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Evaluation of Efficacy of 2 Extended-release Bupivacaine Products in a Porcine Model of Incisional Pain

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Extended-release (ER) local anesthetics are often incorporated in multi-modal analgesia or as an alternative when the effect of systemic analgesics may confound research. In this study, we compared the analgesic efficacy of 2 ER bupivacaine anesthetics with different ER mechanisms, a slow-release bupivacaine-meloxicam polymer (BMP) and a sucrose acetate isobutyrate bupivacaine (SABER-B) system. We used a full-thickness unilateral skin incision porcine model to evaluate the efficacy of these 2 ER bupivacaine analgesics. Eighteen male swine were randomized into 3 groups: control (saline; *n* = 6), bupivacaine:meloxicam (10 mg/kg, 0.3 mg/kg; *n* = 6), and SABER-B (10 mg/kg; *n* = 6). After surgery, pigs were assessed for changes in body weight, salivary cortisol level, and response to von Frey testing at 1, 3, 6, 24, 48, 72, 96, 120, and 168 h. Body weight and salivary cortisol levels were not significantly different between groups. Based on the von Frey testing, the pigs that received analgesics showed a significantly higher withdrawal threshold of nociceptive stimulus than those that received saline at 1, 3, 6, and 24 h after the surgery. At 48 h after surgery, the SABER-B group had a significantly higher withdrawal threshold than the saline group. The withdrawal threshold was not significantly different from the baseline measurement on intact skin at 3 and 6 h after surgery in the BMP group or 1 and 3 h for the SABERB group. The analgesic effects of BMP were greatest at 3 and 6 h after surgery and that of SABER-B as 1 and 3 h SABER-B provided an earlier onset of analgesia and longer analgesia duration than did BMP. This study demonstrates that ER bupivacaine can provide pigs with 24 to 48 h of analgesia for incisional pain.

Abbreviations and Acronyms: BMP, bupivicaine-meloxicam polymer; ER, extended release; FDA, Food and Drug Administration; MNT, mechanical nociceptive thresholds; NSAID, non-steroidal anti-inflammatory drug; SABER-B, sucrose acetate isobutyrate extended-release bupivacaine

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Introduction

Pain management is essential for improving animal welfare and generating sound scientific data. However, both pain and analgesic drugs can alter research outcomes. Pain can affect an animal's emotional, physiological, and behavioral responses.[8,34] In addition, unrelieved acute pain can develop into chronic pain, which can induce maladaptive stress, activate the hypothalamic–pituitary–adrenal gland axis, disrupt sleep, impair functional and immune system performance, and alter social interactions.[7] Depending on the mechanism of an analgesic and the animal model, systemic analgesics have been reported to alter neuroinflammatory processes and reduce lesions in a mouse traumatic brain injury model,[44,46] promote or suppress immunomodulation resulting in inconsistent tumor growth,[39,43] and change physiology to cause significant decreases in cortisol concentration,[3] hematologic alterations,[2] prostaglandin E_2 plasma alterations, and variances in the expression of serum amyloid A2 (SAA2) and CD1.[33] Caution should be taken to choose an analgesic that has minimal impact on the research model while also providing adequate analgesia for the animal.

Commonly used analgesics include NSAIDs, opioids, local anesthetics, and drugs that target neuropathic pain (for example, gabapentin). Local anesthetics (for example, lidocaine and bupivacaine) are commonly used for multimodal analgesia. The short duration of bupivacaine and lidocaine precludes their use as a sole single-dose agent for prolonged analgesia. However, extended-release (ER) local anesthetics provide longer periods of analgesia. They also reach target tissues by local infiltration (as compared with systemically) and allow a lower dosing frequency while maintaining therapeutic plasma concentrations. The use of ER local anesthetics can reduce time, labor costs, and animal stress from handling. These advantages are particularly significant for use in swine, as the size and temperament of swine add to the challenge of frequent dosing of analgesics. Furthermore, in contrast to controlled substances such as opioids, the use of local anesthetics does not require licensing, registration, or rigorous standards for inventory, storage, disposal, and record-keeping.

