Journal of the American Association for Laboratory Animal Science
Vol 61, No 6
November 2022
Pages 595–602

A Review of Long-acting Parenteral Analgesics for Mice and Rats

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Appropriate analgesia is a crucial part of rodent postoperative and postprocedural pain. Providing appropriate analgesia is an ethical obligation, a regulatory requirement, and an essential element of obtaining quality scientific results and conducting reproducible data. Meeting these requirements is facilitated by practical, efficient and safe delivery methods for providing analgesia. Over the last decade, long-acting analgesics have gained widespread use in research animal medicine to avoid or treat postoperative or postprocedural pain while minimizing handling-related time and stress. Long-acting formulations of analgesics suitable for rodents are available for opioids, NSAIDs, and local anesthetics. The goal of this review is to summarize the currently available long-acting formulations of analgesics for rodents and to provide recommendations to veterinarians and researchers regarding their use.

Abbreviations: Bup-HCL, buprenorphine hydrochloride; Bup ER-LAB, extended-release buprenorphine by ZooPharm; Ethiqa-XR, extended-release buprenorphine by Fidelis Animal Health, Inc.; Bup-LHC, long-lasting highly concentrated buprenorphine; Meloxicam-ER, sustained-release meloxicam

DOI: 10.30802/AALAS-JAALAS-22-000061

Introduction

Treatment of pain is an ethical imperative, scientific necessity, and regulatory obligation when working with rodents in research.[34,52] Pain management involves appropriate surgical technique; the selection of effective anesthesia and analgesia regimens (including the dose, administration technique, and frequency of administration).[10,34,67] Long-acting analgesic formulations offer significant refinement for the care of rodents used for biomedical research including decreased labor and dosing time, decreased handling stress, consistent plasma drug concentrations, and clinical analgesia.[18,70] In addition, long-acting formulations also help to eliminate issues with compliance and variability in analgesic administration. Over the last 10+ years, numerous reports have studied the efficacy and duration of analgesia provided by various long-acting analgesic formulations for rodents.[2,8,20,28,30,38-40,42,53,62]

This review provides an overview of the different, but commonly used, long-acting parenteral analgesic drugs currently available for management of postoperative or procedural related pain in rats and mice in a biomedical research setting. When discussing studies evaluating the efficacy of long-acting formulations, we include the pain modality test used, sex and strain of the mouse or rat, as all of these factors can influence the analgesic efficacy of a drug.[60,68] In this review, many of the cited studies use stimulus-evoked pain testing with thermal and/or mechanical hypersensitivity testing to determine whether a long-acting analgesic formulation reduces the hypersensitivity response. Many studies use the Hargreaves test to evaluate thermal hypersensitivity. This is done by measuring the time interval between exposure of a paw to a thermal stimulus and withdrawal of the paw, which is called the heat latency response.[12] The heat latency times before (baseline) and after paw surgery and analgesic drug administration, are measured and compared.[12] A faster thermal latency after surgery indicates thermal hypersensitivity. Cited studies that evaluate mechanical hypersensitivity use the Von Frey test. In this test, the number of paw responses to a filament are measured and compared before (baseline) and again after surgery and administration of an analgesic. If the number of paw responses is higher after surgery, this is called mechanical hypersensitivity.[12] Other methods of evaluating the clinical analgesic effect of long-acting analgesic formulations include behavioral evaluation and measures of pain that are not stimulus-evoked (grimace scales, burrowing, nest building).[12]

This review is organized by the drug class and focuses on the treatment of pain with commonly used long-acting analgesics (opioids, NSAIDs, and local anesthetics) that offer analgesic coverage ranging from 12 to 96 h. For each drug class section, we provide the manufacturer's recommended dosage, current dosages evaluated in the literature, and the authors' recommendations. Specifically, the analgesics that we reviewed are extended-release buprenorphine LAB (Bup ER-LAB), extended-release buprenorphine (Ethiqa-XR), long lasting highly concentrated buprenorphine (Bup-LHC), extended-released meloxicam (Meloxicam-ER), and liposomal bupivacaine.

