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High-dose Meloxicam Provides Improved Analgesia in Female CD1 Mice: A Pharmacokinetic and Efficacy Study

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Meloxicam is a nonsteroidal anti-inflammatory analgesic drug that is often used in mice. However, doses of 1 to 5 mg/kg given twice daily were recently reported to provide inadequate analgesia. Some studies suggest that doses of up to 20 mg/kg may be necessary for adequate pain management. We investigated the analgesia provided by a high-dose of meloxicam in female CD1 mice. Pharmacokinetic analyses demonstrated that a subcutaneous injection of 10 mg/kg or 20 mg/kg of meloxicam produced therapeutic plasma concentrations for at least 12 h. Ovariectomies via ventral laparotomy were performed to assess analgesic efficacy. Mice were treated immediately before surgery with a high-dose of 10 mg/kg, a low-dose of 2.5 mg/kg, or saline, followed by every 12 h for 36 h. At 3, 6, 12, 24, and 48 h after surgery, mice were assessed for pain based on the following behaviors: distance traveled, time mobile, grooming, rearing, hunched posture, orbital tightening, and von Frey. Initially, some mice received a 20-mg/kg loading dose followed by 10 mg/kg every 12 h. This regimen caused severe morbidity and mortality in 2 mice. Subsequently, this regimen was abandoned, and mice assigned to the high-dose group received 10 mg/kg every 12 h. Mice that received the 10-mg/kg dose after surgery showed less orbital tightening between 3 to 6 h and reduced frequency of hunched posture for 48 h compared with mice that received either the low-dose or saline. However, mice were significantly less mobile for 6 to 12 h after surgery regardless of treatment. These data indicate that a meloxicam dose of 10 mg/kg every 12 h provides better analgesia than a 2.5-mg/kg dose but does not completely alleviate pain.

Abbreviations: NSAID, nonsteroidal anti-inflammatory drug

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Introduction

Mice are one of the most common species in biomedical research and they frequently receive meloxicam therapy for postprocedural pain in various models. Although the literature on pain assessment and analgesia in mice has increased, additional information is necessary to assure the effectiveness and safety of nonsteroidal anti-inflammatory drugs (NSAID) in mice. The NSAID meloxicam preferentially inhibits cyclooxygenase-2, thereby reducing inflammation and providing analgesia.[15] However, meloxicam must be used cautiously due to potential toxicities, including gastric or duodenal ulceration, liver and kidney damage, and skin lesions associated with cyclooxygenase inhibition.[8,17,18,23] The dosage range between analgesic effectiveness and toxicity has not been well established.

Commonly used dosing regimens for meloxicam in mice are 1 to 5 mg/kg SC or PO every 12 h.[4] However, these dosing regimens do not achieve therapeutic levels developed by extrapolation of values from dogs (390 ng/mL)[7] or humans.[2] Male and female C57BL/6 mice dosed at 1.6 mg/kg SC mice achieve the purported therapeutic level for up to 12 h.[2] Similarly, female CD1 mice given 1 mg/kg SC maintain purported therapeutic levels for up to 8 h.[9] A 2-mg/kg dose given SC once daily for 3 d appears to produce effective analgesia in male C57BL/6 mice after a partial hepatectomy.[25] More recent studies found that a single injection of 20 mg/kg did not provide analgesia to male BALB/c mice after laparotomy.[21] However, C57BL/6 mice treated with 20 mg/kg of meloxicam after vasectomy had lower fecal corticosterone levels and fewer behavioral indices of pain.[27] These findings indicate that current doses may be insufficient, and higher doses may be needed.[3,4]

The current study was performed to evaluate both the pharmacokinetics of meloxicam given at 10 and 20 mg/kg SC and its analgesic efficacy after laparotomy in mice. The first aim was to determine whether these doses of meloxicam would achieve and maintain the purported therapeutic level (390 ng/mL) over time.[7] The second aim was to determine whether 10 mg/kg of meloxicam would provide better analgesia after laparotomy than would a dose of 2.5 mg/kg.

Materials and Methods

Mice. Female Crl:CD1(ICR) (mean weight 33.8 g; age 8 to 10 wk) were obtained from Charles River Laboratories (Wilmington, MA). Sixty-three mice were used for the pharmacokinetic study and 60 for the experimental laparotomy study. Mice were free of Sendai virus, mouse hepatitis virus, minute mouse virus, mouse parvovirus, mouse norovirus, Theiler murine encephalitis virus, rotavirus, *Mycoplasma pulmonis*, pinworms, and ectoparasites. Mice were fed ad libitum with Teklad Irradiated Diet 2918 (Envigo, Madison, WI) and filter-sterilized water. Mice were housed in a 14:10-h light:dark cycle at 21 °C and 24 °C in individually ventilated cages (Thorne number 9,

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19.6 cm × 30.9 cm × 13.3 cm, Thorne Caging Systems, Hazleton, PA). Maximum housing densities were 3 mice per cage for the pharmacokinetic study. For the experimental laparotomy study, mice were initially housed with 4 per cage. Three days before surgery, mice were separated and housed individually on a static rack in a dedicated room. All experimental procedures, including use of a group that received laparotomy without postsurgical analgesia, were approved by the Institutional Animal Care and Use Committee and conducted at an AAALAC International–accredited facility.

