Journal of the American Association for Laboratory Animal Science
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Vol 61, No 5
September 2022
Pages 468–474

Pharmacokinetics of Sustained-release and Extended-release Buprenorphine in Mice after Surgical Catheterization

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The *Guide for the Care and Use of Laboratory Animals* strongly encourages the use of pharmaceutical-grade chemicals and analgesics. Sustained-release buprenorphine (SRB) is administered extralabel to rodents to mitigate moderate to severe pain. An FDA-indexed buprenorphine formulation—extended-release buprenorphine (XRB)—has recently become available and is currently the only pharmaceutical-grade slow-release buprenorphine formulation approved for use in mice and rats. However, no studies have directly compared the pharmacokinetic parameters of SRB and XRB in surgically catheterized mice. To this end, we compared the plasma buprenorphine concentrations and pharmacokinetic parameters of SRB and XRB in mice after surgical catheterization. We hypothesized that mice treated before surgery with SRB or XRB would have circulating buprenorphine concentrations that exceeded the therapeutic threshold for as long as 72 h after surgery. Male and female C57Bl/6J mice were anesthetized, treated with a single dose of either SRB (1 mg/kg SC) or XRB (3.25 mg/kg SC), and underwent surgical catheterization. Arterial blood samples were collected at 6, 24, 48, and 72 h after administration. Weight loss after surgery (mean ± SEM) was similar between groups (SRB: males, 12% ± 2%; females, 8% ± 2%; XRB: males, 12% ± 1%; females, 8% ± 1%). Both SRB and XRB maintained circulating buprenorphine concentrations above the therapeutic level of 1.0 ng/mL for 72 h after administration. Plasma buprenorphine concentrations at 6, 24, and 48 h were significantly greater (3- to 4-fold) with XRB than SRB, commensurate with XRB's higher dose. These results support the use of either SRB or XRB for the alleviation of postoperative pain in mice. The availability of FDA-indexed XRB increases options for safe and effective pharmaceutical-grade analgesia in rodents.

Abbreviations: MASA, mouse antenna for sampling access; SRB, sustained-release buprenorphine; XRB, extended-release buprenorphine

DOI: 10.30802/AALAS-JAALAS-22-000025

Introduction

Surgical procedures are often a major component of biomedical research and are commonly performed in laboratory animals.[1,2,15,22,31,44] The AALAS position statement on *Alleviating Pain and Distress in Laboratory Animals* states that, "The avoidance and minimization of pain and distress in laboratory animals is an ethical obligation that preserves the welfare of animals used in research …".[1] Pain-relieving measures often include adequate training of personnel, environmental modifications, social housing, acclimation to stressful procedures, and the use of anesthetic and analgesic drugs.[1,2,22,34] Analgesic drugs are a major approach to the alleviation of postsurgical pain because of their ability to reduce the necessary dose of anesthetic[38] and to prevent the development of central sensitization (that is, the 'wind-up phenomenon').[15,31,44] Furthermore, according to the *Guide for the Care and Use of Laboratory Animals*, "Successful surgical outcomes require appropriate attention to … [the] use of analgesics … during all phases of a protocol involving surgery and postoperative care."[2] Therefore, analgesics are an essential component of pain alleviation during and after otherwise painful surgical procedures.

Opioids are one of the most common classes of systemic analgesics used in rodent research.[37] They have become analgesics of choice in mice and rats because of their high therapeutic index,[41] minimal side effects (when used appropriately),[30] and effectiveness in alleviating moderate to severe pain.[4,12,15,23,25,44] Buprenorphine is a partial μ-opioid receptor agonist that is safe and effective in alleviating postsurgical pain in rodents.[4,12,15,23,25,33,37,41] Currently 3 formulations of buprenorphine are commonly used for this purpose: buprenorphine HCl, sustained-release buprenorphine (SRB), and extended-release buprenorphine (XRB). The short duration of action of buprenorphine HCl presents a major limitation to its use[12] and requires handling and dosing of rodents every 3 to 12 h.[16,24,28] Alternatively, the slow-release buprenorphine formulations, SRB and XRB, reportedly provide analgesia for as long as 72 h in mice after a single subcutaneous injection.[12,16,25,28,30,41] The newly available XRB is included on the FDA's Index of Legally Marketed Unapproved New Animal Drugs for Minor Species ('the Index') and thus is currently the only slow-release buprenorphine drug that is legally approved for use in mice.[25,41,43] SRB is a compounded sterile preparation that uses USP-verified pharmaceutical-grade compounds in strict compliance with USP 797 guidelines.[29] However, because SRB has not undergone FDA approval or indexing, it is considered

Submitted: 23 Mar 2022. Revision requested: 29 Apr 2022. Accepted: 15 Jul 2022.
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an unapproved nonpharmaceutical-grade drug. Unapproved drugs have not been reviewed by the FDA for manufacturing standards, purity, potency, stability, or assurance of safety and efficacy.[5] Nonetheless, many studies have independently evaluated SRB, demonstrating its analgesic efficacy and safety in rodents.[4,15,16,23,28,30,46] The *Guide* strongly encourages the use of pharmaceutical-grade substances to avoid unexpected adverse side effects;[2] thus, many institutions may consider switching from SRB to the FDA-indexed XRB. However, no studies have yet directly compared the pharmacokinetic parameters of SRB and XRB in mice undergoing surgical catheterization.

