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Inappropriate Post-Operative Analgesia is Achieved Using Recommended Doses of Sustained-Release Meloxicam in Mice

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Abstract

Meloxicam is an analgesic NSAID commonly used in mice. Anecdotal evidence supports the use of alternative delivery methods for analgesics including injectable sustained release (SR) formulations as ways to achieve long-term analgesia with less frequent handling; potentially minimizing pain and distress. However, objective data regarding efficacy of these alternative approaches is lacking in the literature. We conducted a pilot study evaluating the efficacy and blood levels of Meloxicam-SR administration in a surgical model (osmotic pump placement), using the manufacturer's recommended dosing of 4 mg/kg per 72 h. Mice exhibited signs of pain and plasma drug levels were undetectable 4 h after dosing. Meloxicam-SR failed to deliver adequate pain control at the currently recommended dosing; suggesting further studies are needed to determine effective dosing for mice.

Keywords: Mouse Grimace Scale; Liquid chromatography; Mass spectrometry; Non-steroidal anti-inflammatory; Specific pathogen free

Introduction

The reduction or elimination of pain and distress experienced by animals on research protocols is a top priority for biomedical researchers [1], lab animal care personnel, veterinarians and IACUCs in support of federal mandates for the humane care and use of laboratory animals (AWA/R [1], PHS Policy [2,3], The Guide [4]. In many instances, refinement is imaginable; however, frequently there is a void of scientific evidence to ensure that the addition of analgesic compounds offers necessary pain control. Without such assurance, the use of analgesia may be challenged by the investigator or by peer-review mechanisms.

In addition to being effective, analgesia in rodents must also be practical. Administration of pain relieving medication must be balanced with minimizing animal stress due to repeated handling and the potential pain or discomfort associated with repeated injections. For these reasons, long-acting, single dose compounds and the use of medicated feed and water supplements, have been proposed to offer the benefits of analgesics without the stress of handling [2,5]. These methods are proposed to be convenient, cost-effective and less stressful than repeated injectable doses. However, there is very little evidence of their therapeutic effects.

Non-steroidal anti-inflammatory (NSAID) compounds are commonly used in the lab animal environment to provide analgesia for mild painful stimuli such as minor surgery. NSAIDs carry several advantages over controlled substances as they are readily available in multiple formulations, easily stored and do not require registration with the DEA. Meloxicam, a COX-2 selective NSAID of the acidic enolcarboxamide class [6], is gaining popularity in lab animal medicine due to fewer side effects [7-10] and once per day dosing in target species, dogs and cats (Metacam package insert, Boehringer Ingelheim Vetmedica, Inc.). However, reported pharmacokinetics vary greatly across species resulting in substantial differences in recommend dose and frequency [3,11-13]. In particular, PK data in mice indicates a much shorter half-life (approximately 5 h) at higher doses (10 mg/kg) than those recommended for dogs and cats (0.2 mg/kg and 0.3 mg/kg respectively) [6,14] as described in the Metacam insert. Therefore, several authors suggest that mice likely require higher doses of meloxicam more frequently than current practice [14-17].

Sustained-release formulation of meloxicam (Meloxicam-SR, ZooPharm, CO) may be a better option for providing prolonged, single-dose analgesia in mice. The manufacturer recommends 4 mg/kg SQ every 72 h for mice (ZooPharm document). Studies of Mel-SR PK confirmed detectable levels (>1 ng/ml) at 24 h following dose of 6 mg/kg SQ [14], suggesting Mel-SR may provide long-acting analgesia in mice however, further investigation is required to determine true duration of action as well as extent of analgesia provided.

Our study aimed to compare Meloxicam-SR levels before and after a mild surgical stimulus by objectively quantifying physiologic and behavioral responses as well as plasma drug levels. We hoped to duplicate the dosing regimens that most investigators would attempt. Given previous reports, we hypothesized that Meloxicam SR would provide consistent and effective postoperative analgesia. We found, however, that at the manufacturer's recommended dose, it failed to reach reported therapeutic plasma levels or provide appropriate postoperative pain control.

Materials and Methods

Ethical statement

All animals were housed and monitored under standard husbandry conditions and with veterinary supervision at the University of Arizona's conventional vivarium. The animals were obtained and the

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work was performed following review and approval by the University of Arizona's Institutional Animal Care and Use Committee.