Formulation Technology

Long-acting formulations are available for several different analgesic classes and use different controlled-release delivery systems. These delivery systems are identified by a variety of terms, including sustained-release, extended-release, delayed-release, prolonged action, long acting, and slow release.[46] These terms are inconsistently used, named or described.[46] Most long-acting analgesic formulations use polymeric materials to provide a controlled release of the active drug so that the drug concentration remains in the therapeutic index over a

Submitted: 10 Jun 2022. Revision requested: 21 Jul 2022. Accepted: 23 Sep 2022.
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longer time period.[43,46] Plasma drug level is also affected by the differences in degradation of the polymeric vehicles and the drug formulation used. For example, ZooPharm's extended-release formulations (Bup-ER and Meloxicam-ER) use a liquid polymer dissolved in a biocompatible organic solvent.[76] After injection, the drug encapsulated in the liquid polymer is released over time as the polymer undergoes biodegradation via erosion of the polymer, hydrolysis, and drug diffusion.[16] For Ethiqa-XR, another extended-release formulation of buprenorphine, buprenorphine is bound in a lipid capsule and suspended in a medium chain fatty acid triglyceride that is degraded by lipase and esterase activity.[16,49] Bup-LHC is similar to buprenorphine hydrochloride (Bup-HCL), but Bup-LHC has a higher concentration than Bup-HCL (1.8 mg/mL and 0.3 mg/mL, respectively). In addition, the vehicle for Bup-LHC consists of anhydrous dextrose, parabens, glacial acetic acid, water, and hydrochloric acid or sodium hydroxide.[45] Liposomal bupivacaine is comprised of multivesicular liposomes that encapsulate an aqueous core and bupivacaine and break down gradually over time.[24,63] Because each long-acting drug formulation has a different release technology and vehicle, the dosage and duration of analgesia provided for each formulation must be individually evaluated. To our knowledge, the vehicles for these formulations have not been tested, as companies may not be willing to make the vehicles available for research.

Long-acting buprenorphine formulations. Buprenorphine is commonly used to prevent postsurgical pain in rodents.[16,22,58] Buprenorphine is a partial μ-agonist and partial k-antagonist that relieves pain by binding to and activating the μ-opioid receptors in the central nervous system.[46] Benefits of buprenorphine administration include alleviation of moderate pain,[58] reduction in the minimum alveolar concentration (MAC) of isoflurane,[40] and a wide safety margin for rodents.[58] The primary disadvantage of Bup-HCL use is its short efficacy window, which requires repeated doses to provide extended analgesic coverage. Repeated high doses of buprenorphine can have side effects, including respiratory depression,[55,71] cardiovascular depression,[47,57] decreased blood pressure,[44,57] pica,[9,69] and decreased water consumption.[31,32,72] Bup-HCL has minimal effects on lymphoproliferation, T-cell or macrophage function, and splenic cytokine production.[11,61] Long-acting formulations of buprenorphine are currently available in an extended release formulation from ZooPharm (Bup ER-LAB), an extended release formulation from Fidelis Animal Health (Ethiqa-XR), and the Bup-LHC formulation.

Bup ER-LAB: Extended-release buprenorphine. Buprenorphine ER-LAB (Bup ER-LAB, previously labeled Bup SR-LAB) is a sustained-release buprenorphine formulation that is currently available from ZooPharm (Windsor, CO) for use in mice and rats at a concentration of 0.5 mg/mL or 1 mg/mL.[75,76] The manufacturer, ZooPharm, recommends a single SC injection for mice at 0.5 to 1 mg/kg and for rats at 1 to 1.2 mg/kg, which should provide analgesia for 72 h.[75] In the literature, the effective dose and duration of analgesia provided by Bup ER-LAB for mice ranges from 0.6 to 1.5 mg/kg every 12 to 48 h depending on the strain, sex, and pain assay (Figure 1).[5,28,39] In male Swiss-Webster mice, hot plate and tail flick assays found that 1.5 mg/kg of Bup ER-LAB provided effective analgesia for 48 h.[28] In male BALB/c and SWR/J mice tested using the hot plate assay, 1 mg/kg of Bup ER-LAB provided clinically effective analgesia for 12 h.[5] In female ICR mice undergoing an experimental laparotomy, 0.6 mg/kg of Bup ER-LAB prevented pain related behaviors and provided adequate analgesia for at least 72 h.[39]