Pharmacokinetic study. Sixty-three mice were used to assess the pharmacokinetics of meloxicam. Three mice that did not receive meloxicam were euthanized and blood was collected to provide baseline values for plasma. The remaining mice were injected subcutaneously in the interscapular region with a single dose of meloxicam (Boehringer Ingelheim, Ridgefield, CT) at either 10 mg/kg (n = 30) or 20 mg/kg (n = 30). The 10 mg/kg meloxicam was diluted with sterile saline (0.9%, Hospira, Lake Forest, IL) to 2.5 mg/mL, and the 20 mg/kg meloxicam was administered undiluted at 5 mg/mL. Three mice were euthanized by carbon dioxide at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, and 48 h after treatment, and blood was collected via cardiocentesis. Blood samples were placed in heparinized microcentrifuge tubes (Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 3,200 × g for 15 min. Plasma was collected and stored at −80 °C until analyzed.

Measurement of meloxicam plasma concentrations. Calibration curves and negative control samples were prepared fresh for each quantitative assay. The calibration curve was prepared by dilution of the meloxicam working standard solution (Sigma Aldrich, St. Louis, MO) with drug-free mouse plasma to provide concentrations ranging from 5 to 80,000 ng/mL. Quality control samples (mouse plasma with meloxicam added at 3 concentrations [15, 250, and 50,000 ng/mL] within the standard curve) were included as an additional check of accuracy.

Prior to analysis, 20 µL of plasma was diluted with 200 µL of acetonitrile (ACN):1M acetic acid (9:1, v:v) containing 0.02 ng/µL of piroxicam (internal standard; Sigma Aldrich, St. Louis, MO), to precipitate proteins. The samples were vortexed for 2 min to mix, refrigerated for 20 min, vortexed for an additional 1.5 min, and centrifuged in a Sorvall ST 40R centrifuge (Thermo Scientific, San Jose, CA) at 3830 × g for 10 min at 4 °C. A 30-µL aliquot was then injected into a liquid chromatography tandem mass spectrometry (LC-MS/MS) system.

Quantitative analysis of plasma was performed on a TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific) having an 1100 series liquid chromatography system (Agilent Technologies, Palo Alto, CA). The system was operated using positive electrospray ionization (ESI(+)). The spray voltage was set at 3,500 V, sheath gas and auxiliary gas were 45 and 25 respectively (arbitrary units), and the vaporizer temperature was 350 °C. Product masses and collision energies were optimized by infusing the standards into the mass spectrometer. Chromatography employed an ACE 3 C18 10 cm × 2.1 mm × 3 mm column (Mac-Mod Analytical, Chadds Ford, PA) and a linear gradient of ACN in water, with 0.2% formic acid, at a flow rate of 0.35 mL/min. The initial ACN concentration was held at 5% for 0.33 min, ramped to 95% over 5.0 min, and held at that concentration for 0.1 min, before re-equilibrating for 3.17 min at initial conditions.

Detection and quantification were conducted using selective reaction monitoring (SRM) of the initial precursor ion for meloxicam (mass to charge ratio *(m/z)* 352.0) and the internal standard piroxicam (*(m/z)* 332.0). The response for the product ions for meloxicam (*m/z* 73.0) and the internal standard (*m/z* 78.3, 95.2), were plotted, and peaks at the proper retention time integrated, using Quanbrowser software (Thermo Scientific). Linear regression analysis using the Quanbrowser software was used to generate calibration curves and quantitate analytes in all samples. A weighting factor of 1/X (X, concentration) was used for all calibration curves.

Pharmacokinetic analysis. Noncompartmental analysis for sparse data was performed on plasma meloxicam concentrations using commercially available software (Phoenix Winnonlin v8.2, Certara, Princeton, NJ). Plasma drug concentrations from all mice at each timepoint were analyzed simultaneously, which permitted estimation of the standard errors for C_{max} and AUC_{last}. The standard error of the mean AUC_{last} and C_{max} values were calculated as described previously.[6,19] The 2 meloxicam dose groups were analyzed separately.

Laparotomy model. A laparotomy model was used to determine the analgesic effectiveness of high-dose meloxicam after surgery. Mice were randomly assigned to one of 6 treatment groups: 1) surgery followed by treatment with high-dose meloxicam (SX-10MEL), 2) surgery followed by treatment with low-dose meloxicam (SX-2.5MEL), 3) surgery followed by treatment with saline (SX-SAL), 4) anesthesia-only followed by treatment with high-dose meloxicam (A-10MEL), 5) anesthesia-only followed by treatment with low-dose meloxicam (A-2.5MEL), and 6) anesthesia-only followed by treatment with saline (A-SAL). A meloxicam loading dose of 20 mg/kg was used undiluted at 5 mg/mL, the 10-mg/kg dose was diluted to 2.5 mg/mL, and the 2.5-mg/kg dose was diluted to 0.5 mg/mL, both with sterile saline. The first dose was administered immediately after anesthetic induction, and then repeated every 12 h for 36 h. Injections were given subcutaneously in the interscapular region. The saline volume was equivalent to the LDM volume of approximately 0.13 mL.