Experimental animals

21 male Swiss Webster mice were obtained from the University of Arizona's sentinel breeding colony in Tucson, AZ. All mice were barrier housed and specific pathogen free. Routine quality control monitoring for mice in the original colony is aimed at exclusion of M. pulmonis, MPV, MHV, MVM, MNV, EDIM, TMEV, Sendai, PVM, Reo-3, LCM, Ectromelia, Polyoma, MAD-1 and 2, Helicobacter, ecto- and endoparasites and pathogenic bacteria. The mice were transferred from the barrier facility in Tucson, AZ to a conventional facility in Phoenix, AZ and allowed to acclimate to the facility for 7 to 14 days prior to their participation in the study. Mice were housed 3 to a group in disposable cages on Innovive IVCs racks with ad-lib access to water and standard rodent chow. Facility staff performed cage changes at two week intervals per IACUC-approved exception for IVC housed mice. Mice were maintained on a 14:10 light: dark cycle, at a temperature of 69°F-72°F and humidity of 40%-60%.

Study design

Mice were randomly assigned to one of 7 cages, with three mice per cage, on arrival. Baseline measures for weight, behavioral scores and grimace scale scores were obtained following acclimation. Cage 1 was designated for observations only and these mice did not undergo blood collection procedures following Meloxicam SR administration. Each of the other cages was then randomly assigned a particular time-point for blood collection, so that all of the animals in the same Cage 3 were bled at the same time following Meloxicam administration, before and after the surgical stimulus (Meloxicam SR groups: Cage 2, 4 h; Cage 3, 12 h; Cage 4, 48 h; Cage 5, 24 h; Standard Meloxicam groups: Cage 6, 2 h; Cage 7, 4 h). Following baseline measurements and observations, mice were administered a standard dose of Meloxicam SR (Cages 1-5). Meloxicam SR groups were dosed with Meloxicam starting three days prior to surgery (Day-3; baseline) and then euthanized by isoflurane overdose three days following surgery (Day 3).

Meloxicam dosing

Meloxicam SR-2 mg/mL-was purchased from Zoopharm (Windsor, CO) and administered to 15 mice (cages 1-5) according to the manufacturers recommendations. The dose of meloxicam SR administered per mouse was calculated using average adult mouse weight estimates (30 gm) and 4 mg/kg/72 h. Therefore, each mouse received 0.12 mg (0.06 mL) at each dosing. We chose this method of dosing because it most likely mimics what an average investigator is giving in the field. Based on the actual body weights measured, doses ranged from 3.2 to 6 mg/kg. Meloxicam SR was administered three days prior to surgery and again pre-operatively, 72 h later. Meloxicam SR injections were given in the subcutaneous area of the inguinal region to avoid interference with the osmotic pump, which was surgically implanted in the interscapular area. The first injection was given in the right inguinal region and the second injection was given in the left inguinal region. Both sites were monitored for injection site reactions throughout the study.

Six additional mice (cages 6, 7) received standard injectable Meloxicam and had blood sampled at 2 h and 4 h post-treatment without undergoing surgery, as a positive control group. Standard Meloxicam (Metacam-5 mg/mL-manufactured by Boehringer Ingelheim Vetmedica, Inc.) was administered subcutaneously at the recommended dosage (5 mg/kg) based on a 30 g weight. Mice in these control groups received 0.15 mg (0.03 mL) of Meloxicam once. To ensure administration of the appropriate dose, Meloxicam was diluted in sterile water 1:10 prior to administration, adjusting the injected volume to 0.3 mL.

Surgical stimulus

To mimic a minor surgical procedure, osmotic pumps filled with sterile saline were placed in each animal as our pain stimulus (Day 0). Mice were anesthetized with isoflurane and placed on a nose cone during the procedure. Mice were placed in sternal recumbence and the interscapular region was clipped and aseptically scrubbed using alternating Betadine/alcohol solutions three times. 2% lidocaine was diluted with sterile saline and injected at the incision site prior to an incision being made. Once a steady plane of anaesthesia was reached, (verified *via* toe pinch) a small 1 cm incision was made between the scapulae, sterile hemostats were then used to tunnel caudally in the subcutaneous tissue another 1.5 cm until the pump could be placed easily under the skin. The osmotic pumps (from ALZET; Cupertino, CA) were placed subcutaneously in the interscapular region. The incision was closed using 3-0 monofilament non-absorbable sutures in an intradermal/subcuticular pattern. Animals were placed back in their cage on a heating pad and monitored closely until fully recovered.

Behavioral observations

Mice were observed each morning between 9:00-10:00 am, prior to being weighed, receiving treatment or having blood collected. Multiple parameters were measured to assess the efficacy of Meloxicam SR. Observation scores including components described in the mouse grimace scale, nest complexity, activity level, hair coat quality, body condition and a subjective illness score were recorded for each mouse/cage. The observer (a female, post-graduate student) was blinded to all treatment groups, but not the surgical procedure. Baseline scores for all parameters were recorded three days prior to surgery (Baseline), following Meloxicam treatment, prior to surgery (Day-2, 1), on the day of surgery (Day 0-pre-op), on the day of surgery after recovery (Day 0-post-op) and for three additional days after surgery (Days 1, 2, 3).