Obtaining sufficient blood for analysis in mouse pharmacokinetic studies typically requires terminal blood collections at various time points after drug administration.[20] However, blood sampling from different mice at various time points can introduce variability in interanimal drug responses and also increase the amount of drug and number of mice needed to complete a study.[20,39] Ideally, permanent catheterization and repeated blood sampling from the same mouse would minimize variability and enhance the reproducibility of preclinical drug trial results.[7,8,39] Furthermore, permanent carotid catheterization in mice has not resulted in changes in behavioral parameters, physiologic parameters, or animal welfare assessment scores (including degree of eye opening, appearance, fur quality, body posture and movement, and natural behavior).[21] Using catheters to assess pharmacokinetic parameters and therapeutic drug efficacy is a refinement that decreases data variations due to differences between mice and reduces the number of mice needed.[39] Thus, the goal of the current study was to compare the pharmacokinetic parameters and therapeutic efficacy of SRB and XRB in mice after permanent carotid artery and jugular vein catheterization. We hypothesized that mice treated with either SRB or XRB would have circulating buprenorphine concentrations that exceeded the therapeutic threshold for as long as 72 h after drug administration.

Materials and Methods

All animal procedures were performed in an AAALAC-accredited facility and approved by the City of Hope IACUC. Male (n = 14) and female (n = 14) 6-wk-old C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and allowed to grow and acclimate in the vivarium until catheterization surgery at 13 to 15 wk of age. Prior to surgery, all mice were group-housed in individually ventilated cages (Optimice, Animal Care Systems, Centennial, CO) on corn-cob bedding (Bed-o'-Cobs $\frac{1}{8}$-in., The Andersons, Maumee, OH), with a cotton square and PVC tube provided for enrichment. Beginning at 3 d before surgery, mice were handled daily for approximately 3 min and provided with diet gel (Dietgel Recovery, Clear H_2O, Westbrook, ME) for acclimation; the gel was available for all mice for up to 3 d after surgery. After surgical implantation of the right carotid and left jugular catheters, mice were singly housed to eliminate the risk of catheter port destruction and were assessed daily for clinical signs of pain. Mice were allowed free access to rodent chow (no. 5053, LabDiet, St Louis, MO), diet gel, and reverse-osmosis–purified water and were maintained on a 12:12-h light:dark cycle with recessed, water resistant fluorescent lighting fixtures and lux measurements ranging from 35 to 44 lux at the level of the cage. Based on dirty bedding exposure of sentinel animals followed by assessment with serology and PCR testing, mice were designated as SPF for mouse rotavirus, Sendai virus, pneumonia virus of mice, mouse hepatitis virus, minute virus of mice, mice parvovirus, Theiler murine encephalomyelitis virus, mouse reovirus type 3, mouse norovirus, lymphocytic choriomeningitis virus, mouse thymic virus, mouse adenovirus types 1 and 2, mouse cytomegalovirus, polyoma virus, K virus, ectromelia virus, Hantavirus, LDH–elevating virus, *Pneumocystis* spp., *Bordetella* spp., *Corynebacterium bovis*, *Corynebacterium kutscheri*, *Campylobacter* genus, *Helicobacter* spp., *Klebsiella* spp., *Streptobacillus moniliformis*, *Salmonella* spp., *Staphylococcus aureus*, *Streptococcus pneumonia*, *Beta Streptococcus* spp., CAR *bacillus*, *Encephalitozoon cuniculi*, and *Mycoplasma pulmonis*, *Helicobacter* spp., and *Clostridium piliforme* and were free of endo- and ectoparasites.