Anesthesia was induced and maintained using isoflurane (Fluriso, VetOne, MWI Veterinary Supply, Boise, ID). Once mice were anesthetized, their abdomens were shaved and prepared aseptically using alternating chlorohexidine surgical scrub and 70% isopropyl alcohol. A 2.0-cm cutaneous incision was made along the abdominal midline, followed by a 1.5-cm incision through the abdominal muscle wall. The ovaries were excised bilaterally after cauterizing the ovarian pedicles and distal uterine horns. The abdominal wall was closed using 5-0 PDO suture (Ethicon, Johnson and Johnson, New Brunswick, NJ) and the skin was closed using 9-mm wound clips (Braintree Scientific, Braintree, MA). Mice that received anesthesia alone were anesthetized for a similar duration as mice that underwent surgery. Their abdomens were also shaved to create similar von Frey conditions. Mice were returned to their home cages after surgery or anesthesia and monitored until fully recovered.

Surgeries were performed in cohorts one week apart. Mice in the first SX-10MEL cohort (C1-SX-LOAD) received an undiluted 5-mg/mL loading dose of 20 mg/kg followed by 10 mg/kg every12 h. Mice were randomized in the first cohort as follows: C1-SX-LOAD (n = 4), SX-2.5MEL (n = 2), SX-SAL (n = 0), A-10MEL (n = 0), A-2.5MEL (n = 2), and A-SAL (n = 4). Because greater morbidity was associated with the 20-mg/kg loading dose in C1-SX-LOAD mice, the subsequent SX-10MEL cohorts received 10 mg/kg both before and every 12 h after surgery without a loading dose. The remaining treatments were equally distributed with 2 mice per treatment per cohort. The C1-SX-LOAD mice were analyzed independently of the other cohorts, giving final total sample sizes of SX-10MEL (n = 8), SX-2.5MEL

(n = 10), SX-SAL (n = 8), A-10MEL (n = 8), A-2.5MEL (n = 10), and A-SAL (n = 12).

Behavioral assessments. Baseline behavioral assessments were obtained 24 h before anesthesia and surgery (timepoint 0), and at 3, 6, 12, 24, and 48 h after surgery. Timepoints 0 h and 3 h were performed at approximately 1100. Home cages were placed in an ANY-maze (Stoelting, Wood Dale, IL) video tracking apparatus; mice were then given 5 min to acclimate. After acclimation, overall activity was assessed using ANY-maze video tracking software, which identified the head, abdomen, and tail base and used them to track the whole body's movement for 5 min. Distance traveled and duration of activity data were collected from the ANY-maze software results. During the ANY-maze's recordings, 2 female observers who were blind to the treatment groups performed cageside assessments and tallied the incidences of grooming, rearing, hunched posture (that is, arched back), and orbital tightening every 30 s. The cumulative tallies and scores are presented per timepoint. The presence or absence of a hunched posture was noted and scored (0, no hunched posture; 1, hunched posture). Orbital tightening scores were based on a modified facial grimace scale which correlated to the degree of pain (0, no orbital tightening; 1, moderate orbital tightening; 2, severe orbital tightening).[1,10] Wound licking durations were determined retrospectively from the ANY-maze recorded videos. Interrater consistence was not determined.

A different female observer used an electronic von Frey analgesiometric device (IITC Life Science, Woodland Hills, CA) to test mechanical pain by using a filament capable of measuring up to 80 g. Mice were placed in a plantar test glass stand (IITC Life Science, Woodland Hills, CA) with a perforated base to allow ventral access to their midline incisions without manipulation of the mice. Von Frey tests were performed at each timepoint after a 5-min acclimation period, immediately after ANY-maze observations. After each acclimation, the observer applied increasing pressure in a single motion onto the incision, or midline abdominal region for nonsurgical and baseline measurements, until the mouse withdrew. The maximal pressure was recorded in grams. Von Frey measurements were collected 3 times, with one minute between measurements, and the average force (g) reported.

Histopathology. After the 48-h measurements were completed, mice were euthanized via asphyxiation with carbon dioxide. A diagnostic gross necropsy was performed, and tissue was collected to assess meloxicam toxicity. Interscapular skin at the injection site, heart, lungs, liver, kidneys, stomach, and duodenal-pyloric junction were collected, fixed with 10% buffered formalin, and processed and stained with hematoxylin and eosin.

Statistical analysis. Behavioral data analysis was performed using JMP (v. 15.0.0, SAS, Cary, NC). Full-factorial repeated measures ANOVA and student t tests were performed to identify significant differences between treatment groups across timepoints. Active time, rearing, and von Frey responses were assessed for normality. Distance traveled and wound licking data were log x+1 transformed, and grooming data were log 10 transformed to a gaussian distribution. Descriptive statistics including mean and SD were used to assess orbital tightening and hunched posture. Descriptive data are expressed as mean ± SD. A P value of less than 0.1 was considered statistically significant.

Results

Pharmacokinetics. The standard curve for meloxicam was linear and gave correlation coefficients of 0.99 or better. Accuracy was reported as percent relative to the known concentrations and precision as the percent relative standard deviation. For meloxicam, accuracy was 114% for 15 ng/mL, 107% for 250 ng/mL, and 97% for 50,000 ng/mL. Precision was 4% for 15 ng/mL, 8% for 250 ng/mL, and 4% for 50,000 ng/mL. The assay was optimized to provide a limit of quantitation of 5 ng/mL and a limit of detection of approximately 2 ng/mL for meloxicam.