In rats, the dosage and duration of analgesia of Bup ER-LAB are reported to range from 0.3 to 1.2 mg/kg for 48 to 72 h (Figure 1).[8,20,54,62] In male Sprague–Dawley rats undergoing unicortical orthopedic surgery and hypersensitivity testing, 1.2 mg/kg of Bup ER-LAB provided analgesia for 48 to 72 h.[20] In male Sprague–Dawley rats, 0.3 mg/kg of Bup ER-LAB attenuated thermal hypersensitivity for 48 h mechanical hypersensitivity for 72 h and 1.2 mg/kg of Bup-SR attenuated both mechanical and thermal hypersensitivity for 72 h).[8] Female Sprague–Dawley rats that were treated with 1.2 mg/kg of Bup ER-LAB had effective analgesia for 48 h after a laparotomy procedure.[54] In neonatal rats (Sprague–Dawley, postnatal day 3), both low (0.5 mg/kg) and high (1 mg/kg) doses of Bup ER-LAB attenuated thermal hypersensitivity for at least 8 h in an incisional pain model.[3]

The therapeutic plasma level of buprenorphine has not been established for rodents, but plasma levels that are near 0.5 to 1 ng/mL are generally considered to be antinociceptive.[15,26,35,73] The effective therapeutic plasma level of buprenorphine was evaluated in C57BL/6J female mice using a hot plate assay; withdrawal latency was prolonged when the plasma concentration was just below 0.5 ng/mL at 4 h after injection.[35] By 8 h after injection, when the plasma concentration was 0.2 ng/mL the withdrawal latency was similar to that of saline treated mice.[35] A pharmacokinetic study of Bup ER-LAB at a dose of 0.6 mg/kg in female CD-1 mice indicated that therapeutic plasma levels of 1 ng/mL were maintained for the first 24 h but that the level fell to below 1 ng/mL by 48-h.[37] In male Sprague–Dawley rats given 0.9 mg/kg Bup ER-LAB, therapeutic plasma levels were maintained for at least 72 h. Based on the pharmacokinetic and pharmacodynamic data, we recommend dosing Bup-ER LAB at 0.6 to 1.2 mg/kg every 48 to 72 h for mice and 0.3 to 1.2 mg/kg every 48 to 72 h for rats.

Although Bup ER-LAB offers the benefits of reduced dosing frequency and a sustained analgesia level as compared with Bup-HCL, its use has different challenges. Bup ER-LAB can only be obtained with a prescription from a DEA-licensed veterinarian and cannot be ordered on a researcher's DEA license.[76] Therefore, dispensing and securing Bup ER-LAB is more complicated for the institution, researcher, and veterinarian.[51] Another challenge is obtaining the correct dosing volume, as the drug formulation is viscous, concentrated, and cannot be diluted; these factors make it difficult for researchers to administer appropriately volumes to mice.[51] Based on their findings, the authors recommended administering Bup ER-LAB with a 22-gauge needle and Leur-lock 1-mL syringe, and pinching the injection site for at least 5 s after injection to prevent drug leakage.[51] Bup ER-LAB is provided in a multidose vial and as such has been labeled with a 28-d expiration after the first puncture, even though the formulation has a 6-mo expiration date.[4] However, a recent study has found that when stored securely and accessed with aseptic technique, the vial can be maintained in a sterile state for 6 mo in a research setting.[4] Bup ER-LAB has been associated with clinical side effects that are similar to those of Bup-HCL, especially when a high dosage is used. Bup ER-LAB administration in mice has been associated with decreased GI motility,[28] increased locomotor activity (approximately 17% of mice receiving Bup ER-LAB had hyperreactivity at 24 h),[28] decreased respiratory rate,[28] and injection site lesions.[5,20] Bup ER-LAB administration in rats has also been associated with skin irritation,[20] injection site reactions,[54] pica,[54] and mild sedation.[8] Compared with Bup-HCL, Bup ER-LAB does not alter plasma levels of inflammatory cytokines MCP1 and IL6.[29] Despite the potential challenges in administration of Bup ER-LAB, its use,

