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Authors

Fuetsch, Stephanie R
Stewart, Leslie A
Imai, Denise M
et al.

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Injection Reactions after Administration of Sustained-release Meloxicam to BALB/cJ, C57BL/6J, and Crl:CD1(ICR) Mice

Stephanie R Fuetsch,[1] Leslie A Stewart,[2] Denise M Imai,[3] Laurel A Beckett,[4] Yueju Li,[4] K C Kent Lloyd,[5] and Kristin N Grimsrud[2,6,*]

The sustained-release formulation of meloxicam (MSR) is a compounded NSAID that may provide pain relief for as long as 72 h after administration. MSR injection-site skin reactions have occurred in several species but have not previously been observed in mice. We investigated the development and progression of localized skin reactions after a single injection of MSR in Crl:CD1(ICR), C57BL/6J, and BALB/cJ mice. Each mouse received a subcutaneous injection of MSR (*n* = 60), standard-formulation meloxicam (MEL; *n* = 24) or saline (control; SC; *n* = 24) and was scored daily according to a 5-point system for erythema and mass characteristics. Mice were euthanized at either 7 or 14 d after injection and underwent postmortem analysis. MSR-treated mice had more erythematous and mass reactions than did MEL and SC mice. Mass lesions developed in 49 MSR mice (82%; 95% CI, 70% to 90%), 5 MEL animals (21%; 95% CI, 7% to 42%), and 1 SC mouse. MSR-treated BALB/cJ developed erythematous lesions less frequently than similarly treated Crl:CD1(ICR) or C57BL/6J. Lesions often were ventrolateral to the injection site. The median times to the appearance of mass and erythematous lesions were 2 d and 3 d, respectively. Histologically, the erythematous and mass reactions correlated with necrotizing to pyogranulomatous injection-site panniculitis. Inflammation severity scores at 7 and 14 d after injection were greater in the MSR-treated group than the other 2 groups. No strain- or sex-associated differences emerged except that inflammation severity scores at day 14 were higher in Crl:CD1(ICR) females than males. The character of the inflammatory response in MSR-treated mice did not differ between 7 and 14 d after injection, indicating that MSR-induced inflammation is slow to resolve. The ventral migration and delayed onset of MSR injection-site reactions could result in their being attributed to another cause or not being identified. Researchers and clinicians should be aware of the potential for slowly resolving injection-site reactions with MSR.

Abbreviation: MEL, standard-formulation meloxicam; MSR, sustained-release meloxicam; SC, saline control

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NSAID are commonly used for analgesia in laboratory animals.[4,10] Standard-formulation meloxicam (MEL) is an NSAID that can be provided to mice via several routes (for example, subcutaneous, oral) and is typically administered every 12 to 24 h,[10] but recent studies have shown that, due to rapid clearance, mice require more frequent dosing. A formulation that prolonged the dosing interval could be particularly beneficial.[10] According to the manufacturer, sustained-release meloxicam (MSR) is a compounded formulation that might provide as long as 72 h of analgesia after a single subcutaneous dose in rats and mice.[18] MSR maintains higher plasma drug concentrations for longer durations than MEL in mice, although not for a full 72 h; thus MSR may be preferable when analgesia is warranted for extended periods.[9] A sustained-release formulation would reduce the amount of handling stress, risk of postoperative injury, and labor needed in animals undergoing an analgesic regimen.[4,9,10]

Previously published reports focus on drug efficacy and have not assessed or characterized potential adverse reactions to MSR in rodents or other species, despite reactions after the administration of standard formations of meloxicam.[12,13] However, Hispaniolan parrots and cynomolgus macaques are reported to have adverse reactions at MSR injection sites.[1,7] A sustained-release formulation of the opioid buprenorphine has caused erythematous lesions in several species,[3] including mice[2] and rats.[6] Despite reports of injection-site reactions to sustained-release formulations, this possible complication is not mentioned in the data sheet or information sheet for MSR.[18] Elements of the proprietary vehicle, described by the compounder as a biodegradable liquid polymer matrix,[18] may stimulate the immune system or mechanically irritate or damage tissues. In addition, previous studies have been conducted within the prescribed 72-h drug efficacy range, such that potential injection-site reactions initially arising beyond 72 h may not be observed or linked to MSR injections.[1,2,7,9,13] Regardless of the exact etiology, adverse reactions pose a threat to the assurance of animal welfare and potentially could interfere with research outcomes, particularly when increased inflammation may alter study results.