The mouse grimace score is based on a developed standardized facial coding system that includes evaluating orbital tightening, nose bulge, cheek bulge, ear position and whisker changes [18]. Animals are given a score of 0 to 2, with 0 denoting changes not present and 2 being the most severe. Nest complexity was evaluated on a scale of 0 to 5 (0 indicating no nest built and 5 indicating a complex nest) [19]. Nestlets were added to each cage at the beginning of the study (Day 3) and again when placed in a new cage following surgery. Hair coat was evaluated subjectively using a 1 to 3 scale with 1 indicating a ruffled/unkempt hair coat and 3 indicating a well-groomed animal. Body condition score was evaluated on a 1 to 5 scale with 1 being emaciated and 5 being obese. A subjective illness score was also given to represent a subjective measure similar to what an animal care technician may observe or record during routine husbandry checks, using a scale of 0 to 4, with 0 being non-painful and fully active and 4 being severe pain or a moribund condition. Mice deemed to score a 3 or higher on two consecutive observations would be removed from the study.

Plasma drug levels

Blood collection: Blood was collected from mice in treatment groups (n=3 per group per time point) at 4 h, 12 h, 24 h and 48 h following Meloxicam SR administration before and after surgery. Mice in the control groups (n=3 per group per time point) had blood sampled at 2 h and 4 h following standard Meloxicam injections. Mice were

manually restrained and blood was collected from the submandibular vein into a lithium-heparinized tube. At the end of the study all animals were euthanized by isofluorane overdose followed by exsanguination *via* cardiocentesis. Cervical dislocation was subsequently performed to confirm death by a secondary method. Blood samples were centrifuged at 10,000 xg for 5 min and plasma was collected into micro-centrifuge tubes and stored at -8°C until analysed.

Sample preparation: Proteins and lipids were removed from plasma samples with Captiva ND Lipid filter plates (Agilent) using a modified protocol provided by the supplier. Briefly, a 96-well collection plate, Captiva collar and Captiva ND Lipids 96-well plate were stacked. Methanol (0.35 mL) spiked with 0.001 mg/ml meloxicam-d3 (Sigma Aldrich) was applied to each well followed by 0.1 mL blood and mixed *via* aspiration five times. The samples were subsequently filtered under vacuum and collected in the 96-well collection plate.

LC/MS analysis: The 96-well plate containing the spiked plasma samples was placed in the autosampler rack of an Agilent 1260 HPLC. HPLC separations of 0.005 mL injections of the spiked plasma were performed on a 2.1 mm × 50 mm Zorbex EXTEND-C18 column at a flow rate of 0.5 mL/min. The mobile phase consisted of eluent A (water with 0.1% formic acid) and eluent B (methanol). An isocratic elution of 50:50 (A:B) was started for 2 min and then a linear gradient was applied from 50:50 to 0:100 over 2.5 min, followed by an isocratic wash of 0:100 for 2.5 min before a re-equilibration of 50:50 for 3 min. Typically, a back pressure of ~300 bar was observed at 50:50 (A:B). During separation, the UV absorbance of the eluate was monitored by DAD and the accurate mass measured on an Agilent Accurate-Mass 6530 Q-TOF. All samples were analysed using MassHunter Software (Agilent). A standard curve of known meloxicam quantities was used to determine plasma concentrations.

Sample size and statistical analysis

5 cages with 3 mice per cage are represented in the study data, unless otherwise noted. Body weight, plasma drug levels and individual behaviour observations including orbital tightening, nose bulge, cheek bulge, ear position, whisker changes, activity level, subjective illness score and hair coat quality were recorded daily and compared between cages and between time-points in mice that received Meloxicam SR (n=15). Nest quality, food consumption and water consumption were measured daily per cage (n=5). Control mice (2 cages, 3 mice per cage) that received standard meloxicam injections were only used for plasma drug level measurements. The experiment was planned so that only 1 cage of mice had surgery on any given day and Meloxicam dosing was scheduled accordingly. 2 Way ANOVA testing was performed to evaluate differences between treatment groups (cages) and different time-points (baseline, Day-2, Day-1, pre-op, post-op, Day 1, Day 2, Day 3). When statistically significant differences were noted, post-hoc analyses using Tukey's multiple comparisons tests were used to determine differences between each group or time-point for each measured outcome. Analysis was performed on GraphPad Prism 7.00. The majority of plasma samples obtained for drug level tests had undetectable levels of Meloxicam precluding statistical analysis. When measurable levels were detected results are represented for individual mouse samples.