Formulation and reference	Strain, species, sex	Experimental model and testing modality	Dose, route, and minimum duration of analgesia	Clinical findings
Bup ER-LAB Zoopharm Recommendations[56]	Mouse Rat		0.5–1 mg/kg SC, 72h 1–1.2 mg/kg SC, 72 h	Injection site reactions Injection site reactions
28	Male Swiss-Webster Mice	No surgery: Hot plate and tail flick assay	1.5 mg/kg SC, 48 h	Decreased GI motility until 4 h, increased activity at 4 h, reduced respiratory rate until 48 h.
6	Male BALB/cJ and SWR/J Mice	No surgery: Hot plate assay	1 mg/kg SC, 12 h	Injection site lesions
39	Female ICR Mice	Experimental laparotomy	0.6 mg/kg SC, 48 h	None
37	Female CD-1 mice	No surgery: Pharmacokinetics study	0.6 mg/kg SC, 24 h	None
20	Male Sprague–Dawley rats	Unicortical tibial defect and thermal nociception	1.2 mg/kg SC, 48 to 72 h	Skin irritation
9	Male Sprague–Dawley rats	Paw incision	0.3 and 1.2 mg/kg SC, 48–72 h	Mild sedation
20	Male Sprague–Dawley	No surgery: Pharmacokinetic study	0.9 mg/kg SC, 72 h	Skin irritation
62	Male Sprague–Dawley rats	Paw incision	1.2 mg/kg SC, 96 h	None
54	Female Sprague–Dawley rats	Laparotomy	1.2 mg/kg SC. 48 h	Pica and injection site reactions
4	P3 male and female Sprague–Dawley rats	Incisional pain	0.5 or 1 mg/kg, 8 h	None
Ethiqa-XR Fidelis Animal Health Recommendations[16]	Mouse Rat		3.25 mg/kg SC, 72 h 0.65 mg/kg SC, 72 h	Signs of nausea, including self-licking, self-gnawing and eating wood chip bedding
53	Male C57BL/6J mice	Incisional pain and pharmacokinetics	3.25 or 6.5 mg/kg SC, 48 h	Hyperreactivity
8	Male and female C57BL/6J mice	Laparotomy and pharmacokinetics	3.25 mg/kg SC, 24–48 h	None
3	Male Sprague–Dawley rats	Incisional pain and pharmacokinetics	0.65 mg/kg or 1.3 mg/kg SC, 48 h	Sedation
42	Male and female Sprague–Dawley rats	Pharmacokinetics	0.65 mg or 1.3 mg/kg SC, 72 h	Injection site reaction
Bup-LHC Zoetis Recommendations[74]	Cat		0.24 mg/kg SC, 24 h	Hyperactivity in cats
38	Male C57BL/6J and female CD1 mice	Pharmacokinetics of B6 and CD1 mice, experimental laparotomy for CD1 females	0.9 mg/kg SC, 6 h	None
51	Male and female C57BL/6NCrl mice	Pharmacokinetics	1 mg/kg SC, 12 h	Ataxia, Straub tail reaction and tiptoe gait
30	Male and female Sprague–Dawley rats	Mechanical pain testing, laparotomy, and pharmacokinetics	0.5 mg/kg SC, 12–24 h	Sedation, coprophagy
Meloxicam-ER Zoopharm Recommendations[75]	Rat		4 mg/kg SC, 72 h	GI distress
37	Female CD-1 mice	Pharmacokinetics	6 mg/kg SC, 12 h	None

Figure 1. Summary of the cited studies evaluating the efficacy of long-acting analgesics for mice or rats.