Plasma meloxicam concentrations were determined across 48 h for female CD1 mice given one dose of 10 mg/kg or 20 mg/kg meloxicam SC. The peak plasma concentration for the 10 mg/kg dose occurred at 60 min after injection (C_{max} 28,496 ± 12,208 ng/mL), and plasma concentrations remained above the purported therapeutic level of 390 ng/mL[7] for 12 h, at which time the concentration was 559 ± 69 ng/mL (Figure 1). The peak plasma concentration for the 20 mg/kg dose occurred at 30 min after injection (C_{max} 54,857 ± 21,708 ng/mL), and remained above the purported therapeutic level[7] for 12 h, at which time the concentration was 983 ± 80 ng/mL. Values for both doses were below 390 ng/mL at the 24-h timepoint. A noncompartmental analysis showed a half-life of each of 4 to 5 h (Table 1).

Laparotomy and postoperative behavioral assessments. The analgesia provided by HDM was assessed after laparotomy by using ANY-maze and behavioral observations. The C1-SX-LOAD mice received a 20 mg/kg meloxicam loading dose followed by a dose of 10 mg/kg every 12 h. Although the data from the C1-SX-Load group were not analyzed for statistical significance due to the low sample size, mice in this group frequently had numerically higher orbital tightening scores and hunched posture incidence, lower incidence of rearing, grooming, and licking their surgical sites, and numerically less traveling and mobile time than did the other SX-10MEL cohorts (Table 2). At the 48-h timepoint, one mouse was found dead, and another was euthanized. Necropsy revealed an intestinal perforation and gas distention, respectively. Due to this toxicity, the 20-mg/kg loading dose was eliminated for the remaining SX-10MEL mice, and instead their meloxicam treatment consisted of 10 mg/kg meloxicam before and every 12 h after surgery. The C1-SX-LOAD cohort was not included in the final analysis.

Painful behaviors were frequently not significantly different in the SX-10MEL group as compared with the SX-2.5MEL and SX-SAL groups (Table 3). Mice that received SX-10MEL groomed

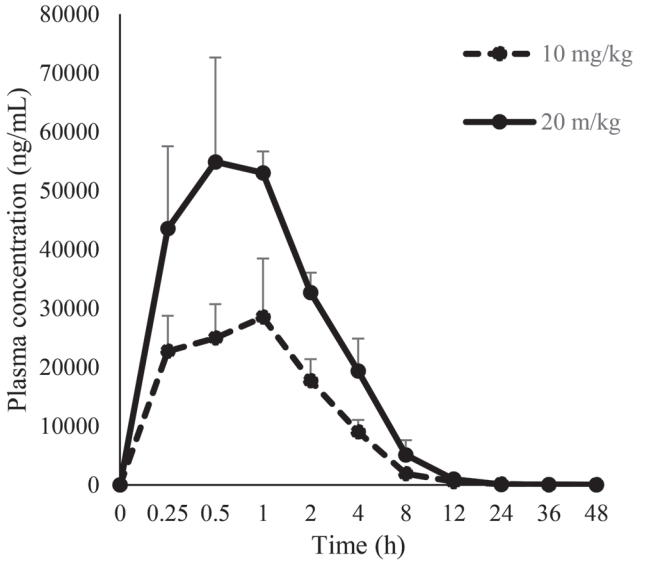

Figure 1. Mean plasma meloxicam concentrations (ng/mL) after 10-mg/kg SC dose in female CD1 mice.

Table 1. Noncompartmental analysis of plasma meloxicam concentrations after subcutaneous administration at 10 or 20 mg/kg to female CD1 mice.

Parameter	Units	10 mg/kg	20 mg/kg
λz	1/h	0.14	0.17
HL λz	h	5.0	4.0
T_{max}	h	1.0	0.5
C_{max}	μg/mL	29	55
C_{max} SE	μg/mL	7.0	12.5
AUC_{last}	h* μg/mL	1,739	3,467
AUC_{last} SE	h* μg/mL	134	254
AUC 0→∞	h* μg/mL	1,744	3,469
$AUC_{\%Extrap}$	%	0.25	0.05

λz, elimination rate constant; HL λz, terminal half-life; T_{max}, time of maximum concentration; C_{max}, maximum concentration; C_{max} SE, standard error of C_{max}; AUC_{last}, area under the concentration-time curve from the time of dosing to the last measurable concentration; AUC_{last} SE, standard error of AUC_{last}; AUC 0→∞, area under the concentration time curve from time 0 extrapolated to infinity; $AUC_{\%Extrap}$, percentage of AUC0→∞ due to extrapolation from the last measured timepoint to infinity.

more frequently than the SX-SAL group ($P = 0.07$) at 3 h. They also licked their surgical sites longer than the SX-SAL at 6 h ($P = 0.08$). The SX-2.5MEL group licked their surgical sites longer than either the SX-10MEL or SX-SAL group at 24 h ($P < 0.001$ and $P = 0.003$, respectively). Mice in the SX-2.5MEL group traveled further than the SX-SAL at 3 h ($P = 0.04$). The SX-2.5MEL group also traveled further than the SX-10MEL at 6 h ($P < 0.10$).

Several metrics suggested that mice that underwent surgery experienced more pain than did anesthesia-only mice, regardless of their meloxicam dose. Mice in the SX-10MEL and SX-2.5MEL groups were less mobile than anesthesia-only mice at 3 h ($P < 0.01$) and 6 h ($P < 0.01$). At 12 h, SX-2.5MEL mice were less mobile than anesthesia-only mice (A-10MEL $P = 0.012$; A-2.5MEL $P < 0.01$; A-SAL $P = 0.049$), while SX-10MEL mice were less mobile than A-10MEL ($P = 0.042$) and A-2.5MEL mice ($P < 0.01$). SX-10MEL and SX-2.5MEL mice did not show significant differences in rearing, distance traveled, and or von Frey responses ($P > 0.1$) as compared with each of the anesthesia-only groups.