Experimental design. The experimental workflows of the 2 treatment groups are presented in Figure 1. SRB (SR-LAB, 1 mg/mL, 5-mL vial) was a patented product prepared by and acquired from Zoopharm (Windsor, CO). XRB (Ethiqa XR, 1.3 mg/mL, 3-mL vial) was a proprietary pharmaceutical-grade formulation prepared by and acquired from Fidelis Pharmaceuticals (North Brunswick, NJ). Beginning 3 d before surgery, mice were weighed daily, clinically evaluated at cage side, and assessed for signs of pain. Mice in each same-sex grouping were assigned to either the SRB (n = 6 male, n = 7 female) or XRB (n = 8 male, n = 7 female) treatment group. On day 0, surgical

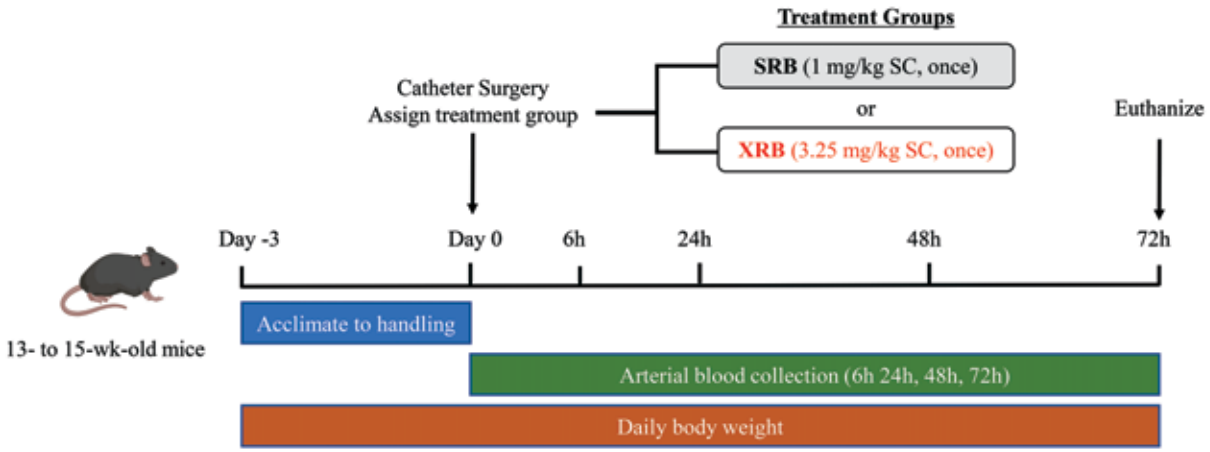

Figure 1. Experimental design. After 3 d of acclimation, mice were anesthetized, treated with either SRB (1 mg/kg SC) or XRB (3.25 mg/kg SC), and surgically implanted with catheters in the left carotid artery and right jugular vein (the right jugular catheter was not used in the current study). Blood samples were collected from the left carotid artery at 4 time points (6, 24, 48, and 72 h after drug administration) and analyzed for plasma buprenorphine by using LC–dual MS.

catheterization was performed (see next section), and each mouse received either SRB (1 mg/kg SC, Hamilton syringe, 23-gauge needle) or XRB (3.25 mg/kg SC, Hamilton syringe, 23-gauge needle) in the right rear flank during the anesthesia induction period. The time of analgesic administration was recorded and used to determine subsequent blood collection time points. Before being injected, both SRB and XRB were mixed well by inversion to produce homogenous suspensions. After surgery, mice were injected with 0.9% sodium chloride (10 mL/kg SC) in the left rear flank and allowed to recover in a clean cage placed on a heating pad. At 6, 24, 48, and 72 h after administration of SRB or XRB, arterial blood samples were collected, and mice were assessed for clinical signs of pain (eye squinting, coat quality, coordination, and overall condition). After blood collection at 72 h, all mice were euthanized by CO_2 asphyxiation followed by cervical dislocation.

Surgical procedure. The left carotid artery and right jugular vein were surgically catheterized, and a mouse antenna for sampling access (MASA; made inhouse) was implanted subcutaneously, as previously described.[7] Only the left carotid artery was used for blood collection; the right jugular catheter remained undisturbed. Briefly, isoflurane-anesthetized mice were aseptically prepared for surgery at the ventral cervical and interscapular regions. A 5-mm longitudinal incision was made just over the left common carotid artery, slightly lateral to the midtrachea. By using blunt dissection, the left common carotid artery was isolated, proximal and distal ligatures were placed, and a silastic–polyethylene 10 catheter[7] (Fischer Scientific, Waltham, MA), prefilled with 100 U heparin–saline lock solution, was secured approximately 8 to 10 mm into the vessel lumen. A second 5-mm incision was made adjacent to the right jugular vein, and ligatures and tubes were placed in the same fashion as just described for the left carotid catheter. The free end of each catheter was tunneled subcutaneously toward the back of the mouse, exteriorized through a small 5-mm incision in the interscapular region, and connected to the MASA device,[7] which sits under the skin. The MASA device was then secured with suture, making blood sampling ports easily accessible from the interscapular region of the mouse.