48	Male Swiss-Webster	Surgical osmotic pump implantation and pharmacokinetics	4 mg/kg SC, was not efficacious	Injection site reactions
62	Male Sprague–Dawley rats	Paw incision	4 mg/kg SC, 96 h (mechanical hypersensitivity only)	None
66	Male and female Sprague–Dawley rats	N/A	N/A	Injection site reactions
Liposomal bupivacaine Pacira Biosciences Recommendations[56]	Human		Max dose based on size of surgical site or 4 mg/kg for pediatric patients	Nausea, constipation, and vomiting
25	Male Swiss-Webster mice	No surgery: electrical sensory stimulus to the abdomen	0.2 mL of 0.5%, 1% and 2% local infiltration in a 26-g mouse provide analgesia for 3, 6 and 26 h	None
Exparel[36]	Male Sprague–Dawley Rats	Paw incision	1 mg/kg local infiltration, 96 h	None
Nocita Elanco Recommendations[11]	Dogs		5.3 mg/kg local infiltration, 72 h	Discharge, inflammation, vomiting

Figure 1. (Continued)

as compared with Bup-HCL, offers a significant refinement for rodent analgesia by providing more consistent long-lasting analgesia with limited administration challenges or untoward clinical effects.

Ethiqa-XR: Extended-release buprenorphine. Extended-release buprenorphine (Ethiqa-XR) from Fidelis Animal Health is an FDA-indexed long-acting buprenorphine that is available at a concentration of 1.3 mg/mL.[16] The dose recommendations of Ethiqa-XR for mice and rats differ from that of Bup ER-LAB, as the 2 formulations use different technologies that affect the rate of drug release (Figure 1). The manufacturer's recommended dose is 3.25 mg/kg for mice and 0.65 mg/kg for rats every 72 h.[14] Recent studies have supported these dosage recommendations for mice and rats. A study that evaluated the clinical efficacy of Ethiqa-XR using an incisional pain model in male C57BL/6J mice at 3.25 or 6.5 mg/kg found effective attenuation of mechanical hypersensitivity and therapeutic plasma levels of at least 1 ng/mL for 48 h.[53] Another study evaluated the efficacy of Ethiqa-XR at 3.25 mg/kg using a laparotomy model in male and female C57BL/6J mice and found that Ethiqa-XR treatment mice had lower ethogram scores at 6 and 12 h after surgery.[7] In this same study, pharmacokinetic analysis of male C57BL6/J mice found that plasma buprenorphine concentration was above 1 ng/mL from 30 min to 48 h after administration.[7] A study using male Sprague–Dawley rats in an incisional pain model found that mechanical hypersensitivity was attenuated for at least 48 h after a dose of 0.65 or 1.3 mg/kg of Ethiqa-XR.[2] The same study found that a dose of 0.65 or 1.3 mg/kg of Ethiqa-XR provided a buprenorphine plasma concentration above 1 ng/mL for 24 h and above 0.5 ng/mL for 72 h.[2] Another pharmacokinetic study in male and female Sprague–Dawley rats given a dose of 0.65 or 1.3 mg/kg also reported buprenorphine plasma concentration above 1 ng/mL at 24 h and above 0.5 ng/mL for 72 h in both male and female rats, although female rats had lower mean buprenorphine plasma concentrations as compared with males.[42] Taking into account the manufacturer's recommended dose and literature results, we recommend dosing Ethiqa-XR SC at 3.25 mg/kg for mice and 0.65 mg/kg for rats every 48 to 72 h.

Ethiqa-XR has many of the same usage challenges described above for Bup ER-LAB. Like Bup ER-LAB, Ethiqa-XR cannot be diluted and has a small administration volume for mice (25-g mouse = 0.07 mL for a 3.25 mg/kg dose). Even though Ethiqa-XR is less viscous than Bup ER-LAB, we recommend injecting it with a 22-gauge needle and a Leur-lock 1-mL syringe. The Ethiqa-XR vial should be mixed before dosing to ensure even drug distribution.[16] Like Bup ER-LAB, the injection site should be pinched for 5 s after administration to prevent drug leakage.[53] A benefit of Ethiqa-XR as compared with Bup ER-LAB is that Ethiqa-XR can currently be purchased from distributors with a researcher DEA license. The only untoward side effect reported after administration of Ethiqa-XR to mice is hyperreactivity (over 50% of study mice exhibited hyperreactivity at 24 h).[53] Untoward clinical effects reported in rats included mild sedation and injection-site reactions.[2,42] Like Bup ER-LAB, Ethiqa-XR also offers a significant refinement over Bup-HCL, and because it is available with a researcher DEA license, procurement and management may be easier distribution as compared with Bup ER-LAB.