Histopathology. No gross abnormalities were seen at the surgical and injection sites. Mild-to-moderate dermatitis at the injection site was observed histologically in all treatment groups (10MEL 4 of 16; 2.5MEL 11 of 16; SAL 7 of 16). In addition, 4 of 16 mice in the 10MEL group had more severe dermatitis with crusting and ulceration. We found no other histologic evidence of meloxicam toxicity.

Discussion

We characterized the pharmacokinetics and analgesic effects of high-dose meloxicam in female CD-1 mice, expanding on low-dose pharmacokinetic data.[9] The pharmacokinetics indicate that a single 10 or 20 mg/kg dose of meloxicam provides therapeutic plasma concentrations for 12 h. As expected, the C_{max} and total plasma concentration over time (AUC) was greater in mice given 20 mg/kg as compared with 10 mg/kg. Both doses showed similar half-lives (as expected), and both fell below the purported therapeutic level[7] by 12 h after administration. These findings suggest that the clearance rates are similar. Conducting a pharmacokinetic analysis with multiple doses of meloxicam could help to determine whether dosing every 12 h may have a cumulative effect, resulting in higher plasma concentrations than predicted by the single-dose analysis. The experimental laparotomy model demonstrates that subcutaneous meloxicam

Table 2. Postoperative behavioral scores (mean ± SD) in female CD1 mice treated with a loading dose of 20 mg/kg of meloxicam preoperatively, then 10 mg/kg every 12 h.

	Time (h)	C1-SX-LOAD survivors	C1-SX-LOAD nonsurvivors*
No. in group		2	2
Orbital Tightening	−24	0 ± 0	0 ± 0
	3	0.5 ± 0.7	8.5 ± 12.0
	6	2.3 ± 0.4	6.8 ± 8.8
	12	1.0 ± 1.4	0.5 ± 0.7
	24	0 ± 0	8.0 ± 7.1
	48	0.3 ± 0.4	22
Hunched Posture	−24	0 ± 0	0 ± 0
	3	1.0 ± 0.7	11.5 ± 12.7
	6	0.3 ± 0.4	9.3 ± 6.7
	12	0 ± 0	7.0 ± 0.7
	24	0.3 ± 0.4	5.3 ± 1.8
	48	2.0 ± 2.8	22
Rearing	−24	29 ± 32.5	58.8 ± 10.3
	3	17.3 ± 15.2	15.3 ± 13.1
	6	7.3 ± 3.2	14.3 ± 13.8
	12	8.5 ± 6.4	14 ± 0
	24	14.8 ± 7.4	4.8 ± 6.7
	48	9.3 ± 5.3	0
Wound licking duration	3	95.7 ± 72.7	8.8 ± 1.8
	6	77.0 ± 75.6	12.3 ± 9.5
	12	63.8 ± 51.5	11.3 ± 1.1
	24	22.6 ± 25.0	1.8 ± 2.5
	48	0.8 ± 0.8	0
Grooming	−24	4.3 ± 3.2	2 ± 0
	3	2.7 ± 2.5	1.3 ± 1.8
	6	7.3 ± 7.4	5.5 ± 1.4
	12	8.8 ± 8.1	3.3 ± 3.2
	24	4.8 ± 1.8	0.8 ± 1.1
	48	3.8 ± 1.1	0
Distance Traveled (m)	−24	3.9 ± 4.7	8.7 ± 1.1
	3	2.8 ± 3.2	2.0 ± 2.2
	6	0.6 ± 0.9	3.1 ± 0.8
	12	1.2 ± 1.5	1.6 ± 0.1
	24	2.1 ± 0.7	6.7 ± 5.9
	48	2.3 ± 1.3	5.9
Time mobile (s)	−24	140 ± 129	236 ± 18
	3	79 ± 86	82 ± 78
	6	182 ± 166	94 ± 20
	12	33 ± 39	79 ± 15
	24	72 ± 52	159 ± 139
	48	84 ± 42	220

*One nonsurvivor died prior to 48 h and the other was euthanized immediately after the final behavior assessment.

Table 3. Postoperative behavior scores (mean ± SD) in female CD1 mice that underwent surgery (SX). Mice were treated with high-dose meloxicam (10MEL, 10 mg/kg), low-dose meloxicam (2.5MEL, 2.5 mg/kg), or saline (SAL). Mice that received anesthesia (A) only plus 10MEL, 2.5MEL or SAL.

