Original Article

Extended-release buprenorphine with bupivacaine effectively provides postoperative analgesia in a mouse laparotomy model

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Abstract

Multimodal analgesia is widely encouraged for major survival surgery. This facilitates lower doses of individual drugs, thereby reducing side effects. Extended-release analgesics offer sustained analgesia coverage, prevent drug dosing nadirs, and reduce stress. Studies evaluating multimodal and extended-release analgesia combinations for major survival surgery in mice are limited. We compared the analgesic effect of bupivacaine and extended-release buprenorphine (Bupi-ER) to bupivacaine and buprenorphine HCl (Bupi-HCl). We hypothesized that preoperative Bupi-ER would provide more effective analgesia than Bupi-HCl. Mice were randomly assigned to 1 of 2 treatment groups: bupivacaine (2 mg/kg, local infiltration, once) with either 1) extended-release buprenorphine (0.6 mg/kg, SC once, n=6, Bupi-ER), or 2) buprenorphine HCl (0.1 mg/kg SC, BID, 2 days, *n*=5, Bupi-HCl). Animals underwent a laparotomy on day 0. Behavioral evaluations were performed twice a day by 2 blinded evaluators on day -1, 0, 1, and 2 post-operatively utilizing a modified Mouse Grimace Scale (mMGS) and behavioral scoring. On day 3, mice were sacrificed and weighed for experimental tissue collection. The mMGS and behavioral scores observed on D1 and D2 showed no significant difference compared to those on D-1 or D0. Our findings indicate that both Bupi-ER and Bupi-HCl provide effective multimodal analgesia in a mouse laparotomy model.

Keywords: bupivacaine, buprenorphine HCl, extended-release buprenorphine, laparotomy, mice, multimodal analgesia

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Introduction

An essential component of research animal veterinary medical care is the prevention or alleviation of pain associated with procedural and surgical protocols" (National Research Council, 2011). Pain can lead to distress and negative physiological consequences, including immune dysfunction (Bartolomucci *et al.*, 2007; Blackburn-Munro and Blackburn-Munro, 2001) and behavioral disturbances (Karas *et al.*, 2001) affecting animal welfare and scientific outcomes (National Research Council, 1992; Oliver *et al.*, 2018). Effective pain control techniques employing preventative and multimodal analgesia are most likely to offer pain prevention for animals (Flecknell, 2018). Preventative analgesia minimizes noxious stimuli sensitization arising over the perioperative period (Rosero and Joshi, 2014). Multimodal analgesia involves the use of two or more analgesic agents to provide additive analgesia. When combining drugs of different analgesic classes, they affect different nociceptive pathways and provide a synergistic effect, reducing individual drug doses and associated side-effects (Guneli *et al.*, 2007; Cannon *et al.*, 2011).

Buprenorphine is one of the most used rodent analgesics (Pergolizzi *et al.*, 2010; Stokes *et al.*, 2009). Buprenorphine, an opioid analgesic synthesized from thebaine, acts as a mu receptor agonist and kappa receptor antagonist, although its primary effect is at the mu opioid receptor (Kögel *et al.*, 2005). It is 25-40 times more effective than morphine at providing analgesia, likely due to its greater affinity for the mu receptor (Kögel *et al.*, 2005). It is frequently used pre- and post-operatively to treat mild or moderate acute or chronic visceral pain (Gades *et al.*, 2000). A typical buprenorphine analgesic regimen for treating mouse post-surgical pain involves three to four subcutaneous injections per day for a period determined by the expected pain duration (Flecknell and Waterman-Pearson, 2000). Extended-release buprenorphine (Bup-ER, formerly SR) provides an injectable, extended-release dose over 72 hours (Wedgewood, 2025). This allows for decreased handling and fewer injections, thereby reducing the stress of analgesic administration. The recommended dosage and duration of action of Bup-ER in mice have been evaluated by several groups using pharmacokinetic analysis, thermal nociception, and laparotomy models (Kendall *et al.*, 2014; Clark *et al.*, 2014; Carbone *et al.*, 2012; Healy *et al.*, 2014; Jirkof *et al.*, 2014).