Blood collection. At each blood collection time point, 80 to 100 µL of arterial blood was collected from the carotid catheter of conscious, unrestrained mice via the MASA. Briefly, a nonserrated hemostat was used to occlude and connect a custom catheter extension tube to the left carotid artery port of the MASA. Care was taken to connect ports quickly, to discard approximately 10 µL of the heparin–saline lock solution, and to avoid the introduction of air bubbles into the arterial catheter line. Blood was transferred from a heparin-coated syringe to a 0.5-mL sterile microfuge tube (Fisher Scientific, Waltham, MA) on ice and then centrifuged at 4 °C and 2500 × *g* for 10 min to obtain plasma.[9] Plasma was transferred to individual sterile glass vials, immediately frozen by using liquid nitrogen, and stored at –80 °C until analyzed.[19] After removal of plasma, RBC were suspended in sterile saline and centrifuged; the supernatant was removed, the pellet was resuspended in sterile saline, and the twice-washed RBC were returned to each mouse via slow infusion into the left carotid catheter in order to avoid anemia. Fresh heparin lock solution (100 U) was infused into the catheter to maintain patency. At the 72-h time point, blood was collected from the arterial catheter, and mice were euthanized.

Pharmacokinetic analysis. Buprenorphine concentrations were measured in mouse plasma by using a LC-dual MS assay established in the City of Hope analytical Pharmacology Core Facility. After precipitation of plasma proteins with acetonitrile containing 3.5 ng/mL buprenorphine-D_4 (Cerilliant, Round Rock, TX) as an internal standard, the sample was vortex mixed for 2 min and centrifuged for 10 min at 21,100 × *g* and 4 °C. A 50-µL aliquot of the resulting supernatant was further diluted 1:4 with 40% methanol, and 5 µL was injected onto the column. Analyte separation was achieved on a Kinetex 2.6 µm C18, 50 × 2.1 mm analytical column (Phenomenex, Torrance, CA) by using gradient separation. The retention time was 3.6 min for buprenorphine and buprenorphine-D_4, and the total run time was 7 min. Detection was performed by using a Xevo TQ-XS Triple Quadrupole Mass Spectrometer (Waters, Milford, MA) with electrospray ionization and operating positive-ion mode. The precursor→product ion combinations at m/z 468.38→396.24 for buprenorphine and 472.38→400.24 for buprenorphine-D_4 were used in multiple-reaction monitoring mode, and MassLynx version 4.2 software (Waters) was used to acquire and analyze data. The lower limit of detection for buprenorphine was 0.3 ng/mL.[30]

Pharmacokinetic parameters were calculated by using PK Solver 2.0 with a noncompartmental analysis linear up-log down method.[45] Parameters included in the analysis were half-life, time to maximum concentrations, peak concentrations, $AUC_{0\text{-last}}$, and clearance. For statistical analysis, 2-way ANOVA followed by Bonferroni multiple-comparisons testing was used to compare differences in buprenorphine concentrations at the 6, 24, 48, and 72-h sample time points. All statistical analyses were completed by using Prism version 9.2.0 for Windows (GraphPad Software, San Diego, CA).

Results

Overall animal health. During the acclimation period, all mice were handled for approximately 3 min daily. No significant changes in body weight occurred during the preoperative period. Preoperative days 3, 2, 1 and day 0 body weights for SRB-treated male mice were 30.0 ± 0.5 g, 29.9 ± 0.6 g, 30.0 ± 0.6 g, and 30.1 ± 0.6 g, respectively, and for XRB-treated male mice were 29.0 ± 0.5 g, 29.0 ± 0.5 g, 29.1 ± 0.5 g, and 29.3 ± 0.5 g, respectively (mean ± SEM, $P > 0.05$). Preoperative days 3, 2, 1 and day 0 body weights for SRB-treated female mice were 21.8 ± 0.7 g, 22.3 ± 0.8 g, 22.6 ± 0.8 g, and 22.8 ± 0.7 g, respectively, and for XRB-treated female mice were 21.5 ± 0.6 g, 21.9 ± 0.6 g, 22.0 ± 0.5 g, and 22.3 ± 0.5 g, respectively (mean ± SEM, $P > 0.05$). Prior to surgery, the body weights of the 2 drug groups within each sex were not significantly different (Figure 2A). After surgery, SRB- and XRB-treated mice had similar declines in body weight. Compared to preoperative values, body weights fell significantly during postoperative days 1 through 3 in male mice (SRB, 28.0 ± 0.8 g, 27.1 ± 1.2 g, and 25.2 ± 0.9 g; XRB, 26.5 ± 0.3 g, 25.3 ± 0.5 g, and 23.8 ± 0.6 g, postoperative days 1 through 3, respectively) and during days 2 through 3 in female mice (SRB, 19.7 ± 0.8 g and 19.0 ± 0.4 g; XRB, 19.4 ± 0.6 g and 19.2 ± 0.7 g, postoperative days 2 through 3, respectively, $P < 0.05$, Figure 2 A). However, changes in body weight were not different between SRB- and XRB-treated mice (Figure 2 B). As compared with preoperative values, body weights in male mice fell by 12% ± 2% in the SRB group and by 12% ± 1% in the XRB group ($P = 0.72$); body weights in female mice fell by 8% ± 2% in the SRB group and by 8% ± 1% in the XRB group (Figure 2 B, $P = 0.83$). No mouse in any treatment group showed gross signs of adverse reactions at the subcutaneous injection site, signs of infection at the catheter implantation site, or differences in cage side monitoring for clinical signs of pain. Anecdotally, XRB-treated mice displayed hyperactive behavior upon recovery from anesthesia, but the SRB-treated mice did not.