	Time (h)	SX-10MEL	SX-2.5MEL	SX-SAL	A-10MEL	A-2.5MEL	A-SAL
No. per group		8	10	8	8	10	12
Orbital Tightening	−24	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	3	1.1 ± 1.6	3.3 ± 4.6	8 ± 6.2	0 ± 0	0 ± 0	0 ± 0
	6	0.1 ± 0.2	0.7 ± 1.3	3.1 ± 4.5	0 ± 0	0 ± 0	0 ± 0
	12	0.1 ± 0.2	0.1 ± 0.3	0.5 ± 1.0	0 ± 0	0 ± 0	0 ± 0
	24	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	48	0 ± 0	0.6 ± 1.9	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Hunched Posture	−24	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	3	9.3 ± 3.2	11.6 ± 6.4	14.7 ± 4.8	0 ± 0	0.1 ± 0.2	0.1 ± 0.2
	6	7.3 ± 3.4	10.4 ± 5.6	10.8 ± 3.8	0.1 ± 0.2	0 ± 0	0.6 ± 0.9
	12	4.9 ± 3.8	6.7 ± 4.6	5.9 ± 4.0	0.1 ± 0.2	0 ± 0	0.2 ± 0.6
	24	3.6 ± 2.8	5.5 ± 3.4	4.1 ± 3.3	0.1 ± 0.2	0 ± 0	0.5 ± 1.3
	48	2.3 ± 3.4	4.5 ± 4.5	3.3 ± 4.0	0.1 ± 0.4	0.3 ± 0.8	0 ± 0.1
Rearing	−24	49.2 ± 17.0[a,d]	61.1 ± 10.9[c]	55.3 ± 17.4[d]	49.5 ± 11.7[a,d]	62.1 ± 17.3[b,c,e]	54.5 ± 9.5[d]
	3	24.5 ± 18.4[c,d,e]	23.0 ± 10.8[c,d,e]	17.2 ± 19.0[c,d,e]	46.8 ± 16.3[a,b,d]	58.5 ± 12.3[a,b,c,e]	50.0 ± 17.5[a,b,d]
	6	18.2 ± 11.4[c,d,e]	22.8 ± 9.0[d,e]	22.6 ± 19.2[d,e]	31.7 ± 10.6[d]	53.2 ± 11.9[a,b,c,e]	42.3 ± 15.7[a,b,d]
	12	21.0 ± 13.3[d,e]	17.5 ± 10.2[c,d,e]	25.6 ± 19.0[d]	31.6 ± 13.0[a,d]	43.7 ± 9.5[a,b,c]	35.9 ± 17.2[a]
	24	31.5 ± 16.2[b,c,d,e]	32.8 ± 12.3[c,d,e]	43.8 ± 12.4	45.6 ± 15.5[a]	53.6 ± 11.1[a,b]	45.3 ± 16.0[a]
	48	34.3 ± 13.4[b,d]	40.0 ± 19.9	46.3 ± 13.3	39.9 ± 11.7	42.3 ± 15.5	41.9 ± 15.0
Wound licking duration (s)	−24	0	0	0	0	0	0
	3	89.0 ± 50.2[c,d,e]	90.3 ± 39.8[c,d,e]	72.6 ± 42.4[c,d,e]	2.5 ± 5.0[a,b]	0.7 ± 1.6[a,b]	1.2 ± 4.0[a,b]
	6	124.0 ± 47.9[b,c,d,e]	79.8 ± 31.6[c,d,e]	63.5 ± 41.1[c,d,e]	1.1 ± 1.6[a,b]	0.4 ± 1.2[a,b]	2.8 ± 5.4[a,b]
	12	46.2 ± 33.1[c,d,e]	65.5 ± 74.0[c,d,e]	70.5 ± 48.0[c,d,e]	2.6 ± 7.0[a,b]	2.4 ± 5.2[a,b]	3.9 ± 8.8[a,b]
	24	5.3 ± 9.8[a,d]	50.1 ± 60.2[b,c,d,e]	12.7 ± 14.3[a,c,d,e]	1.0 ± 2.0[a,b]	0.0 ± 0.0[a,b]	0.7 ± 1.6[a,b]
	48	1.1 ± 2.8	2.1 ± 2.5[c]	3.2 ± 3.5[c,d,e]	0.0 ± 0.0[a,b]	0.3 ± 0.9[b]	0.3 ± 0.6[b]
Grooming	−24	0.6 ± 0.5	1.0 ± 4.4	1.1 ± 1.1	0.4 ± 0.9	0.4 ± 0.6	1.0 ± 0.8
	3	4.9 ± 3.7[b,d,e]	3.1 ± 2.9[d,e]	2.5 ± 3.0	1.8 ± 4.0	1.1 ± 1.3[a]	1.3 ± 1.8[a]
	6	3.9 ± 3.3[c,d]	2.8 ± 2.7[d]	1.8 ± 1.4	0.8 ± 0.9	0.8 ± 1.1[a]	1.7 ± 2.0
	12	4.1 ± 4.2[e]	2.5 ± 1.6	2.7 ± 1.7	0.6 ± 1.4	1.0 ± 1.6	1.6 ± 2.3
	24	1.0 ± 1.3	1.8 ± 1.9	0.9 ± 0.9	1 ± 1.9	1.0 ± 1.5	0.9 ± 0.9
	48	0.9 ± 0.9	0.7 ± 0.5[e]	1.9 ± 1.8	0.9 ± 0.6	0.9 ± 1.2	1.3 ± 1.4[a]
Distance Traveled (m)	−24	7.1 ± 2.4	7.7 ± 2.0	8.7 ± 2.4	7.8 ± 3.2	8.8 ± 1.9	7.4 ± 1.9
	3	3.2 ± 1.4[c,d,e]	3.5 ± 1.5[b,c,d,e]	2.5 ± 2.2[a,c,d,e]	7.0 ± 1.5[a,b]	7.6 ± 1.3[a,b]	6.3 ± 1.5[a,b]
	6	2.5 ± 1.4[a,c,d,e]	3.6 ± 1.6[d,e]	3.4 ± 2.3[c,d,e]	4.9 ± 1.8[b]	6.4 ± 2.5[a,b]	5.3 ± 2.1[a,b]
	12	3.0 ± 1.1[c,d]	2.8 ± 1.3[c,d,e]	3.3 ± 2.2[c,d]	4.9 ± 2.0[a,b]	6.5 ± 2.6[a,b,e]	4.2 ± 1.6[a,d]
	24	5.2 ± 1.8	5.3 ± 2.4	6.7 ± 2.9	6.6 ± 2.7	5.9 ± 2.4	5.2 ± 1.7
	48	5.4 ± 2.3	5.6 ± 3.3[e]	6.1 ± 2.7	6 ± 1.6	5.7 ± 1.3	4.5 ± 1.5[a]
Time mobile (s)	−24	237 ± 45	239 ± 35	253 ± 28	238 ± 31	263 ± 22	241 ± 24
	3	113 ± 39[c,d,e]	115 ± 52[c,d,e]	103 ± 72[c,d,e]	220 ± 41[a,b]	235 ± 40[a,b]	201 ± 31[a,b]
	6	84 ± 40[b,c,d,e]	114 ± 37[c,d,e]	130 ± 83[c,d,e]	181 ± 74[a,b]	205 ± 71[a,b]	180 ± 42[a,b]
	12	117 ± 49[c,d]	108 ± 46[c,d,e]	113 ± 55[c,d]	169 ± 52[a,b]	197 ± 53[a,b,e]	151 ± 48[a,d]
	24	200 ± 50	174 ± 72	213 ± 41	213 ± 63	208 ± 79[e]	198 ± 32[d]
	48	193 ± 64	198 ± 74	203 ± 39	213 ± 25[e]	218 ± 48[e]	168 ± 17[c,d]
Von Frey (g)	−24	8.3 ± 5.0	5.4 ± 3.0	5.1 ± 1.5	7.8 ± 7.0	5.9 ± 3.1	5.1 ± 2.8
	3	5.90 ± 3.4[c]	5.5 ± 4.0[c]	5.3 ± 2.7[c]	11.2 ± 7.1[a,b]	8.4 ± 8.7	7.9 ± 6.3
	6	4.0 ± 1.2[c]	3.7 ± 3.7[c]	5.5 ± 5.7[c]	9.7 ± 8.4[a,b,d]	3.3 ± 1.7[c]	5.1 ± 2.8
	12	2.6 ± 1.7[c,e]	4.7 ± 3.7	4.2 ± 2.2	8.2 ± 7.8	3.9 ± 1.5	6.5 ± 2.9
	24	2.8 ± 1.3[c,d]	2.6 ± 1.5[c,d,e]	2.6 ± 0.9[c,d]	8.3 ± 6.8[a,b]	5.1 ± 2.1[a,b]	5.2 ± 4.2[a]
	48	2.6 ± 1.4[c,d,e]	3.4 ± 2.6[c,e]	3.8 ± 2.1[c,e]	9.5 ± 5.7[a,b,d]	4.0 ± 1.2[c]	7.4 ± 4.1[a,b]