Results

Study populations

21 male Swiss Webster mice completed the study in good health conditions. There were no animals lost to fight wounds, illness, or other clinical changes. Due to randomization, differences in mean body weights were observed between cages at the onset of this experiment. Figure 1A depicts body weights for each mouse in each cage at baseline (Day 3). All of the mice gained weight and remained active throughout the course of the study. On average, body weights were significantly increased on Day 1, Day 2 and Day 3 after surgery when compared to baseline (Figure 1B). There was a significant difference in the amount of weight gained between cage 4 (which had blood collection times at 48 h post-meloxicam dosing) and cage 5 (which had blood collection times at 24 h post-meloxicam dosing). Injection site reactions were noted in three of fifteen mice that received Meloxicam-SR and these animals were the group sampled at 48 h prior to surgery (cage 4). Injection site reactions were observed prior to surgery in the right inguinal region where the first dose of Meloxicam-SR had been given 3 days prior. At the injection site a firm red 0.5 cm mass was noted on all three animals. Mice did not appear painful when masses were palpated and no exudate was noted. Just in case this injection site reaction was due to bacterial contamination we discarded the opened bottle of Meloxicam-SR and used a new bottle for the rest of the study, no other injection site reactions were seen. The lesions resolved spontaneously before the end of study. Whether the difference in weight gain was attributable to associated systemic effects of this injection site reaction is unknown. Importantly, differences in weight gain between cage 4 or cage 5 and cages 1, 2 and 3 did not reach statistical significance (Figure 1C). On average, all mice experienced increases in food and water consumption over time (data not shown). These changes correspond with normal growth and weight gain.

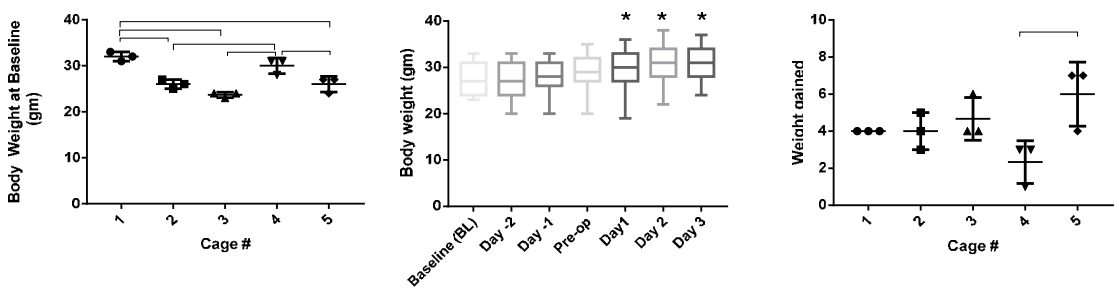

Figure 1: (A) The body weight of each mouse at baseline is depicted. Brackets show statistically significant differences in the average body weight of mice in each cage. (B) Mean and standard deviation of body weights for all mice in the study are depicted using box and whisker plots. A statistically significant change in body weight from baseline was observed for all post-operative time points. (C) The amount of weight gained (g) by each mouse is depicted by cage number. Animals in cage number 4 exhibited statistically lower weight gains than those in cage number 5.

Behavioral observations

There were significant differences in behavioral pain parameters measured for all study groups when preoperative and postoperative scores were compared, demonstrating that the placement of an osmotic pump was enough to elicit a significant pain response in mice. Mouse grimace score criteria were evaluated during visual monitoring through the cage wall by a single observer once daily. Mice received a score from 0 to 2 for each parameter and scores for each parameter were added together for each mouse so that ten (10) would be the maximum composite mouse grimace score possibly obtained, indicating the most severe pain. Mouse grimace scores increased significantly in all parameters tested individually and in the composite score postoperatively when compared to baseline preoperative values ($p<0.05$). In addition, ear position scores and the composite mouse grimace score were significantly higher than baseline on the day following surgery (Day 1) potentially indicating unalleviated pain for up to 24 h following Meloxicam SR dosing (Figure 2). Activity levels were scored from 0 to 3 with 3 being the most active (normal) value. Activity scores were significantly decreased in mice after surgery when compared to baseline preoperative values. Activity levels are normalized by day 1 post-surgery (Figure 3A). Subjective illness scores were assigned using a scale of 0 to 4, with 0 being non-painful and fully active and 4 being severe pain or a moribund condition. Subjective illness scores were significantly increased in mice receiving Meloxicam SR postoperatively and at Day 1 when compared to baseline preoperative values; and they returned to baseline by day 2 after surgery (Figure 3B). Hair coat was evaluated subjectively using a 1 to 3 scale with 1 indicating a ruffled/unkempt hair coat and 3 indicating a well-groomed animal. Hair coat quality scores were significantly decreased in mice receiving Meloxicam SR postoperatively and at Day 1 when compared to baseline preoperative value (Figure 3C). Nest complexity scores did not differ significantly between time-points when compared to baseline preoperative values (Figure 3D). While the changes observed did not indicate a large degree of compromise, the results suggest that Meloxicam SR may not sufficiently control pain in mice.