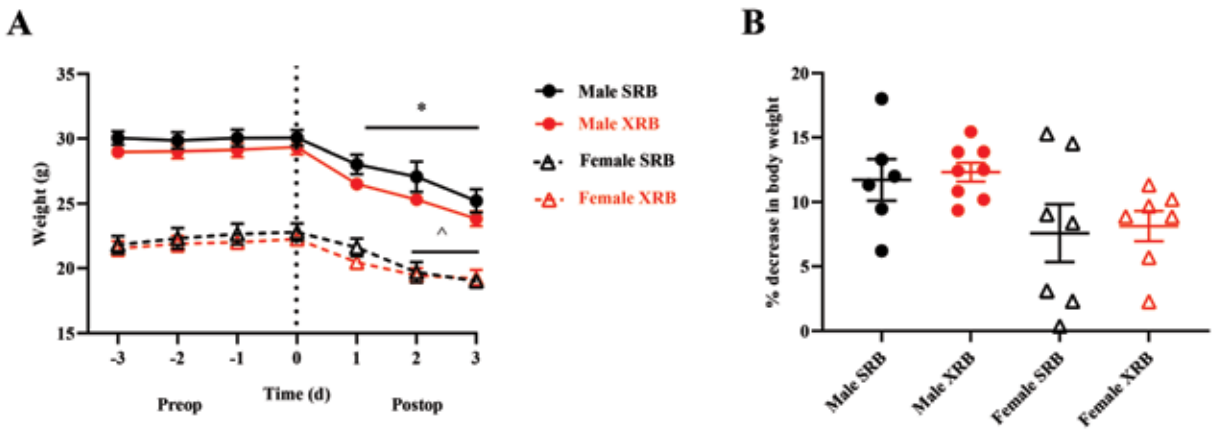

Figure 2. All mice lost body weight after surgery (Postop), with similar losses in both drug groups. (A) Compared to preoperative values, body weights of XRB- and SRB-treated mice fell significantly ($P < 0.05$) 1–3 d (*) and 2–3 d (^) postoperatively in males and females, respectively, but were not different between XRB- or SRB-treated groups. (B) Compared to preoperative values, percent decreases in body weight were similar between sexes treated with SRB or XRB. Data are presented as mean ± SEM. Dashed line in panel A indicates the day of surgery.

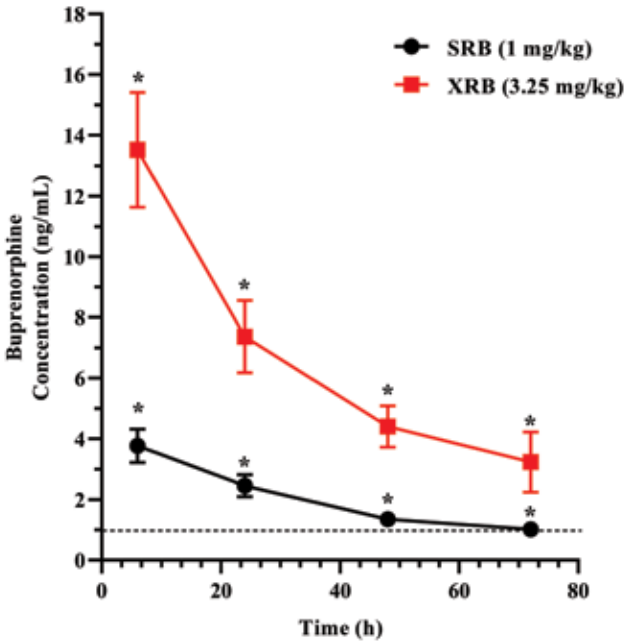

Figure 3. Plasma buprenorphine concentrations in mice treated with SRB (1 mg/kg SC, black line) or XRB (3.25 mg/kg SC, red line) significantly (*, $P < 0.05$) exceeded the therapeutic threshold (1 ng/mL, dashed line) for up to 72 h after administration. Data are presented as mean ± SEM.