Bupivacaine is a local anesthetic that elicits analgesia by blocking voltage-gated Na+ channels, preventing sensory pain pathway activation (Pedersen *et al.*, 1996). Although local anesthetics only provide a short duration of action (Lemke and Dawson, 2000; Kang *et al.*, 2017), including local anesthetics in a multimodal analgesia regimen can decrease inflammation, pain, and need for additional post-operative analgesics (Pedersen *et al.*, 1996; Bisgaard *et al.*, 1999; Rasmussen *et al.*, 1998; Jin and Chung, 2001).

Previous studies evaluating the efficacy of Buprenorphine HCl as an analgesic for experimental mouse laparotomy procedures indicated that repeated dosing at eight-hour intervals may lead to insufficient analgesia at times (Gades *et al.*, 2000; Jirkof *et al.*, 2014). A study examining the clinical efficacy of Bup-ER for an experimental laparotomy procedure in female CD1 mice determined that while 0.6 mg/kg of Bup-ER provided adequate analgesia, buprenorphine HCl at 0.1 mg/kg every 12 h did not (Kendall *et al.*, 2016). There is a dearth of information regarding the efficacy of a bupivacaine-buprenorphine multimodal combination. The aim of this study was to investigate whether bupivacaine with buprenorphine extended release (Bupi-ER) reduces postoperative pain more effectively than bupivacaine with buprenorphine HCL (Bupi-HCl) in a mouse laparotomy model.

Materials and Methods

Ethical statement: All animal experiments were approved by Stanford University's Administrative Panel for Laboratory Animal Care (Animal Welfare Assurance Number: D16-00134 (A3213-01), expires 05/31/2025). All mice were treated in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011). All authors complied with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines 2.0.

Animals and husbandry: B6;129P*Upk1b*^{tm1Pzg}/ J transgenic mice (16.9 ± 3.0 weeks; n=11, females) bred from stock originally purchased from Jackson Laboratories were used. Sentinel mice were free of minute virus of mice, mouse hepatitis virus, mouse rotavirus, Theiler's murine encephalomyelitis virus, Sendai virus, murine adenovirus 1 and 2, ectromelia virus, lymphocytic choriomeningitis virus, pneumonia virus of mice, respiratory enteric virus 3 (Reovirus 3), *Mycoplasma pulmonis,* endo- and ectoparasites, and pinworms. Mice were housed in conventional caging on pre-filled corncob bedding, fed a commercial diet (Teklad Global 18% Protein Rodent Diet 2018, Harlan Laboratories, Madison, WI), provided water filtered by reverse osmosis, and provided a nestlet, in addition to corncob bedding, as enrichment. Rooms were maintained on a 12:12-h dark:light cycle at 68-79°F (20-26°C) and 30-70% relative humidity.

Study design: Mice were randomly assigned to one of two experimental groups based on preoperative analgesic: 1) Bupi-HC1 (n=5): 0.5% bupivacaine (Hospira, Inc., Wake Forest, IL), 2 mg/kg once at the incision site and 0.1 mg/kg Buprenorphine HCl (0.3 mg/mL; Hospira, Lake Forest, IL) twice daily (07:00 and 19:00) for 2 consecutive days (subcutaneous route, SC); or 2) Bupi-ER (*n*=6): 0.5% bupivacaine, 2 mg/kg once at the incision site and 0.6 mg/kg Bup-ER (1 mg/mL; Zoopharm, Fort Collins, CO; now labeled as Buprenorphine ER-Lab) once, SC pre-operatively.

Surgery: General anesthesia was induced with isoflurane (3-5% in 100% O_2) using an induction chamber. Mice were maintained via a mask with isoflurane (1.5-2.5%) in 100% O_2 (0.5-1 L/min). Sterile eye lubrication was applied upon anesthetic induction, and mice were placed in dorsal recumbency on a circulating warm-water blanket throughout. Cefazolin (30 mg/kg SC) and warm balanced fluid (0.9% NaCl, 5