Bup-LHC: Long-lasting highly concentrated buprenorphine. Long-lasting highly-concentrated formulation Bup-LHC (commercially available as Simbadol) is FDA-approved and labeled as providing 24 h of analgesia for cats.[74] According to the manufacturer (Zoetis), the recommended dose for cats is 0.24 mg/kg once daily for up to 3 d.[74] Bup-LHC is considered an immediate-release product, which means that buprenorphine is quickly absorbed after subcutaneous injection.[74] Based on the literature, we recommend dosing mice at 0.9 to 1 mg/kg SC every 6 to 12 h (Figure 1).[38,51] Bup-LHC at 0.9 mg/kg provided at least 6 h of decreased pain behaviors in female ICR mice after experimental laparotomy.[38] In male and female C57BL/6NCrl mice, Bup-LHC at 1 mg/kg maintained therapeutic plasma levels of buprenorphine for 12 h, but was below the therapeutic level at 16 h.[51] Based on the literature, we recommend dosing rats with Bup-LHC at 0.5 mg/kg every 12 to 24 h. In male and female Sprague–Dawley rats, Bup-LHC at 0.5 mg/kg provided clinical analgesia for at least 12 h, as evaluated by mechanical pain tolerance and experimental laparotomy.[30] A buprenorphine plasma concentration above

1 ng/mL was maintained for just under 24 h in male and over 24 h in female Sprague–Dawley rats.[30]

Bup-LHC is available from veterinary drug distributors and can be purchased with a researcher DEA license. Although Bup-LHC is highly concentrated, the solution is not viscous like Bup ER-LAB or Ethiqa-XR so it can be dosed with a 25-gauge needle. Behavior changes noted in mice after Bup-LHC administration include mild ataxia, Straub tail reaction, and a tiptoe gait.[51] In rats, untoward clinical effects included sedation and coprophagy.[30] Although the therapeutic plasma concentration and clinical efficacy of Bup-LHC last longer than that of Bup-HCL, both Bup ER-LAB and Ethiqa-XR formulations provide longer periods of analgesia and practical, compliance, and welfare advantages as compared with Bup-LHC.

Long-acting NSAIDs. Several NSAIDs are available for analgesia in rodents with varying durations of effect, including carprofen (12 to 24 h), meloxicam (12 h), ketoprofen (24 h), ibuprofen (not determined), and acetaminophen (not determined).[17,19] NSAIDs exert their main effect by inhibiting the action of the enzyme cyclooxygenase (COX) and mediating the process of inflammation.[17] Typically, NSAIDs are considered to provide less analgesic strength than opioids, but are useful for managing mild pain and inflammatory conditions (such as arthritis), and as part of a multimodal analgesic regimens with opioids.[17] A review of recent literature[19] suggests that commonly used dosing regimens for NSAIDs in rodents likely do not match the duration of effective analgesia or therapeutic plasma levels.[19] For example, one study found that carprofen given to mice at 5 mg/kg SC provided effective analgesia for 6 to 12 h, but the dosing interval often used is every 24 h.[19,37,59] NSAID use, especially at a higher dosing frequency, can increase the risk of untoward clinical effects[17] including gastrointestinal disturbances, nephrotoxicity, interference with platelet function, blood dyscrasias, liver toxicity, ulceration,[64] and hemorrhage.[64] Long-acting NSAIDs are attractive as they offer a sustained therapeutic concentration without the risk of adverse effects. Currently, one commercially available long-acting NSAID, sustained-release meloxicam, is available for use in rodents.