Pharmacokinetics. Plasma buprenorphine concentrations were measured at 6, 24, 48, and 72 h after administration of SRB or XRB (Figure 3). Concentrations (mean ± SEM) for SRB-treated mice were 3.8 ± 0.5 ng/mL at 6 h, 2.4 ± 0.4 ng/mL at 24 h, 1.3 ± 0.2 ng/mL at 48 h, and 1.0 ± 0.2 ng/mL at 72 h, as compared with 13.5 ± 1.9 ng/mL at 6 h, 7.4 ± 1.2 ng/mL at 24 h, 4.4 ± 0.7 ng/mL at 48 h, and 3.2 ± 1.0 ng/mL at 72 h for XRB. Plasma buprenorphine concentrations were significantly ($P < 0.05$) higher for XRB than SRB at 6, 24, and 48 h but not at 72 h after administration and did not differ between sexes. These concentrations remained above the therapeutic threshold (1 ng/mL;[25,30] dashed line, Figure 3) for 48 to 72 h after the administration of SRB or XRB. Based on noncompartmental analysis, the slope of elimination for plasma buprenorphine was exponential over time for both SRB ($R^2 = 0.98$) and XRB ($R^2 = 0.96$) and plasma concentrations at any given time can be assessed by using the following equations:

$$\text{SRB Concentration} = 4.03e^{-0.02(time[h])}$$

$$\text{XRB Concentration} = 13.69e^{-0.021(time[h])}$$

Pharmacokinetic parameters for SRB- and XRB-treated mice are presented in Table 1. Half-life and clearance were not significantly different ($P = 0.79$ and $P = 0.99$, respectively) between mice treated with SRB (half-life, 37.8 h; clearance, 5.1 µL/h/kg) and XRB (half-life, 40.3 h; clearance, 5.1 µL/h/kg). Although the peak plasma concentration occurred at 6 h after administration for both SRB and XRB, the peak buprenorphine concentration was 3 to 4 times greater in XRB-treated mice (13.5 ng/mL) than in SRB-treated mice (3.8 ng/mL, $P < 0.01$). In addition, $AUC_{0\text{-last}}$, a measure associated with the systemic distribution of a drug, was significantly higher in XRB-treated mice (452 h×ng/mL) than SRB-treated mice (139 h×ng/mL, $P < 0.05$).

Discussion

In the current study, we surgically implanted indwelling carotid arterial and jugular vein catheters in mice and assessed the pharmacokinetics of subcutaneously administered SRB and XRB. This study design enabled comparison of plasma buprenorphine concentrations for 72 h after drug administration. We found that SRB and XRB had similar half-lives and maintained circulating buprenorphine concentrations above the therapeutic level (1.0 ng/mL) for 72 h after administration. Furthermore, we determined that the maximum concentration and AUC of plasma buprenorphine was 3 to 4 times greater in XRB-treated as compared with SRB-treated mice, which was likely due to the higher recommended dose for XRB (3.25 mg/kg) as compared with SRB (1 mg/kg). The results support our hypothesis that mice treated with either SRB or XRB have circulating buprenorphine concentrations that exceed the therapeutic threshold for as long as 72 h.

The goal of this study was to directly compare the pharmacokinetic parameters of the newly available XRB with the commonly used SRB in mice. In human clinical reports, buprenorphine concentrations of at least 1.0 ng/mL provided pain

Table 1. Pharmacokinetic parameters of SRB and XRB were calculated from a noncompartmental pharmacokinetic analysis[45] of averaged buprenorphine concentrations at each time point (6, 24, 48, and 72 h after administration)

	SRB	XRB
Half-life (h)	37.8	40.3
Time to peak concentration (h)	6	6
Peak concentration (ng/mL)	3.8	13.5
$AUC_{0\text{-last}}$ (h × ng/mL)	139	452
Clearance (µL/h/kg)	5.14	5.08

$AUC_{0\text{-last}}$ = area under the concentration-time curve

relief;[14,42] however, therapeutic levels in rodents have not been established definitively and may not correlate with adequate analgesia.[6,25,30,36,41] Two recent studies revealed attenuated mechanical—but not thermal—hypersensitivity in mice[36] and rats[6] with plasma buprenorphine levels of at least 1 ng/mL. The authors of those previous studies attributed this difference to a possible opioid-induced hypersensitivity that can occur after low doses of opioids or to differences in mechanical and thermal pain thresholds. In other experiments, buprenorphine plasma levels of 0.5 ng/mL provided pain relief in approximately half of the mice studied.[25] Although we did not measure mechanical or thermal hypersensitivity associated with surgery and SRB or XRB treatment, we detected no significant difference in cage side assessment of clinical signs of pain, including eye squinting, coat quality, coordination, and overall condition, over the 72-h period. The SRB 72-h buprenorphine concentrations teetered along the 1.0-ng/mL therapeutic threshold level[28] (Figure 3), suggesting that additional dosing of SRB might be indicated for more painful surgical procedures.[28] Our current study reveals that buprenorphine levels of at least 1 ng/mL provide adequate levels of analgesia based on the absence of clinical signs of pain in mice after surgical catheterization. However, more studies are necessary to elucidate therapeutic buprenorphine levels for various strains and procedures.