Most clinical data on ER local anesthetics are tailored to human use, and information on species-specific efficacy is lacking for animals. The anatomy and physiology of swine and humans share many similarities in cardiovascular, urinary, integumentary, and digestive systems.[35,42] Publications using swine as a surgical model have increased markedly in the past 2 decades. As swine become increasingly used as models for

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preclinical research, toxicologic testing, and surgical research and training, evaluating the efficacy of ER bupivacaine in a swine model can refine postoperative pain management.

This study was designed to evaluate 2 different ER bupivacaine analgesics: a fixed ratio bupivacaine and meloxicam polymer (BMP; Zynrelef) and SABER-B using a well-described swine incision model.[4] BMP is an ER local analgesic that was recently approved by the FDA for managing pain after soft tissue and orthopedic surgeries in humans. In human clinical trials, BMP significantly reduced postoperative pain and opioid use as compared with bupivacaine hydrochloride and placebo in patients undergoing bunionectomy, herniorrhaphy, or total knee arthroplasty.[23,30,45] BMP uses ER polymer technology to provide local tissue anesthesia for as long as 72 h while minimizing systemic distribution, absorption, metabolism, and the potential for toxicities. Meloxicam was included in the formulation to reduce inflammation associated with tissue damage after surgery and to provide a neutral-to-basic tissue environment that potentiates the effect of bupivacaine.[32] Inflammation potentially can lower the pH of affected tissues, thus reducing the ability of local anesthetics to penetrate neurons.[32] SABER-B (sucrose acetate isobutyrate ER bupivacaine; Posimir) is FDA-approved for use in human arthroscopic subacromial decompression, inguinal hernia repair, hysterectomy, laparotomy, laparoscopic cholecystectomy, laparoscopically assisted colectomy, and appendectomy.[13,17,22,36,41,47] The delivery platform (organic esterified sugar-derived matrix and sucrose acetate isobutyrate) provides ER of bupivacaine.[17] Benzyl alcohol is included as an antimicrobial solvent. After the instillation of SABER-B into the surgical incision before skin closure, the solvent and bupivacaine diffuse over time into local tissues over time due to the controlled-release matrix.[17]

Our study evaluated the efficacy of ER local anesthetic swine for postsurgical incisional pain management in swine. Our hypothesis was that both ER formulations, BMP and SABER-B, would provide comparable analgesia for incisional pain in swine.

Materials and Methods

Animals and housing. All animal experiments were approved by the University of Michigan Institutional Animal Care and Use Committee and performed at the University of Michigan, an AAALAC-accredited institution. This study adhered to the principles in the *Guide for the Care and Use of Laboratory Animals*. Power analysis was not conducted; the animal numbers were based on previously published data using the same model.[4–6] Swine were acquired through an IACUC-approved vendor and acclimated for 5 d before experimentation. The study design is depicted in Figure 1. A saline control group was included due to the lack of published data on using ER bupivacaine in pigs. If an animal displayed signs of pain that interfered with normal behavior, an injectable NSAID such as meloxicam was administered as rescue analgesia.

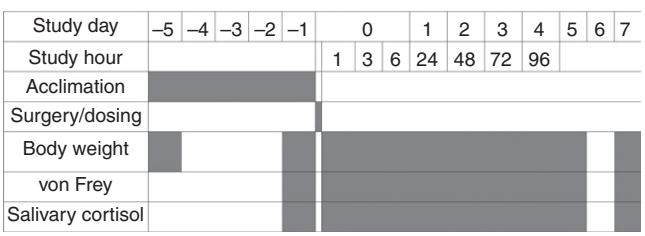

Figure 1. Study design. The figure shows the study activity over 13 d.