Meloxicam-ER: Extended-release meloxicam. Extended-release meloxicam (Melox-ER) is a sustained-release meloxicam that is available from ZooPharm at a concentration of 2 mg/mL.[76] ZooPharm recommends a dose of Meloxicam-ER of 4 mg/kg every 72 h for rats. One study has evaluated Meloxicam-ER at 4 mg/kg in male Sprague–Dawley rats; this study found that this therapy attenuated mechanical hypersensitivity (but not thermal hypersensitivity) for 96 h in an incisional pain model.[62] Injection site reactions have been reported as an untoward clinical effect of Meloxicam-ER in Sprague–Dawley rats.[66] ZooPharm does not have a label recommendation for the dose of Meloxicam-ER in mice.[76] One study[1] evaluated the clinical efficacy of 4 mg/kg of Meloxicam-ER during surgical osmotic pump placement in male Swiss Webster mice and found that the mice showed behavioral indications of pain and insufficient plasma drug concentration at 4 h after administration.[48] Injection site reactions occurred in some of the mice that received Meloxicam-ER.[48] Injection site reactions were also noted and slowly resolved after Meloxicam-ER administration in multiple strains of mice (Crl:CD1(ICR), C57BL/6J, and BALB/cJ).[21]

The therapeutic plasma level of meloxicam is currently unknown, but based on studies in cats,[23] dogs,[33] and horses[41] it is estimated to be between 390 and 911 ng/mL. In Sprague–Dawley rats, 4 mg/kg of Meloxicam-ER provided a plasma concentration that was highest on day 1 (1,800 ng/mL), lower on day 2 (500 ng/mL), and subsequently lower thereafter.[62] In female CD1 mice, Meloxicam-ER dosed at 6 mg/kg provided a plasma level above 1,000 ng/mL for the 12 h after administration.[37] Another study[1] also evaluated a dose of 6 mg/kg of Meloxicam-ER in female CD1 mice and found that plasma levels remained above the estimated therapeutic level (390 to 911 ng/mL) for the first 12 h without any incidence of clinical signs.[37]

Meloxicam-ER can also be administered using a slow-release technology consisting of a lipid polymer encapsulation of the drug.[76] This lipid polymer produces a highly viscous solution, making drug preparation and injection more difficult.[62] We recommend administering Meloxicam-ER with a 22-gauge needle and Leur-lock 1-mL syringe and pinching the injection site for at least 5 s after administration to prevent drug leakage. Further evaluation of the clinical efficacy of Meloxicam-ER in mice is needed, as the plasma concentration measurements indicate that the therapeutic level is maintained for 12 h, but pharmacodynamic studies present conflicting results. For rats, we suggest using Meloxicam-ER at a dose of 4 mg/kg every 96 h.

Long-acting local anesthetics. Local anesthetics provide a quick onset of action, minimal side effects, large therapeutic index, reduced intraoperative bleeding with vasoconstriction, and a predictable duration of action.[65] A review of preemptive analgesic practices[50] notes that the disadvantage of local anesthetics is typically that they offer only a short therapeutic window (< 8 h) that does not extend for long into the postoperative period after a surgical procedure.[50] Local anesthetics with a long duration can decrease the postoperative pain and, in some situations, may be the only analgesic required or may be a critical component of a multimodal analgesic regimen.

Liposomal bupivacaine. Liposomal bupivacaine is a slow-release bupivacaine formulation that can provide extended postoperative analgesia after single-dose administration.[24,27,63] An experimental drug formulation of liposomal bupivacaine (0.2 mL of 0.5, 1, or 2% liposomal bupivacaine) was evaluated on a marked location of the abdomen in male Swiss-Webster mice.[25] At 24 h after injection, approximately 20% to 30% of the initial concentration remained at the injection site and could still be detected at 48 h.[25] The duration of analgesic response time as determined by monitoring the vocalization response to an electrical stimulus was 3, 6, and 26 h for mice injected with 0.2 mL of 0.5, 1, and 2% liposomal bupivacaine, respectively.[25] No untoward clinical signs were noted in mice.[25] Exparel is a commercially available formulation of liposomal bupivacaine (13.3 mg/mL) that was first approved by the FDA in 2011 for postsurgical pain management in humans; the recommended dose for human patients under the age of 17 is 4 mg/kg.[13] One study reported that local infiltration of liposomal bupivacaine (Exparel) at a dose of 1 mg/kg SC attenuated postoperative mechanical and thermal hypersensitivity for 96 h in rats with incisional pain.[36] Based on the clinical efficacy of Exparel for the attenuation of hypersensitivity, we recommend dosing liposomal bupivacaine at 1 mg/kg to provide up to 96 h of local anesthesia.