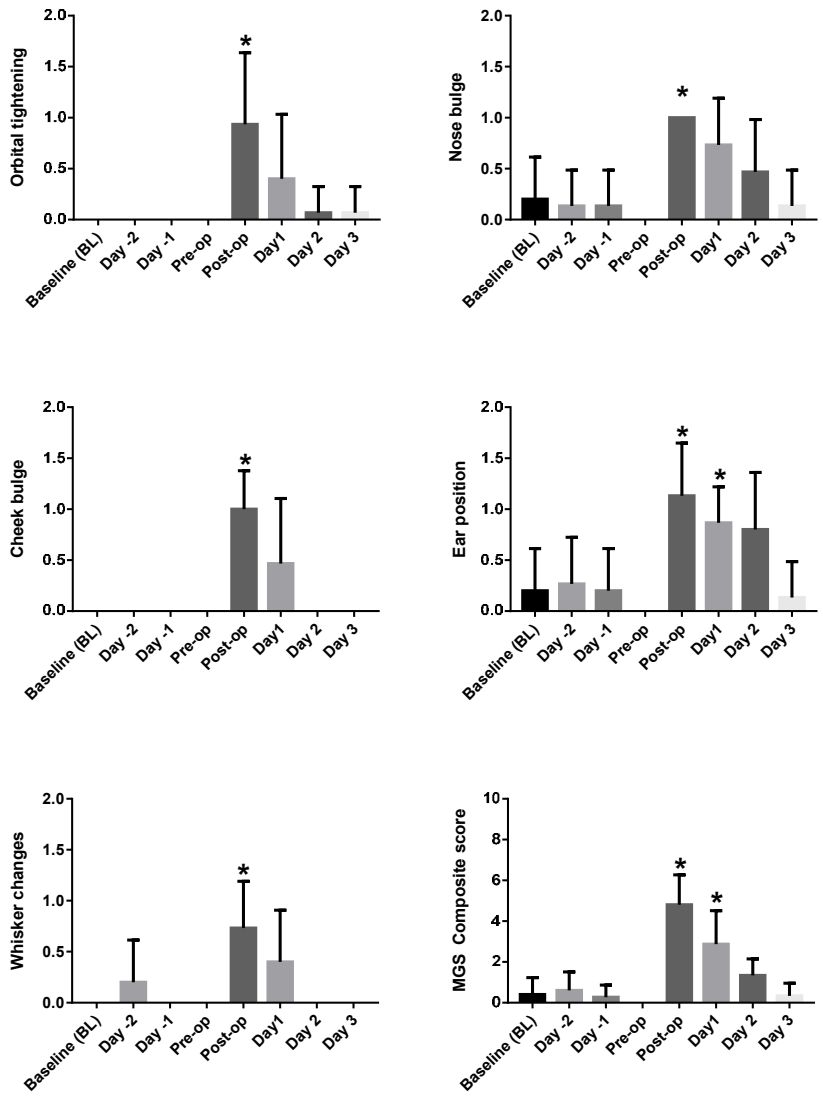

Figure 2: Mouse grimace score parameters were assessed by one female observer for all mice before (baseline) and after administration of Meloxicam. Three days later, mice received another dose of meloxicam, underwent a minor surgical procedure and behavioral parameters were scored for three more days. Bars and brackets depict mean index score and standard deviation (n=15). Asterisks indicate statistically significant changes in scores, from baseline, for each day of observation.