Eighteen male PIC800 sire cross non-SPF Yorkshire-sow pigs were obtained from the Michigan State University Swine Teaching and Research Center. The pigs had been vaccinated for *Erysipelothrix rhusiopathiae* and porcine circovirus type II. Pigs were tested for other agents (for example, porcine epidemic diarrhea virus, transmissible gastroenteritis, porcine reproductive and respiratory syndrome, *Actinobacillus pleuropneumoniae*, and *Mycoplasma pneumonia*) if clinical signs developed. Pigs ranged in weight from 8 to 14 kg and in age from 4 to 6 wk. All pigs received a general physical exam to assess their well-being. No additional diagnostic tests were done to exclude any animals. Environmental conditions were 68 to 72 °F [20 to 22.2 °C], 30% to 50% relative humidity, and a 12:12-h light:dark cycle. Pigs were housed in an open elevated pen measuring 4 × 6 ft for 5 d before the study. The pigs received water ad libitum and were fed twice daily with a starter diet (LabDiet 5080 porcine starter diet; Land O'Lakes).

Acclimation protocol. Upon arrival, the pigs were randomly placed into groups of 3 per pen. A 5-d acclimation period then occurred before surgery. Researchers interacted with the pigs in their home pen for at least 15 min twice a day throughout the acclimation period until the end of data collection. During the acclimation period, the pigs were introduced to novel treats like bananas, apple sauce, plain yogurt, and marshmallows. Positive reinforcement was used to accustom pigs to human touch, the algometer, and the saliva sponge. This training allowed the pigs to become familiar with the observers and the procedures, so restraint was not necessary during the von Frey testing or saliva collection. This acclimation process aimed to reduce stress and minimize false positive reactions during von Frey testing.

Anesthesia and surgery. This study used the porcine skin incision model described in previous literature.[4,6] Eighteen male swine were randomized into 3 groups: control (saline; n = 6), BMP (10 mg/kg:0.3 mg/kg; n = 6), and SABER-B (10 mg/kg; n = 6). Food was withheld for 9 to 12 h before the surgery; water was not restricted. On the day of surgery, pigs were sedated with intranasal midazolam[24] (0.2 to 0.4 mg/kg; Akorn Pharmaceuticals, Gurnee, IL) and maintained at a surgical plane of anesthesia with isoflurane gas (1.5% to 3% in 100% oxygen) using a face mask. The order of treatment was randomized. The skin at the caudal back of the pig was shaved and cleaned with alternating scrubs of 70% ethanol and 4% chlorhexidine solution. Body temperature was regulated within the range of 37 to 38.5 °C with a warming blanket controlled by a feedback system. (Cincinnati SubZero, Blanketrol II). Each pig was placed on a surgical table in a sternal position. ECG, blood oxygen saturation, body temperature, and respiratory rate (Surgivet advisor; Smiths Medical) were monitored throughout the procedure. A 6- to 7-cm-long incision was made through the skin and fascia on the left side, approximately 3 cm lateral to the spine. Based on the treatment group, either ER bupivacaine or saline was injected along the subcutaneous tissue at the incisional site before skin closure. The dose-volume range for BMP and SABER-B was 2.75 to 4.6 mL and 0.76 to 1.1 mL, respectively.

The skin incision was closed using a nonabsorbable monofilament polypropylene suture. The entire procedure was usually completed within 30 min. After the procedure, pigs were placed in a transport cage to recover. During the recovery phase, their respiratory rate, body temperature, and heart rate were monitored every 10 min. They were covered with a towel to maintain warmth. After being placed in the transport cage, they typically recovered within 15 min and were transferred back to their home cages as soon as they could walk on all 4 legs.