A veterinary product, Nocita, was approved by the FDA in 2016 for use in dogs and cats at a dosage of 5.3 mg/kg.[14] Nocita is also formulated with 13.3 mg/mL of bupivacaine.[14] Nocita and Exparel are both currently available in 10-mL or 20-mL, single-use vials.[14] The label states that liposomal bupivacaine can be stored in a syringe at a room temperature of 68 to 77 °F (20 to 25 °C) for a maximum of 4 h.[14] Another study evaluated 5 d of repeated sterile withdrawal from single-use vials and found that Nocita could be used for up to 96 h when stored either under refrigeration or at room temperature.[6] Liposomal bupivacaine should be administered by infiltration of all incised

Drug	Mouse: dosage and duration	Rat: dosage and duration
Bup ER-LAB	0.6–1.2 mg/kg, 48–72 h	0.3–1.2 mg/kg, 48–72 h
Ethiqa-XR	3.25 mg/kg, 48–72 h	0.65 mg/kg, 48–72 h
Meloxicam-ER	Further evaluation needed	4 mg/kg, 72–96 h
Liposomal bupivacaine	1 mg/kg (local infiltration), 96 h	1 mg/kg (local infiltration), 96 h

Figure 2. Summary of the authors' recommendations for dosage and duration of analgesia provided by long-acting analgesics for mice and rats. The dosage chosen will depend on the model being studied, expected pain, strain, sex, and other analgesics or anesthetics used.

tissue layers prior to closure of the surgical site.[14,56] If the dose volume too small to cover the surgical site, an equal volume of normal (0.9%) sterile saline or Lactated Ring's solution[14,56] can be added. Due to the small administration volume, price, and need to discard soon after opening, use of Nocita and Exparel can be financially challenging (a 10-mL vial costs approximately $180 USD). However, for studies in which opioids and/or NSAIDs are contraindicated, Nocita should be considered for analgesia.

Conclusions

Long-acting parenteral analgesics offer the benefits of an extended duration of analgesia, less handling stress, and lower risk of untoward clinical effects. Bup ER-LAB and Ethiqa-XR, extended-release buprenorphine formulations are practical options that have been shown to be clinically efficacious in both mice and rats (Figure 2). An additional benefit of Ethiqa-XR is that it can be procured with a research DEA license instead of a veterinary license. However, Ethiqa-XR is also more expensive than Bup ER-LAB. One dose of Bup ER-LAB (1.2 mg/kg SC) for a 300 g rat costs $8.28 USD, and Ethiqa-XR (0.65 mg/kg SC) costs $18.75 USD. For a 25-g mouse, one dose of Bup ER-LAB (1 mg/kg SC) costs $1.56 US, whereas Ethiqa-XR (3.25mg/kg SC) costs $8.75 USD. Meloxicam-ER has been evaluated less extensively than long-acting buprenorphine formulations; for rats, current data indicate Meloxicam-ER is clinically efficacious, but more evaluation is needed for mice. Nocita and Exparel are the most expensive (at over $180 USD per vial) but are good options for studies that require multimodal analgesia or those for which opioids and/or NSAIDs are contraindicated.

The choice of an analgesic regimen, including analgesic type, dosage regimen, and method of administration, must consider the model, study objectives, anesthetic method, sex, species, and strain. All these factors can affect the dose and duration of analgesia. Additional studies are needed to determine whether long-acting formulations could replace other short-acting opioids (such as morphine, fentanyl, oxymorphone, or butorphanol) or NSAIDs (such as ibuprofen, ketorolac, celecoxib, diclofenac, or parecoxib) for rodents and to determine how long-acting formulations can be used as a part of a multimodal analgesia regimen.

Acknowledgments

We are grateful for the assistance of Janis Atuk-Jones for formatting and reviewing the manuscript.

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