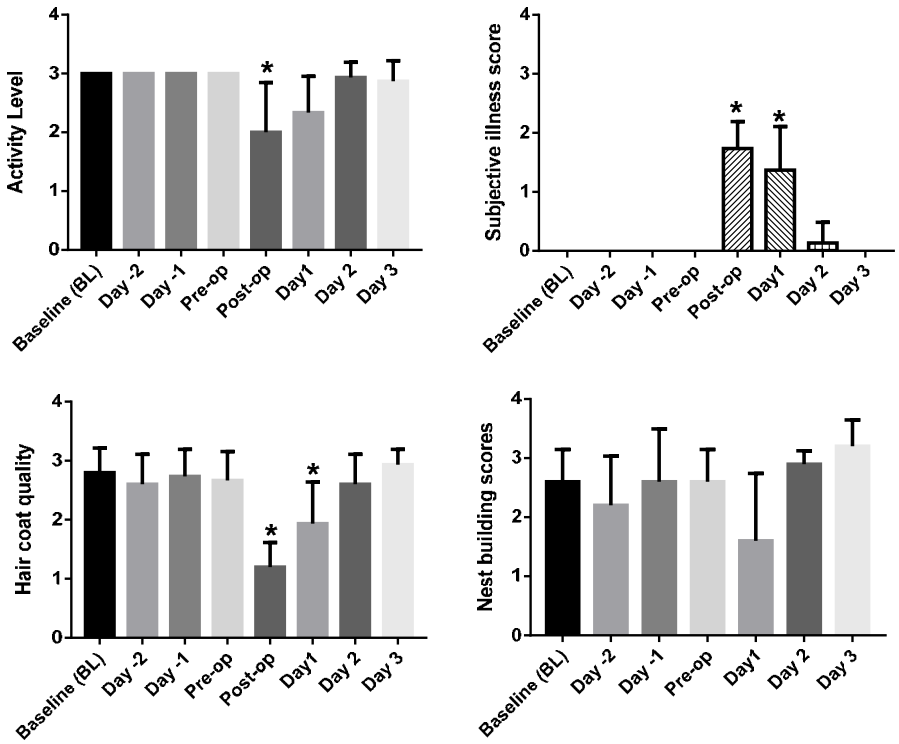

Figure 3: Activity level, a subjective illness score, and hair-coat quality were scored for each mouse (n=15), and the quality of the nest in each cage (n=5) was evaluated before (baseline) and after administration of Meloxicam. Three days later, mice received another dose of meloxicam, underwent a minor surgical procedure and behavioural parameters were scored for three more days. Bars and brackets depict mean index score and standard deviation. Asterisks indicate statistically significant changes in scores, from baseline, for each day of observation.

Plasma drug levels

Meloxicam SR was given three days prior to surgery and again pre-operatively. Plasma was collected following the initial treatment (3 days prior to surgery) at time-points 4 h, 12 h, 24 h and 48 h and again postoperatively at time-points 4 hr, 12 hr, 24 hr and 48 h. Six additional mice received standard injectable Meloxicam and were sampled at 2 and 4 h post-treatment without undergoing surgery, as a positive control group. Plasma concentrations in the control mice receiving standard injectable Meloxicam reached an average of 2519 and 540 ng/mL at 2 and 4 h respectively, which is consistent with previous and expected therapeutic concentrations. In animals that received injectable Meloxicam SR formulation prior to surgery Meloxicam was only detectable at 4 h after administration (925 ng/mL on average). Plasma levels of Meloxicam were undetectable in all other samples tested. Figure 2 depicts plasma concentrations measured in individual mice in the groups with detectable meloxicam levels. The lower limit of detection for the assay was 1 ng/mL. Meloxicam SR delivered inconsistent results (was not measurable in mice undergoing surgery) and exhibited shorter duration of action than expected in this study (Figure 4). Differences in dosing based on body weight, do not account for immeasurable levels.

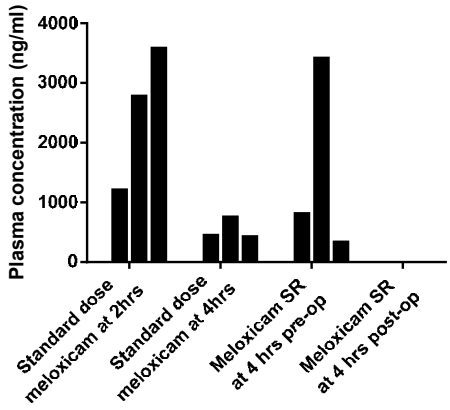

Figure 4: Plasma concentrations of meloxicam measured from individual mice at specific time points. Each bar represents the concentration of meloxicam detected in the plasma sample of one mouse, measured by LC/MS. Plasma samples obtained at 2 h and 4 h after administration of standard meloxicam SQ (positive controls) are compared with plasma samples obtained at 4 h after administration of sustained release meloxicam SQ, before surgery (Day 3) and after surgery (Day 0). Samples measured at all other time points contained undetectable meloxicam levels.

Discussion

The present study was conducted to determine if Meloxicam SR would offer adequate pain control following a minor surgical stimulus at the dose recommended by the manufacturer. Laboratory animal veterinarians and researchers are constantly trying to improve animal welfare and continue to search for better ways to offer pain management in experimental models. The NSAID meloxicam has been shown to be effective in treating mild to moderate pain postoperatively in rodents [17,20]. In this study, however, plasma drug concentrations of Meloxicam SR were inadequate.