[a]Value significantly ($P < 0.10$) different from the surgery with low-dose meloxicam treatment group
[b]Value significantly ($P < 0.10$) different from the surgery with saline treatment group
[c]Value significantly ($P < 0.10$) different from the anesthesia only with high-dose meloxicam treatment group
[d]Value significantly ($P < 0.10$) different from the anesthesia only with low-dose meloxicam treatment group
[e]Value significantly ($P < 0.10$) different from the anesthesia-only with saline treatment group

at a 10-mg/kg dose given every 12 h does not cause toxicity and provides greater analgesia than does a dose of 2.5 mg/kg every 12 h.

Female CD1 mice given 20 mg/kg of meloxicam SC developed gastrointestinal toxicity as evidenced by fecal occult blood and histologic evidence of gastritis; however, the mice remained clinically normal.[8] In a previous study, 20 mg/kg SC given every 24 h for 6 d resulted in gastritis in 1 of 4 C57BL/6 mice and dermatitis in 4 of 4 mice.[23] Based on these findings, our pharmacokinetic profiles, and previous studies,[21,23,27] the initial 4 mice in our C1-SX-LOAD group received a 20-mg/kg meloxicam loading dose immediately before surgery, followed by 10 mg/kg every 12 h. However, one of these mice was found dead prior to the 48 h endpoint, and another was euthanized at 48 h. Both mice had gross evidence of gastrointestinal pathology and their behavior indicated greater pain. However, their wound licking durations were shorter than those of the other surgery mice, which is perhaps due to decreased activity caused by pain. The behaviors of the mice in the first cohort of SX-10MEL which did not show clinical morbidity, were similarly indicative of pain. Daily meloxicam could be toxic at an overall daily dose of 30 mg/kg. In addition, surgery stress could exacerbate the toxic gastrointestinal effects. As in a previous study,[23] we also saw evidence of dermatitis at the injection site in mice that received a higher concentration of meloxicam. Although dermatitis was seen in mice that received low-dose meloxicam diluted to 0.5 mg/mL, similar lesions were seen in mice that received saline even though pathology was not apparent clinically.[2] The dermatitis associated with the higher concentration of meloxicam should be considered when administering it subcutaneously in mice, as dermatitis and its side effects could affect some models.