von Frey testing. Five mechanical nociceptive thresholds (MNTs) measurements were taken at each time point using a handheld 2-mm probe connected to a digital algometer (ProdPlus; Topcat Metrology Ltd.).[28,29] The person who performed the von Frey test was blind to the treatment. The withdrawal threshold was measured in Newtons. The device had been calibrated by the manufacturer. Withdrawal thresholds were recorded before (baseline) and after surgery at 1, 3, 6, 24, 48, 72, 96, 120, and 168 h. The lowest and highest values were excluded, and only 3 of the 5 measurements were used in statistical analysis. Mechanical nociceptive stimulation was administered 1 cm away from the incision site on either side.[4] von Frey testing evaluated pain around the incision site, not generalized pain. A one to 2-min pause was provided between each measurement. Pigs received food or treats during mechanical nociceptive stimulation. No manual restraint was needed. Moving away or vocal cues were considered positive responses to the mechanical nociceptive stimulation.[4] The stimulation force was gradually increased until a positive response was observed. Stimulation was also applied to the intact contralateral side, on which a stronger force was required to elicit a withdrawal response, indicating that due to pain or discomfort rather than fear. The experiment was designed to account for individual variations in pain sensitivity by having each pig to serve as its own control thereby assessing and comparing MNT before and after a painful event.

Saliva collection for salivary cortisol analysis. Saliva samples were taken to measure the levels of salivary cortisol. The person who collected the saliva sample was blind to the treatment. The first sample (baseline) was collected on the morning before the surgery; subsequent samples were taken at 1, 3, 6, 24, 48, 72, 96, 120, and 168 h after surgery. Saliva was collected at the same time every morning until day 5 and again on day 7 after the surgery. At cage side during the acclimation period, the pigs had been trained to chew on the sponge provided in a Salivette kit (Salivette Cortisol, Sarstedt, Germany). The sponge was secured with a clamp on one end and was placed in the pig's mouth for a minimum of 1 min to collect saliva. Personnel held onto the clamp during sample collection to prevent the pig from swallowing the sponge. Food was withheld for an hour before collection to avoid contamination in the saliva sample. Multiple samples were collected if blood was present in the sample.

The saliva-wet sponges were placed in tubes for centrifugation, and the saliva was then frozen at −20 °C until assayed for cortisol concentrations. A validated enzyme-linked immunosorbent assay (Salimetrics, State College, PA) was used to analyze the saliva sample.[21,31]

Statistical analyses. Split-plot ANOVA was used to analyze body weight, von Frey data, and salivary cortisol. The grouping factor included treatment (saline, BMP, and SABER-B) with a single repeat factor (time) and 6 pigs nested in each treatment group. For all the statistical tests, a P value less than 0.05 was considered to indicate statistically significant differences. Normality was assessed using a histogram and normal probability plot. The homogeneity of variances was assessed by means of the Residuals*Yhat plot. Post hoc tests were done by means of Bonferroni for multiple comparisons. Data were described by mean, SD, and SEM. All calculations were made using NCSS 2019 (Kaysville, UT).

Results

Analgesic efficacy study. *Body weight*. No significant differences in body weight were found between any of the 3 groups (Figure 2; $P = 0.4$).

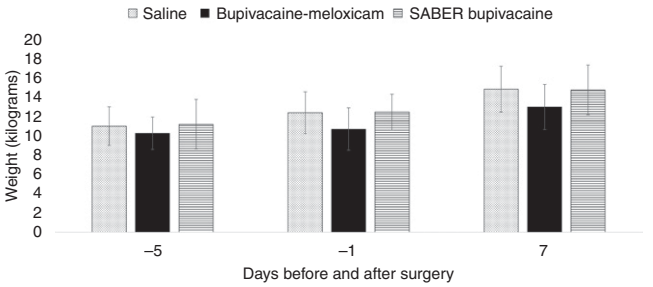

Figure 2. Body weights (mean ± 1 SD) of pigs receiving saline, bupivacaine meloxicam, or SABER bupivacaine. Body weights were taken at 5 d before surgery (arrival), 1 d before surgery, and 7 d after surgery.