Meloxicam SR was dosed according to the manufacturers' recommendations (4 mg/kg) and because, previous studies have shown

that meloxicam-SR demonstrated prolonged plasma levels up to 72 h following administration, we hypothesized that Meloxicam-SR would have similar results in our study. In this study, however, Meloxicam-SR had an average plasma level of 925 ng/mL when measured 4 h following administration of a 4 mg/kg dose in the non-surgical control group, compared to the reported >3000 ng/mL at 4 h which followed administration of a 6 mg/kg dose in a previous study [14]. In another study, rats treated with Meloxicam-SR at a dosage of 4 mg/kg showed the highest plasma concentrations of 18,500 ng/mL at day 1 following administration and detectable plasma levels up to day 4 [21]. Importantly, this and other studies demonstrate that pharmacokinetics of meloxicam vary significantly between mice and other rodents (rats) [6,16,22]. The differences in plasma concentrations between the current study and other studies may be due to such species differences and/or strain differences, or sex differences as previously reported, but our results suggest inconsistent plasma drug levels and a very short duration of action for meloxicam SR in mice.

Another factor we considered was our injection site location; we choose the inguinal region to avoid interference with the placement of the mini osmotic pump. To the best of the authors' knowledge no studies have been conducted evaluating differences in absorption for subcutaneous injection between these locations for sustained release formulations. Additionally, inconsistencies between formulated batches of the drug must be considered when using compounded formulations with less rigorous quality controls. Our ability to detect the drug in a subset of mice prior to surgery and in mice dosed with the standard formulation makes technical measurement errors less likely, however, technical dosing errors cannot be completely ruled out. Interestingly, the group of mice that achieved therapeutic plasma levels of meloxicam SR was also observed to have significantly decreased food consumption levels. Further studies are needed to determine the significance of this finding.

No study, to the best of our knowledge, has evaluated meloxicam-SR in the presence of a minor surgical stimulus and compared behavioural parameters for mice. Other studies have evaluated meloxicam-SR and nociceptive pain based on thermal and mechanical hypersensitivity and found that meloxicam-SR attenuated mechanical but not thermal hypersensitivity in rats [21]. Evaluation of behavioural parameters following meloxicam-SR dosing in our study revealed that mice demonstrated signs of pain following surgical intervention when compared to baseline, preoperative values.

Behavioral and physiological parameters used to evaluate pain included mouse facial grimacing, subjective pain assessment, activity level, hair coat quality, nest building complexity, body condition score, body weight and food and gel consumption. We choose these parameters based on a combination of previously reported ethograms used to assess analgesia wefficacy [2,10,23-25]. We evaluated all groups of mice each day between 900-1000 for about 10 min per cage. While this assessment is practical, as animal technicians are doing their routine health checks around this time each morning, it may have an impact on the observers' evaluation of behavioral and physiological pain, since rodents are more active at night. Only one female observer evaluated all mouse groups throughout the study. The results, therefore, are based on her subjective interpretation of behavioral and physiological pain. While parameters such as the mouse grimace score and nest building complexity are relatively more objective, there is still a level of subjective interpretation involved. However, when compared to thermal and mechanical hypersensitivity assays, it has previously been reported that spontaneous pain is a much better predictor of overall pain in animals.

Conclusion

Our data suggest that meloxicam-SR failed to provide appropriate or long-acting postoperative analgesia at the manufacturer's recommended dosage based on assessed behavioral parameters and plasma concentrations. The therapeutic concentration of meloxicam in mice, however, remains unknown. There is a broad range of dosages reported by different institute formularies ranging from 0.3 mg/kg to 10 mg/kg, as well as reports in the literature of dosing for meloxicam in mice as high as 20 mg/kg. Based on the extensive range of doses that have been reported and are currently being used and on the results of the current study, higher dosages than dose recommended by the manufacturer may be necessary when considering the use of meloxicam as a postoperative analgesic following a minor surgical procedure. Further studies are warranted to evaluate these formulations at higher dosages in order to assess efficacy and side effects in mice. Based on the results of this study a mini osmotic pump placement surgery elicits a pain response in mice and merits appropriate postoperative pain control that extends beyond the use of local anesthetics.