ml/kg SC) were administered. Pre-emptively, all mice received a local bupivacaine surgical site infiltration (0.5% bupivacaine, 2 mg/kg). Five minutes prior to surgery, mice received either Bupi-HCl or Bupi-ER subcutaneously. After aseptic abdominal preparation and surgical draping, a standardized midline laparotomy was performed, as previously described by Fu *et al.* (2012). Through an approximately 1.5 cm incision, the urinary bladder was exteriorized. Next, 50-µl of PBS was injected into the urinary bladder wall with a 29-gauge needle. The urinary bladder was replaced in the abdomen, and the abdominal wall and skin incision were closed separately with 4-0 Vicryl suture. The incision received a topical application of antibiotic ointment. Mice were monitored during recovery in a clean cage. After recovery, mice were returned to their home cage with moistened rodent chow and Nutri-Cal® (Vetoquinol, USA) supplement on the cage floor.

Body weight and clinical appearance: Mice were weighed on D-1 and D3, and observed for clinical appearance (lethargy, rough hair coats, and motility) daily.

Behavioral scores and modified mouse grimace scale: Behavioral scores and modified mouse grimace scale (mMGS) assessments were performed one day prior to surgery (D-1), day of surgery (pre-op, D0), and at 1 (D1) and 2 (D2) days post-operatively. Mice were euthanized (D3) 3 days post-operatively. Observers were blinded to the treatment groups. Evaluations were performed daily in the morning at 09:00 (CP) and mid-afternoon at 13:00 (HC), except for the day of surgery when both were performed in the morning at 8:00 (CP) and 10:00 (HC). Both observers (experienced laboratory animal veterinarians) had previous experience in using both behavioral evaluations and the mMGS.

Behavioral scores included spontaneous behavior; posture; breathing; and coat, eye, and wound condition, and were scored as either 0 or 1 (total score of 7; Table 1) (Guneli *et al.*, 2007). An mMGS, based on the work of Langford *et al.* (2010), was modified to simplify the scale to 0 (not present) or 1 (present). This modified mMGS scale presents a simpler rubric for the observers to determine if facial pain indications were present or not (total score of 5; Table 2). Mice were not scored when sleeping, exploring, sniffing, or grooming.

Statistical analysis: Data were analyzed using repeated-measures (R development Core Team, 2015) to compare behavioral scores, mMGS scores, and weights. The residuals were subsequently examined for normality employing Anderson-Darling statistics. Because the normality assumption was violated, Friedman's statistics, a non-parametric test, was considered for comparing time across each drug, followed by the Wilcoxon signed-rank test for pairwise comparison. Data were expressed as mean ± standard error of the mean (SEM). A *p-value* of <0.05 was considered significant.

Table 1 Behavioral scores. Behavior scores were evaluated as either not painful (0) or painful behaviors (1).

Score	0	1
Spontaneous behavior	Sleeping, resting, digging, running, walking, rearing, climbing, eating, drinking, grooming, sniffing	Sudden movements, backwards movements, transient involuntary muscular contraction of any body part, kicking with hind paws, licking, biting the wound
Posture	Lying, sitting, moving	Hunched, arched back, crouched
Breathing	Regular, undisturbed	Irregular, labor
Coat condition	Clean, smooth, well-groomed	Ruff, dirty, piloerection
Eyes	Clear, bright	Discharge
Body condition	Good	Sunken flank, swollen area, ascites
Wound	Clean, dry, smooth	Dirty, bloody, uncleaned, signs of self-injury, signs of inflammation, redness
Total	**0**	**7**

Table 2 Modified mouse grimace scale. Facial pain expression scored: 0 (not present) or 1 (signs of pain).

Facial Action Unit	0	1
Orbital tightening	Baseline position	Tightly closed eyelid or eye squeeze. A wrinkle may be visible around the eye
Nose bulge	Baseline position	Bulge on the top of the nose. Skin and muscles around the nose will be contracted, creating a round extension of skin visible on the bridge of the nose. There may be wrinkles extending down the side of the nose, and the nose area appears wider
Cheek bulge	Baseline position	Cheeks (below eyes to whiskers) are convex
Ear position	Baseline position, perpendicular to the head	Ears are rotated outwards and away from the face
Whisker change	Baseline position	Whiskers are pulled back or pulled forward as if standing on end
Total	**0**	**5**

Result

Body weight and clinical appearance: Mouse body weights in the Bupi-HC1 and Bupi-ER groups on D3 (26.0 ± 2.6 and 27.8 ± 2.3 g, respectively) were significantly lower than those on D0 (29.2 ± 3.3 and 30.3 ± 2.6 g, respectively) (Fig. 1). No abnormalities were noted during clinical observations.