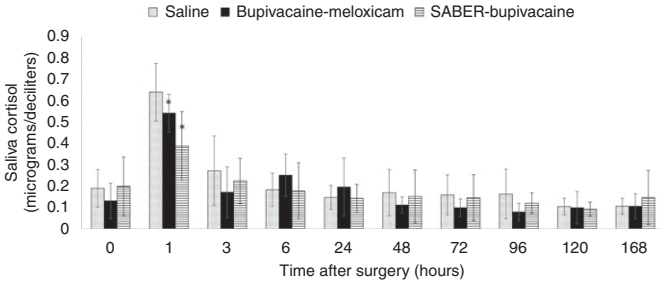

Figure 3. Salivary cortisol (mean ± 1 SD) of pigs receiving saline, bupivacaine-meloxicam, or SABER bupivacaine. Salivary cortisol was taken at baseline before surgery and 1, 3, 6, 24, 48, 72, 96, 120, and 168 h after surgery. Salivary cortisol levels in the SABER bupivacaine group and the bupivacaine-meloxicam group were significantly lower than the saline group in the first hour after surgery ($P = 0.029$ and $P = 0.00001$). The asterisk marks the statistical significant difference.

***Salivary cortisol*.** The 2 analgesic groups showed no significant differences in salivary cortisol levels (Figure 3; $P = 0.62$). The salivary cortisol levels in the SABER-B and BMP groups were significantly lower than those in the saline group in the first hour after surgery ($P = 0.029$ and $P = 0.00001$). No significant differences were detected among the 3 groups at other time points.

***Von Frey testing*.** The BPM and SABER-B showed a significantly higher NMT than did the saline group (Figure 4; $P < 0.0001$) at 1, 3, 6, and 24 h after surgery. At 48 h after surgery, the withdrawal force in the SABER-B group was still significantly higher than that of the saline group. The response to mechanical pain stimulation in the saline group remained significantly below baseline at all time points. Both BMP and SABER-B groups showed higher withdrawal thresholds that began as early as 1 h after surgery and lasted for up to 24 and 48 h, respectively. The response to mechanical nociceptive stimulation for the BMP group differed significantly from baseline at 1, 24, 48, 72, 96, 120, and 168 h after the surgery. Similarly, for the SABER-B group, the response differed significantly from baseline at 6, 24, 48, 72, 96, 120, and 168 h after the surgery. This indicates that BMP was the most effective at 3 and 6 h after surgery, while the effect of SABER-B peaked at 1 and 3 h after surgery.

Discussion

Our data showed that BMP and SABER-B both effectively attenuate mechanical hypersensitivity for 24 and 48 h, respectively, in pigs that have undergone incisional surgery. Pigs in the analgesia groups had lower salivary cortisol levels at 1 h after surgery than did pigs in the saline group. Neither drug affected body weight. Both ER bupivacaine products were effective analgesics for management of postsurgical incisional pain in pigs.

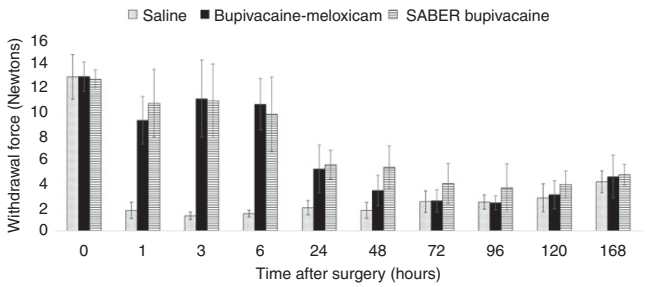

Figure 4. von Frey measurement (mean ± 1 SD) of pigs receiving saline, bupivacaine-meloxicam, or SABER bupivacaine. Data were collected at baseline before surgery and 1, 3, 6, 24, 48, 72, 96, 120, and 168 h after surgery. The withdrawal threshold in bupivacaine–meloxicam and SABER-bupivacaine was significantly higher from the saline group ($P < 0.0001$) at 1, 3, 6, and 24 h after surgery. In addition, the withdrawal threshold in SABER-bupivacaine was significantly different from the saline group at 48 h after surgery.