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References

1. Carbone L (2012) Pain management standards in the eighth edition of the guide for the care and use of laboratory animals. J Am Assoc Lab Anim 51: 322-328.
2. Adamson TW, Kendall LV, Goss S, Grayson K, Touma C, et al. (2010) Assessment of carprofen and buprenorphine on recovery of mice after surgical removal of the mammary fat pad. Journal of the American Association for Laboratory Animal Science 49: 610-616.
3. Bauer C, Frost P, Kirschner S (2014) Pharmacokinetics of 3 formulations of meloxicam in cynomolgus macaques (*Macaca fascicularis*). J Am Assoc Lab Anim Sci 53: 502-511.
4. Institute for Laboratory Animal Research (2011) Division on Earth and Life Studies; The National Academies. Guide for the Care and Use of Laboratory Animals.
5. USDA APHIS (2017) Animal Welfare Act and Animal Welfare Regulations.
6. Busch U, Schmid J, Heinzel G, Schmaus H, Baierl J, et al. (1998) Pharmacokinetics of meloxicam in animals and the relevance to humans. Drug Metab Dispos 26: 576-584.
7. Engelhardt G (1996) Pharmacology of meloxicam, a new non-steroidal anti-inflammatory drug with an improved safety profile through preferential inhibition of COX-2. Br J Rheumatol 1: 4-12.
8. Ratsep MT, Barrette VF, Winterborn A, Adams MA, Croy BA (2013) Hemodynamic and behavioral differences after administration of meloxicam, buprenorphine, or tramadol as analgesics for telemeter implantation in mice. J Am Assoc Lab Anim Sci 52: 560-566.
9. Tachibana M, Inoue N, Yoshida E, Matsui M, Ukai Y, et al. (2003) Anti-inflammatory effect and low ulcerogenic activity of etodolac, a cyclooxygenase-2 selective non-steroidal anti-inflammatory drug, on adjuvant-induced arthritis in rats. Pharmacology 68: 96-104.
10. Tubbs JT, Kissling GE, Travlos GS, Goulding DR, Clark JA, et al. (2011) Effects of buprenorphine, meloxicam, and flunixin meglumine as postoperative analgesia in mice. J Am Assoc Lab Anim Sci 50: 185-191.
11. Toutain PL, Reymond N, Laroute V, Garcia P, Popot MA, et al. (2004) Pharmacokinetics of meloxicam in plasma and urine of horses. Am J Vet Res 65: 1542-1547.
12. Turner PV, Chen HC, Taylor WM (2006) Pharmacokinetics of meloxicam in rabbits after single and repeat oral dosing. Comp Med 56: 63-67.
13. Yuan Y, Chen XY, Li SM, Wei XY, Yao HM, et al. (2009) Pharmacokinetic studies of meloxicam following oral and transdermal administration in Beagle dogs. Acta Pharmacol Sin 30: 1060-1064.

14. Kendall LV, Hansen RJ, Dorsey K, Kang S, Lunghofer PJ, et al. (2014) Pharmacokinetics of sustained-release analgesics in mice. J Am Assoc Lab Anim Sci 53:478-484.

15. Haile M, Boutajangout A, Chung K, Chan J, Stolper T, et al. (2016) The Cox-2 inhibitor meloxicam ameliorates neuroinflammation and depressive behavior in adult mice after splenectomy. J Neurophysiol Neurol Disord 3.

16. Roughan JV, Bertrand HG, Isles HM (2016) Meloxicam prevents COX-2-mediated post-surgical inflammation but not pain following laparotomy in mice. Eur J Pain 20: 231-240.

17. Wright-Williams SL, Courade JP, Richardson CA, Roughan JV, Flecknell PA (2007) Effects of vasectomy surgery and meloxicam treatment on faecal corticosterone levels and behaviour in two strains of laboratory mouse. Pain 130: 108-118.

18. Mittal AM, Lamarre YY, Gupta K (2014) Observer based objective pain quantification in sickle Mice using Grimace scoring and body parameters. Blood 124.

19. Jirkof P, Fleischmann T, Cesarovic N, Rettich A, Vogel J, et al. (2013) Assessment of postsurgical distress and pain in laboratory mice by nest complexity scoring. Lab Anim 47: 153-161.

20. Nunamaker EA, Stolarik DF, Ma J, Wilsey AS, Jenkins GJ, et al. (2014) Clinical efficacy of sustained-release buprenorphine with meloxicam for postoperative analgesia in beagle dogs undergoing ovariohysterectomy. J Am Assoc Lab Anim Sci 53: 494-501.

21. Seymour TL, Adams SC, Felt SA, Jampachaisri K, Yeomans DC, et al. (2016) Postoperative analgesia due to sustained-release buprenorphine, sustained-release meloxicam, and carprofen gel in a model of incisional pain in rats (*Rattus norvegicus*). J Am Assoc Lab Anim Sci 55: 300-305.

22. Chen PH, Boyd KL, Fickle EK, Locuson CW (2016) Subcutaneous meloxicam suspension pharmacokinetics in mice and dose considerations for postoperative analgesia. J Vet Pharmacol Ther 39: 356-362.

23. Christy AC, Byrnes KR, Settle TL (2014) Evaluation of medicated gel as a supplement to providing acetaminophen in the drinking water of C57BL/6 mice after surgery. Journal of the American Association for Laboratory Animal Science 53: 180-184.

24. Molina-Cimadevila MJ, Segura S, Merino C, Ruiz-Reig N, Andres B, et al. (2014) Oral self-administration of buprenorphine in the diet for analgesia in mice. Laboratory Animals 48: 216-224.

25. Hill WA (2015) Public health service policy on humane care and use of laboratory animals.