Behavioral scores: On D-1 and D0 (pre-op), behavioral scores were zero (score=0/7) for all mice (Fig. 2). On D1, behavioral scores were 0.4 ± 0.2 in the Bupi-HCl group and 0.4 ± 0.2 in the Bupi-ER group, and these values did not differ from D-1 or D0 values. Similarly, on D2, behavioral scores (0 ± 0 in Bupi-HCl group and 0.1 ± 0.1 in Bupi-ER group) did not differ significantly compared to D-1 or D0 values.

mMGS: On D-1 and D0 (pre-op), mMGS were zero (score=0/5) in all animals (Fig. 3). On D1, the behavioral scores were 0.2 ± 0.2 in Bupi-HCl group and 0.6 ± 0.3 in Bupi-ER group, and these values did not differ from D-1 or D0 values. Similarly, on D2, behavioral scores (0.1 ± 0.1 in Bupi-HCl group and 0.4 ± 0.2 in Bupi-ER group) were not significantly different compared to D-1 or D0 values.

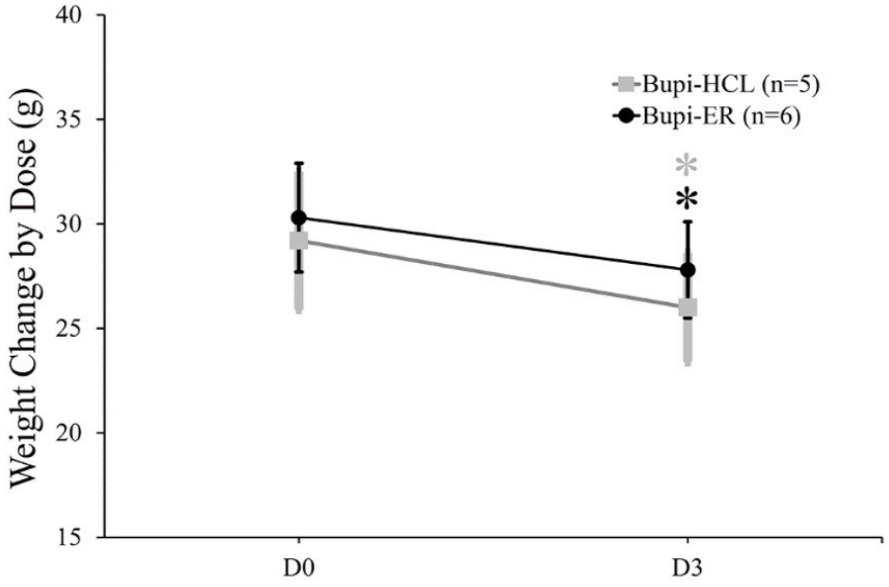

Figure 1 Body weights of mice. Body weights (Mean ± SEM) of mice on D0 (pre-op) and D3. * Significantly ($P < 0.05$) different on D3 vs D0.