Pigs have been used as biomedical animal models to study developmental processes, congenital diseases, and pathogen response mechanisms. They are also used in testing vaccines and drugs and as organ donors for xenotransplantation.[25] The use of pigs has been increasing in the areas of device development, surgical methods, transplantation, and cardiovascular research. The recommended approach to effective analgesic management involves preemptive analgesia, multimodal analgesia, partial intravenous anesthesia, and postoperative analgesia.[8] The choice of analgesics depends on the research objective, the species of the animal, and the procedure involved. The variability of data due to analgesic usage can be reduced by using drugs that align with the research objective. For example, a cardiovascular study might want to avoid using an opioid due to its impact on heart rate, blood pressure, and hematology.[18,37] Species-specific studies are essential to determining the effectiveness of analgesics and providing evidence-based guidelines for pain management.

This study investigated the efficacy of 2 ER local anesthetics approved for human use in a swine incisional pain model. The behavior of pigs was closely monitored after the surgical manipulation. None of the pigs showed changes in their eating, drinking, gait, or activity levels. When they returned to their home cage, they started eating normally. These observations indicate that the small surgical incision did not cause any pain or stress that affected normal behavior. Body weight and salivary cortisol were measured to evaluate the pigs' overall well-being and stress levels.

Salivary cortisol has been used to evaluate stress in many porcine models, including castration surgical procedures in piglets, nose-snare restraint, and prolonged transport.[14,16,40] Given its advantages of stress-free collection, ease of repeated sampling over a short time, easy sample processing, and a good correlation between cortisol in saliva and blood, the use of cortisol measurements in saliva is very suitable in porcine stress research.[10,14,15,26] Several studies have demonstrated that salivary cortisol concentrations are valid indicators of circulating cortisol levels in pigs, and the cortisol concentration is a valid means of evaluating the HPA response to a stressor.[10,40] We minimized the impact of circadian rhythm on salivary cortisol by using consistent sampling time points.[16,38] All saliva samples were taken at the same time in the morning before the husbandry personnel entered the animal room and at designated time points on the day of surgery. In our study, salivary cortisol levels in our study peaked at one hour after surgery but had returned to baseline by the third hour. Our treatment groups had significantly lower salivary cortisol levels than the saline group in the first hour after surgery. This suggests that both of the ER bupivacaine analgesics that we evaluated helped to alleviate stress or pain caused by the surgery. Multiple factors contribute to fluctuating salivary cortisol levels, including the time of day of sample collection and factors such as age, sex, and stress.[38] The stress caused by the skin incision used for this study did not result in a continuous rise in salivary cortisol levels. We speculate that the pain and stress levels the pigs experienced during the study were not sufficient to cause a continuous increase in salivary cortisol levels among the groups. Higher levels of salivary cortisol are correlated with a young age and morning sampling time in pigs.[38] Our data showed that the young pigs whose saliva samples were collected in the morning had a high baseline of salivary cortisol levels. This high baseline made it challenging to detect any increase in cortisol levels due to stress.

von Frey testing is a method to directly measure the response to a painful stimulus. We chose to use young pigs based on a report[4] that their thinner skin and size facilitated von Frey testing. The pigs received treats during the von Frey testing to keep them close to the researcher and minimize the need for physical restraint. Despite being offered treats, the pigs still responded to the von Frey probes by moving away. However, the intact contralateral side required a near-baseline stimulus to elicit the avoidance behavior, indicating that avoidance of the von Frey probe was not due to fear. The von Frey data indicated that the BMP was most effective at 3 and 6 h after surgery, while SABER-B peaked at 1 and 3 h after surgery. At those time points, the withdrawal force to the mechanical nociceptive stimulation was comparable to that at baseline. Both treatments effectively numbed the skin area shortly after surgery and provided relief from mechanical hypersensitivity for an extended period. However, withdrawal threshold values did not return to their initial levels during our study. The skin incision may not have healed completely healed by day 7. In humans, 0.5% bupivacaine takes 5 to 17 min to take effect and can last for 2 to 4 h at a maximum dose of 2 mg/kg.[1,9] Both BMP and SABER-B provided over 4 h of analgesia and had effective durations of 24 and 48 h, respectively. The release rate of SABER-B during the first 48 h is 10 to 20 mg/h.[13] The delayed effect observed in the BMP group may be due to a lower rate of drug release as compared with SABER-B. Our von Frey data indicate that SABER-B has a faster onset and longer duration than BMP for incisional pain in swine at a 10-mg/kg dose.

