.
48. **Roughan JV, Flecknell PA.** 2000. Effects of surgery and analgesic administration on spontaneous behaviour in singly housed rats. *Res Vet Sci* **69**:283–288.

49. **Roughan JV, Flecknell PA.** 2002. Buprenorphine: a reappraisal of its antinociceptive effects and therapeutic use in alleviating postoperative pain in animals. *Lab Anim* **36**:322–343.
50. **Roughan JV, Wright-Williams SL, Flecknell PA.** 2009. Automated analysis of postoperative behaviour: assessment of HomeCageScan as a novel method to rapidly identify pain and analgesic effects in mice. *Lab Anim* **43**:17–26.
51. **Sharp J, Zammit T, Azar T, Lawson D.** 2003. Recovery of male rats from major abdominal surgery after treatment with various analgesics. *Contemp Top Lab Anim Sci* **42**:22–27.
52. **Smith LJ, Valenzuela JR, Krugner-Higby LA, Brown C, Heath TD.** 2006. 206. A single dose of liposome-encapsulated hydromorphone provides extended analgesia in a rat model of neuropathic pain. *Comp Med* **56**:487–492.
53. **Thompson AC, Kristal MB, Sallaj A, Martin LB, Martin T.** 2004. Analgesic efficacy of orally administered buprenorphine in rats: methodologic considerations. *Comp Med* **54**:293–300.
54. **Watson AD, Nicholson A, Church DB, Pearson MR.** 1996. Use of antiinflammatory and analgesic drugs in dogs and cats. *Aust Vet J* **74**:203–210.
55. **Yassen A, Olofsen E, Kan J, Dahan A, Danhof M.** 2008. Pharmacokinetic–pharmacodynamic modeling of the effectiveness and safety of buprenorphine and fentanyl in rats. *Pharm Res* **25**:183–193.
56. **Yu S, Zhang X, Sun Y, Peng Y, Johnson J, Mandrell T, Shukla AJ, Laizure SC.** 2006. Pharmacokinetics of buprenorphine after intravenous administration in the mouse. *J Am Assoc Lab Anim Sci* **45**:12–16.
57. **ZooPharm.** [Internet]. 2010. [Cited 15 October 2010]. Available at: [http://zoopharm.net/documents/Buprenorphine\\_info\\_sheet.pdf](http://zoopharm.net/documents/Buprenorphine_info_sheet.pdf).