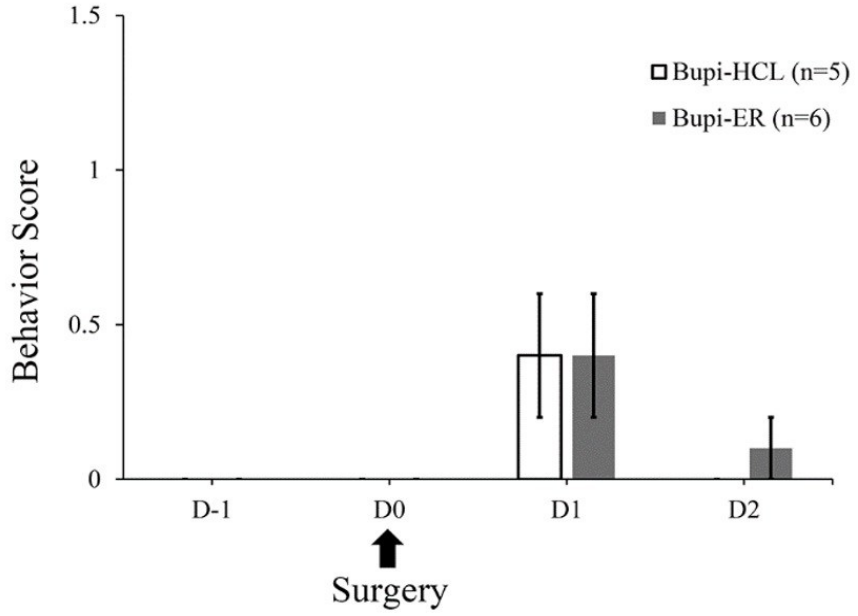

Figure 2 Behavioral scores. Behavioral scores (Mean ± SEM, max total score is 7) were evaluated by 2 blinded observers on D-1, D0 (pre-op), D1, and D2. *Significantly ($P < 0.05$) different from D0 value for the same treatment group

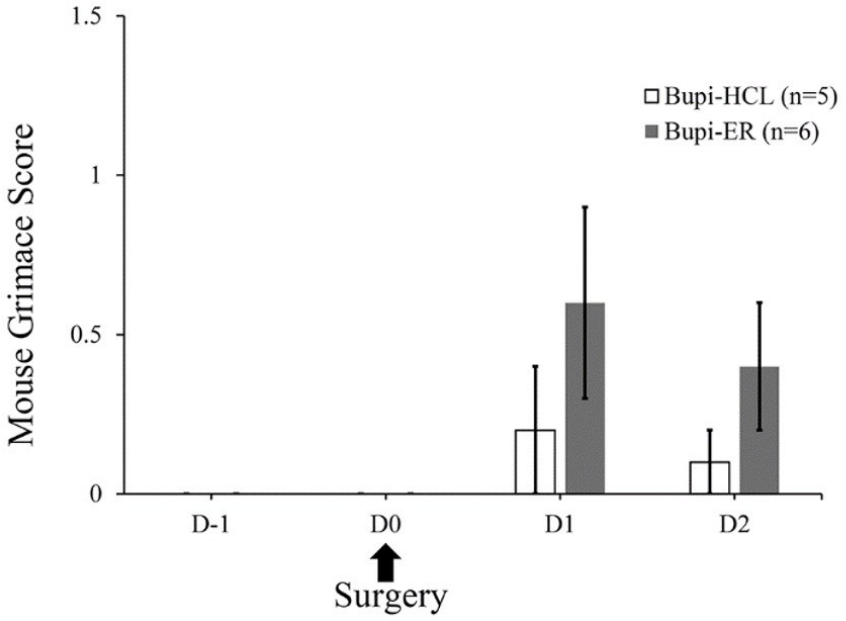

Figure 3 Modified mouse grimace scale. mMGS (Mean ± SEM, max total score of 5) was evaluated by 2 blinded observers on D-1, D0 (pre-op), D1, and D2. *Significantly ($P < 0.05$) different from D0 value for the same treatment group.

Discussion

This study demonstrates that both Bupi-HCl and Bupi-ER effectively prevented pain behaviors and facial expressions of pain for at least 2 days in a mouse laparotomy model. Mouse body weights in both treatment groups were lower on D3. The current data did not support our hypothesis that Bupi-ER reduces postoperative pain more effectively than Bupi-HCl in a mouse laparotomy model.