Our findings were not consistent with the manufacturer's purported analgesic durations. Based on human clinical trials, BMP and SABER-B and both marketed as providing drug delivery and efficacy up to 72 h after administration.[11,13,20,32] The difference in the duration of pain relief between swine and humans can be due to different pain assessment methods and species. Pain was not assessed in response to mechanical stimulation in humans, as we did in swine. Differences in subcutaneous drug permeability and metabolism between humans and swine could also affect the duration of pain relief. A previous study reported that BMP effectively relieved pain for up to 72 h in pigs subjected to the same incisional model.[32] However, the concentration of meloxicam used was 1.8 mg/mL as compared with the commercially available concentration of 0.88 mg/mL. The same study documented a dose-dependent pattern. The duration of release of BMP can last up to 6 d using a high-concentrated formulation of bupivacaine (176 mg/mL) and a high dose of 1.8 mL per pig. The duration of release decreases to 3 d when using a less concentrated

formulation of bupivacaine (29 mg/mL) and a low dose of 3.4 mL per pig.[32] As only a fixed ratio BPM (29.25 mg/mL) formulation is commercially available, we used the human dose to estimate a swine dose (mg/kg) by extrapolation from the manufacturer's dose of 400 to 600 mg for a 60 kg human with an exponent of 0.75. Because SABER-B is labeled as a single 5-mL dose in humans, we used 10 mg/kg for extrapolation. Our calculated doses were 9.6 to 11 mg/kg and 15.8 to 18 mg/kg for BMP and SABER-B, respectively for the 8 to 14 kg pigs used in this study.[27] This dose is comparable to the total dose of liposomal bupivacaine used for onychectomy in both forelimbs of cats.[19]

Several factors must be considered when using ER local anesthetics in pigs. These include drug availability, risk of dehiscence, ease of application, a safe dose, and the potential for toxicity. BMP and SABER-B both have a viscous and oleaginous consistency that makes them difficult to administer and retain at the incision site. The oleaginous nature of these drugs can compromise suture knot security and increase the risk of dehiscence. The drug is designed to fill spaces such as joint cavities in humans and is applied topically over the incision by using a needleless syringe.[12,23,32,45] The drug would likely leak out of the incision if used in the same way in pigs before skin closure. To avoid leakage, we administered the drug by subcutaneous infiltration along the incision before closing the wound. The drug volume used also affects ease of application. BMP has a lower concentration of bupivacaine than does SABER-B (29.25 and 132 mg/mL, respectively). For the size of the incision we used, SABER-B was less likely to leak from the incision because less volume was needed. For a procedure with a larger incision, the overflow of BPM from the incision would be minimal. The primary adverse effects of ER bupivacaine are related to the disruption of sodium channels, which leads to cardiovascular or neurotoxicity after high bolus doses or rapid systemic absorption.[11] No cardiovascular or neurotoxicity was observed during this study.

Our study has a few limitations. First, because we used only male pigs, investigation of sex differences in pain response in pigs would be useful. Second, in our study, surgeries and drug administration were done by 2 surgeons with different experience levels. Although surgeons began the surgery without prior knowledge of the treatment, the surgeons could identify the drug at the time of administration due to the obvious differences in the volume and consistency. Thus, surgeons were not blind to the treatment, although the individual(s) who performed von Frey testing were blind to the pig's treatment group.

In conclusion, ER BMP and SABER-B provided adequate analgesia for pig incisional pain for up to 24 and 48 h, respectively. These drugs can serve as components of multimodal analgesic plans or as alternatives to systemic analgesics. Further study on the safety of high doses, pharmacokinetics of systemic absorption, and dose-dependent response is recommended.

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Conflict of Interest

The author(s) have no conflict(s) of interest to declare.

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