SRB and XRB had similar half-lives, times to peak buprenorphine plasma concentration, and clearance rates (Table 1). The peak plasma concentrations that we measured were found at the 6 h time point for both SRB (3.8 ± 0.5 ng/mL) and XRB (13.5 ± 1.9 ng/mL); however, other studies have found peak plasma concentrations as early as 4 h.[30] We collected our earliest samples at the 6 h time point for consistency with protocols established in the safety and efficacy studies of XRB.[26,41] The peak plasma buprenorphine concentration that we measured in XRB-treated mice (13.5 ± 1.9 ng/mL) was similar to a previously published peak concentration of 16.3 ± 8.3 ng/mL in CD1 mice at 6 h after administration.[41] However, the previous study[41] reported a large decrease in buprenorphine concentrations at 1, 2, and 3 d (4.1, 1.3, and 1.5 ng/mL, respectively) after XRB administration, as compared with buprenorphine levels of 7.4 ± 1.2, 4.4 ± 0.7, and 3.2 ± 1.0 ng/mL, respectively, in our current study. This difference is likely due to the different mouse strains used and the interanimal variability that can occur when using terminal blood collection—and thus different mice—at each time point.[39] A recent study reported buprenorphine levels of 7.4 ± 2.0 ng/mL at 4 h after SRB administration to male C57BL/6J mice, higher than that found in our study (3.8 ± 0.5 ng/mL).[36] These results suggest that the peak buprenorphine concentration in SRB-treated C57BL/6J mice likely occurs closer to 4 h, rather than 6 h, after administration. However, the same previous study[36] further reported peak XRB-associated buprenorphine levels of 11.9 ± 5.1 ng/mL at 4 h, whereas we measured higher levels (13.5 ± 1.9 ng/mL) at 6 h after administration. These results suggest that the peak buprenorphine concentration in XRB-treated C57BL/6J mice occurs close to 6 h after administration. In addition, in the previous study,[6] buprenorphine levels in XRB-treated mice were 1.9 ± 0.4, 2.0 ± 1.0, and 0.4 ± 0.3 ng/mL on days 1, 2, and 3 after administration; these are much lower than what we reported here. This inconsistency is likely due to differences in sample collection, as our study used a larger sample size and collected serial samples from the same mice over 72 h.[36]

The AUC, which reflects the systemic distribution of the drug after administration, depends on the rate of elimination (equivalent between SRB and XRB [5.14 and 5.08 h×ng/mL, respectively]) and the dose administered. The AUC was significantly ($P < 0.05$) higher in XRB- as compared with SRB-treated mice (452 h×ng/mL and 139 h×ng/mL respectively). These results suggest that, due to the higher dose, XRB-treated mice (3.25 mg/kg) had a 3- to 4-fold greater distribution and systemic exposure of buprenorphine than did mice given SRB (1 mg/kg). A previous study of SRB in CD1 mice reported an AUC of 322 h×ng/mL at a lower dose (0.6 mg/kg) than that used in the current study (1.0 mg/kg).[30] This difference could be related to variations between the mouse strains used and emphasizes the need to establish specific pharmacokinetic parameters and efficacious buprenorphine levels in various mouse strains.[12,13]

Because our study focused on comparing the pharmacokinetic parameters of XRB and SRB, we did not assess histopathologic lesions and acknowledge this as a limitation to our study. Higher doses of these long-lasting buprenorphine formulations can result in inflammatory changes, subcutaneous hemorrhage, and necrosis at the site of injection.[12,26,33,36] Variability in the degrees of pain and tissue inflammation after surgery are often attributed to differences in tissue handling, which may require multimodal analgesia that includes an anti-inflammatory drug.[18] We used the drug manufacturer's recommended doses in our study and did not observe any clinical concerns or grossly apparent injection site reactions. To minimize the incidence of potential inflammation at the injection site, we used a Hamilton syringe, injected slowly, and pinched the skin after administration to promote retention and local dispersion of the drug. We recommend this delivery method when administering either SRB or XRB, to avoid inadvertent skin reactions.