The aim of this current study was to refine a multimodal analgesic technique for postoperative pain control by using common analgesics in a mouse laparotomy model. We chose a mouse laparotomy model because it is classified as a moderate-severe pain procedure in mice at our institutions and can serve as a model for surgical situations in the laboratory mouse where analgesic administration is crucial. This surgical procedure causes both somatic pain from the skin, muscle, and related tissue injuries as well as visceral pain from the organ manipulation. Given that humans and animals are expected to experience pain post-laparotomy (Langford *et al.*, 2010; Jirkof *et al.*, 2010; Arras *et al.*, 2007; Cheong *et al.*, 2001), no saline control was employed in our surgical model. Laparotomy and urinary bladder wall injection is a technique developed in our lab (Fu *et al.*, 2012) involving the exposure of the urinary bladder through an abdominal incision, which induces visceral pain that is most severe during the first 24 h (Arras *et al.*, 2007; Ulker *et al.*, 2022). In this current study, the procedure was standardized and performed with minimal variation (i.e., one surgeon, same postoperative evaluators) on many subjects, making it a good choice for an experimental model to assess post-operative pain treatment in mice, specifically, in B6;129 hybrid mice. The B6;129P*Upk1b*^{tm1Pzg}/J mice used have a mutation in the transmembrane protein Uroplakin, which is important for maintaining urothelial integrity and are phenotypically and clinically normal. Changes in uroplakin expression may cause altered sensory processing in the bladder; however, this would have uniformly affected all study animals, and our evaluation included the overall pain caused by a major abdominal procedure. In addition to evaluating different analgesic regimens, evaluating different mouse strains is also critical, as differing strains are reported to have varying analgesic needs and observed behavioral or evoked pain responses (Carbone *et al.*, 2012; Rudeck *et al.*, 2020; Arthur *et al.*, 2022).

The concept of pre-emptive (administering analgesics prior to surgery) and multimodal analgesia (combining several analgesic classes or techniques) is accepted and encouraged whenever possible to provide effective analgesia (Foley *et al.*, 2019; Nir *et al.*, 2016; Lamont, 2008; Hellyer *et al.*, 2007; Navarro *et al.*, 2021a). In this study, 3 commonly used analgesics (bupivacaine, buprenorphine HCl, and Bup-ER) and 2 administration techniques [local infiltration and systemic] were chosen. Bupivacaine, a sodium channel blocker, was locally infiltrated (2 mg/kg) along the surgical site to desensitize the surgical area while buprenorphine formulations were administered subcutaneously. All analgesics were administered pre-emptively to minimize central sensitization. To minimize plasma Bup-HCl fluctuations (Jorkof *et al.*, 2014), a higher dose at 0.1 mg/kg of Bup-HCl (twice daily, SC) was selected, and Bup-ER at 0.6 mg/kg (once, SC) was used. Future refinements to the use of pre-emptive and multimodal analgesia regimens may incorporate the use of other extended-release buprenorphine formulations (Ethiqa-XR), extended-release local anesthetics, and NSAIDs.

Measuring pain in laboratory mice presents obvious difficulties; not only are mice unable to self-report their pain scores, they are also prey animals and can hide signs of pain and distress (National Research Council, 1992; Flecknell, 2016). We chose to assess spontaneous behaviors (and not evoked pain testing) because the authors aimed to mimic a real clinical setting where postoperative cage-side monitoring (for spontaneous/ongoing pain behaviors) would be

performed by staff. These cage-side behavioral assessments require familiarity with normal rodent behavior and appearance in order to identify abnormal clinical signs (Flecknell, 2016). In this study, two experienced veterinarians (HC and CP) performed evaluations for pain. Postoperative behavioral scores and mMGS were not significantly different from baseline values for both Bupi-HCl and Bupi-ER-treated mice. However, more frequent (twice daily) handling and injections were required in the Bupi-HCl mice. The single pre-operative Bupi-ER injection minimizes mouse stress (handling for repeated injections) and decreases the risk of analgesic coverage gaps.

This is the first mouse study demonstrating the effectiveness of Bupi-HCl and Bupi-ER as analgesic regimens for a mouse laparotomy model. A two-day analgesic administration and observation period was chosen based on our previous experience with this specific model and surgeon. Several factors can affect the expected pain intensity, including strain, age, specific surgery model, and surgeon. The local block of bupivacaine blocked afferent input to the spinal level and minimized the requirement for opioid analgesia with buprenorphine post-operatively. A synergistic effect has been noted in humans, as the use of a similar combination (0.5% bupivacaine and 0.003 mg/kg buprenorphine HC1) for oral surgical procedures extends the analgesic duration (Nagpal *et al.*, 2017; Modi *et al.*, 209). Using both bupivacaine (blocking afferent input to the spinal level) and buprenorphine (treating pain at the spinal and supraspinal levels) provided synergistic analgesia to attenuate pain. These results further support providing multimodal analgesia for surgical procedures, even if moderate to severe pain is expected.