Understanding the analgesic effectiveness of high-dose meloxicam requires assessment of the behavioral effects of therapy in conjunction with expected drug plasma concentrations, effects in anesthesia-only mice that do not develop pain, and histopathologic changes. The mouse grimace scale is a common criterion used to assess mouse pain.[13,16] We simplified its application to our study by focusing on orbital tightening which is readily visualized and easy to perform; higher scores indicate more pain.[10,11,24] Hunched posture is also a common indicator of mouse pain.[14] Orbital tightening scores and hunched postures incidence in SX-10MEL and SX-2.5MEL mice were highest 6 to 12 h after surgery, similar to previous reports.[20,22] The mean orbital tightening scores and hunched posture incidences were consistently, but not significantly lower in SX-10MEL mice as compared with SX-2.5MEL mice. SX-10MEL mice also showed numerically, but not statistically, more frequent grooming than did SX-2.5MEL mice. Collectively, these results demonstrate that high-dose meloxicam given at 10 mg/kg every 12 h provides better pain management than does a low-dose at 2.5 mg/kg given every 12 h.

Our study used multiple additional behavioral assessments to evaluate analgesic efficacy in mice.[1,9,26] Distance traveled and active time were measured using ANY-maze, which provides indices of pain (greater postoperative distance traveled and longer durations of mobility correlate with greater analgesia).[5,11,12] We used ANY-maze to measure distance traveled and duration of activity. Greater pain would result in less distance traveled and a shorter duration of activity. These behavioral variables were valuable additions to the other observational metrics of pain; rearing frequency, wound licking duration, and grooming are susceptible to human error, whereas ANY-maze provides more objective metrics. Wound licking is an indicator of postsurgical pain in mice.[10,11] Although we initially recorded the incidence of wound licking during our experiments, substantial individual variation was noted in the durations of wound licking. Therefore, we calculated wound licking duration by using the recorded ANY-maze videos to assess this measure more accurately. Greater pain is expected to reduce rearing and grooming and to promote longer wound licking durations. Comparisons of SX-10MEL and SX-2.5MEL mice showed infrequent and inconsistent statistical differences in rearing, wound licking duration, grooming, distance traveled, and active time. Overall, these metrics do not support differences in analgesia between mice that received high or low-dose meloxicam in our study.

We evaluated potential behavioral effects of meloxicam by using the A-SAL group as a control for comparison to A-10MEL and A-2.5MEL mice. Differences in behavioral scores occurred inconsistently across all timepoints when comparing A-SAL with A-10MEL and A-2.5MEL mice (Table 3). Thus, meloxicam alone did not appear to have consistent effects on mouse behavior. The SX-SAL treatment group was also used to demonstrate the degree and duration of pain and to validate the model. Behavioral differences indicating pain occurred often during the initial 12 h after surgery in the SX-SAL group, demonstrating that laparotomy pain lasts for about 12 h after surgery, as reported in previous studies.[11,16]

Frequent statistical differences between mice that had and did not have surgery indicated incomplete pain management. Mice that experienced surgery were consistently and significantly less active than anesthesia-only mice for the first 6 h after surgery, and were significantly less active than A-10MEL and A-2.5MEL mice at 12 h after surgery. Although only the SX-2.5MEL group was statistically different from A-SAL at 12 h, all mice that had surgery were consistently less mobile on average numerically throughout the most painful period. Frequently, mice that had surgery also significantly reared less, traveled less, and tolerated less abdominal pressure via von Frey as compared with anesthesia-only mice.

Based on the observations of both observers and the institutional veterinary staff, the SX-SAL mice did not display painful behaviors warranting rescue analgesia, as shown in Tables 2 and 3. The C1-SX-LOAD mouse that had clinical signs of pain was euthanized, as recommended by an institutional veterinarian. SX-SAL mice showed changes in orbital tightening and arched posture, but not in wound licking, distance traveled, or active time. These changes varied substantially in magnitude and were not uniform across behaviors. Including a SX-SAL group allowed better assessment of the efficacy of meloxicam for postoperative pain.

Assuming a meloxicam therapeutic level of 390 ng/mL in female CD1 mice, our pharmacokinetic data indicate that therapeutic plasma concentrations are present for 12 h. However, the present study does not correlate with the pharmacokinetics. This divergence suggests that the true meloxicam minimum therapeutic plasma concentration for mice is likely higher than 390 ng/mL. Although HDM provides better analgesia than does LDM, as evidenced by less orbital tightening and hunched posture behaviors in HDM mice, other more objective measurements of pain indicate inadequate analgesia overall. This contrasts with a previous study which suggested that lower doses and less frequent administration provides sufficient postsurgical analgesia.[25] More frequent administration and/or higher doses may further improve pain management.

This study showed that a 20-mg/kg dose has no additional benefit as compared with a 10-mg/kg dose when administered subcutaneously to female CD1 mice; both doses exceed the therapeutic concentration similarly for 12 h, and the 20-mg/kg

loading dose was toxic in at least 2 mice. Giving the 10-mg/kg meloxicam dose every 12 h provides greater analgesia than does 2.5 mg/kg given every 12 h, as demonstrated by orbital tightening and hunched posture, with no toxicities noted. However, 10 mg/kg every 12 h may not provide complete postoperative analgesia based on the other parameters evaluated. The toxicity observed with a daily dose of 30 mg/kg suggests a narrow therapeutic window between analgesia and toxicity. While 10 mg/kg meloxicam every 12 h provides more analgesic benefit than 2.5 mg/kg every 12 h, dosing protocols must continue to be evaluated for clinical efficacy, including an evaluation in other strains and male mice.

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