According to the *NIH–ARAC Guidelines for Blood Collection in Mice and Rats*, the amount of blood that can be safely withdrawn from a single mouse is approximately 10% of the total blood volume every 2 to 4 wk, 7.5% every 7 d, and 1% every 24 h.[3] Following these guidelines is important to prevent anemia, dehydration, and associated pain and distress. However, pharmacokinetic studies may require larger amounts of blood, with 6 to 12 terminal blood samples per drug per time point, thus requiring the use of more mice and contributing to greater variability between samples.[32] In the current study, we used the indwelling carotid catheter to collect multiple blood samples over a 72-h period, returned saline-washed RBC to each mouse, and assessed buprenorphine concentrations in the same mouse at multiple time points. This method is an important refinement in two ways. First, washing RBC and administering them back to the mouse after plasma collection mitigates any anemia that might be associated with collection of larger or more frequent blood volume collections. Second, catheterization allows collection of blood from the same mouse at multiple time points, reducing the number of mice needed as compared with protocols that use terminal blood collection.[20,39,40] Previous studies found that responses to injected drugs varied with regard to both

mouse strain and individual mice; thus, the use of an indwelling catheter reduces intra-animal variation and potentially increases translatability.[7,8,39] Importantly, assessment of pharmacokinetic parameters acquired from the same animal over time more closely mimics preclinical experimental drug trials that are performed in humans and larger animal species.[20,39] Therefore, the use of an indwelling carotid catheter for pharmacokinetic blood collection in mice can be advantageous in studies requiring 3 or 4 blood collections in volumes up to 100 µL.

Although high doses of opioids can cause respiratory depression,[17] prolonged sedation,[30] and weight loss,[11] our findings are consistent with other reports showing that these negative effects are rare if SRB and XRB are used at manufacturers' recommended doses (1 mg/kg and 3.25 mg/kg, respectively).[6,36,41] We anecdotally observed apparent hyperactive behavior in XRB-treated mice. Hyperactivity has previously been documented after opioid administration in rodents.[10,27,35,36] Whether XRB-associated hyperactivity is a negative side effect related to high doses or indicates a superior analgesic efficacy than does the lower dose of SRB remains to be determined. Another study found that both SRB and XRB at low and high doses (3.25 mg/kg and 6.5 mg/kg, respectively) were associated with hyperactivity at 4 and 24 h after treatment.[36] We are currently using indirect calorimetry to assess differences in activity levels in SRB- and XRB-treated mice before and after surgery (manuscript in preparation); our preliminary data suggest that XRB-associated hyperactive behavior occurs between 0 to 12 h after administration. Our observations and those of another study suggest that high opioid doses may lead to transient hyperactivity.[36] The risk of undertreating pain associated with surgical procedures[13] should be weighed against potential opioid-associated hyperactivity when selecting doses for SRB and XRB. Differences in mouse strain, sex and the type of procedure performed may affect both the degree of hyperactivity and therapeutic concentrations of buprenorphine.[12,13] Future studies are necessary to further understand the relationship between hyperactive behavior and buprenorphine plasma levels in various mouse strains. Nonetheless, our results establish that XRB given at 3.25 mg/kg SC during anesthetic induction is comparable to SRB given at 1 mg/kg SC in providing therapeutic buprenorphine concentrations for the alleviation of postsurgical catheterization pain in mice.

Several differences between SRB and XRB may influence the choice of one over the other. SRB is a compounded sterile preparation that uses USP-verified pharmaceutical-grade compounds but is considered an unapproved animal drug that has not been reviewed by the FDA.[29] Administration of SRB in mice is considered extralabel use; however, at appropriate dosages, many studies have shown its safety and efficacy in multiple strains of mice and rats.[4,12,15,16,23,28,30] In contrast, XRB is an FDA-indexed Current Good Manufacturing Practices formulation.[25,26,41] FDA-indexed drugs undergo an FDA approval process designed for species that are too rare or varied to undergo a full approval process.[5] FDA-indexing of drugs takes less time than the standard FDA approval process and reduces costs without compromising the evaluation of the drug efficacy or safety; thus, XRB has a level of FDA certification that SRB currently lacks.[5,29] Nonetheless, both SRB and XRB meet standards set by the *Guide* for ensuring that they do not cause "toxic or unwanted side effects."[2] Our results support the use of either SRB or XRB for the alleviation of postsurgical pain in mice, and the new availability of XRB increases the options for safe and effective analgesia in rodents.

Acknowledgments

We thank the City of Hope Analytical Pharmacology and Animal Resources Cores, which are supported by the National Cancer Institute of the NIH (P30CA033572). We thank the staff of the Comprehensive Metabolic Phenotype Core and the Center for Comparative Medicine. Fidelis Pharmaceuticals provided the XRB used in this study. MS was supported by a National Cancer Institute Cancer Metabolism Training Program Postdoctoral Fellowship (T32CA221709). This work was supported, in part, by intramural funding from Beckman Research Institute, City of Hope.

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