In addition to scoring pain behaviors, clinical well-being was assessed throughout the study. Weight measurements taken on D0 and D3 were another parameter to evaluate overall health (Brennan *et al.*, 2009; Gates *et al.*, 2023). Mice in both Bupi-HCl and Bupi-ER groups exhibited a weight decrease after surgery, which is consistent with previous rat and mouse findings (Hayes *et al.*, 2000; Goecke *et al.*, 2005; Jablonski *et al.*, 2001). The lack of a statistically significant difference in weight between Bupi-HCl and Bup-ER groups (Gates *et al.*, 2023) on day 3 suggests the weight loss seen does not differ with different drug formulations. Although weight loss is a general welfare indicator in any species, using weight loss specifically as a measure of postoperative pain is confounded by a few factors. First, weight loss is a known side effect of buprenorphine administration in rodents. Secondly, weight loss may be observed after any anesthetic event (Gates *et al.*, 2023; Dholakia *et al.*, 2017; Molina-Cimadevila *et al.*, 2014; Navarro *et al.*, 2021a). Additionally, a recent study indicated that diet gel supplementation could help prevent post-operative weight loss for a mouse laparotomy procedure when Bup-ER is used (Gates *et al.*, 2023). Other studies using Bup-ER formulations have reported crusted cutaneous (Arthur *et al.*, 2022), ulcerative (Clark *et al.*, 2014), or scabby (Carbone *et al.*, 2012; Navarro *et al.*, 2021b; Kendall *et al.*, 2021; Traul *et al.*, 2015) lesions in mice and granulomatous inflammation (Foley *et al.*, 2011) or cystic structures (Page *et al.*, 2019) in rats. We did not see any evidence of injection site reactions during the study period or during gross pathological evaluation. When any buprenorphine formulation is used in mice, side effects (respiratory depression, sedation, lethargy, hyperreactivity) may be noticeable (Navarro *et al.*, 2021b); no abnormal clinical signs (opioid-induced sedation or hyperactivity) were observed in this study.

There were several limitations to our study. First, only B6;129 female mice were used. This strain was selected because they were what was a part of our collaborator's research paradigm and IACUC-approved protocols. Results may differ with other mouse strains or with the inclusion of males. Other studies have reported that pain perception may differ between sexes (Vinogradova *et al.*, 2001; 2003). Second, evoked pain testing was not included. Spontaneous or ongoing pain behaviors were only used because evoked pain testing would require more mouse handling, which could affect spontaneous pain behaviors and interfere with the researcher's study objectives. Third, this study did not include a negative control group for welfare reasons because these were real clinical research cases on an approved IACUC protocol. Fourth, we focused only on a single local anesthetic, bupivacaine, and one extended-release buprenorphine formulation. Other analgesic drug classes, such as nonsteroidal anti-inflammatory drugs or other extended buprenorphine (Ethiqa-XR) formulations, were not evaluated. Fifth, the plasma concentration of analgesic drugs was not measured. Sixth, postoperative analgesia was only monitored twice a day and for 2 days following the surgery. Future studies should evaluate multimodal analgesia combinations at additional study timepoints and for an extended period.

In conclusion, results of our study indicate that a single dose of bupivacaine (2 mg/kg pre-operative local infiltration) with either buprenorphine HCl (0.1 mg/kg twice daily for 2 d) or buprenorphine-ER (0.6 mg/kg, once) will provide at least 2 days of postoperative analgesia for a mouse laparotomy model.

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List of abbreviations: Bupivacaine with buprenorphine HCL (Bupi-HCl), Bupivacaine with extended-release buprenorphine (Bupi-ER), Mouse Grimace Scale (mMGS), one day prior to surgery (D-1), day of surgery (pre-op, D0), 1 day after surgery (D1), 2 days after surgery (D2), 3 days after surgery (D3), respiratory enteric virus 3 (Reovirus 3), standard error of the mean (SEM).

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