We hypothesized that adverse injection-site reactions could be influenced by strain or sex and that these reactions could vary histologically in time, acutely or chronically. To test these hypotheses, we designed this study to characterize injection site reactions after treatment with MSR by assessing the presence, frequency, and severity of MSR injection-site reactions in males

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[1]School of Veterinary Medicine, [2]Mouse Biology Program, [3]Comparative Pathology Laboratory, [4]Public Health Sciences, and the Departments of [5]Surgery and [6]Pathology, University of California–Davis, Davis, California
[*]Corresponding author. Email: kngrimsrud@ucdavis.edu

and females of 3 commonly used inbred and outbred mouse (*Mus musculus*) strains (BALB/cJ, C57BL/6J, and Crl:CD1(ICR). These strains have unique genetic backgrounds, which could influence reaction propensity. The C57BL/6J strain was selected because it is a widely used mouse strain for models of disease and is known to be predisposed to skin sensitivities, such as developing ulcerative dermatitis.[4,8,14] Crl:CD1(ICR) are outbred animals and may have more robust or variable immune responses, owing to higher genetic diversity.

The goals of the current research were to determine the extent to which MSR leads to localized tissue reactions at injection sites, determine the characteristics and progression of any reactions, and assess whether strain-associated differences occur. These findings may aid veterinarians and researchers in drug selection.

Materials and Methods

Animals. All procedures were approved by the IACUC of the University of California, Davis, an AAALAC-accredited institution. Animal housing was in accordance with recommendations of the *Guide for the Care and Use of Laboratory Animals*.[5] Mice were SPF from the following pathogens: all ectoparasites and endoparasites, lymphocytic choriomeningitis virus, ectromelia, Theiler disease virus, rotavirus, mouse adenovirus of mice (types 1 and 2), minute virus of mice, mouse parvovirus, mouse hepatitis virus, pneumonia virus of mice, Sendai virus, reovirus 3, murine norovirus, *Bordetella bronchiseptica*, *Salmonella* spp., *Corynebacterium kutscheri, Pseudomonas* spp., *Citrobacter rodentium*, *Klebsiella* spp., *Mycoplasma arthritidis*, *Mycoplasma pulmonis, Pasteurella multocida*, *Pasteurella pneumotropica*, *Streptobacillus moniliformis, Streptococcus* spp., β-hemolytic *Streptocuccus* spp., and *Helicobacter* spp. Age- and sex-matched mice ($n = 108$; age, 9 wk) were procured for the study; Crl:CD1(ICR) mice ($n = 36$, 18 males and18 females) were obtained from the UC Davis barrier facility inhouse colony, and C57BL/6J ($n = 36$, 18 males and 18 females) and BALB/cJ ($n = 36$, 18 males and18 females) mice were obtained commercially from Jackson Laboratories (Sacramento, CA). Mice were cohoused in same-sex groups of 4 or 5 in IVC (Optimice IVC, Animal Care Systems, Centenniel, CO) with cotton squares (Ancare, Bellmore NY) and crinkle paper (Carefresh, HealthyPet, Ferndale WA) enrichment, a 12:12-h light:dark cycle (lights on, 0600), room temperature of 68 to 79 °F (20.0 to 26.1 °C), and standard rodent chow (Harlan 2918, Envigo, Indianapolis, IN). Two cages of male mice (BALB/cJ and C57BL/6J) were separated and singly housed after fighting was observed. Mice were acclimated for a minimum of 7 d before the start of the study, and all animals were 10 wk old when the experimental procedures were initiated.

Study design. Mice were randomly assigned to 3 treatment groups that received a single subcutaneous injection of MSR (Meloxicam SR, ZooPharm, Winsor, CO), MEL (OstiLox, VetOne, Boise, ID), or sterile 0.9% saline (controls [SC]). The number of mice that were treated in each strain was 20 MSR, 8 MEL, and 8 SC, with each group having equal numbers of males and females. Half of the mice from each treatment group were euthanized and analyzed by necropsy and histology at 7 d after injection and the other half at 14 d, except for male C57BL/6J that had deviations in their submissions (MEL, $n = 1$ at 7 d, $n = 3$ at 14 d; SC $n = 3$ at 7 d and $n = 1$ at 14 d).

Injections and dosing. All injections were administered according to manufacturer instructions. MSR and MEL were dosed at 4 mg/kg SC in the interscapular area;[14] SC mice received at an equivalent volume as the MSR dose volume. Injections were performed by a single person to minimize variation in injection technique. The vials of MSR, MEL, and SC used in the study were submitted to a pathology laboratory (Comparative Pathology Laboratory, University of California–Davis, Davis, CA) for sterility testing by aerobic culture on sheep blood agar culture plates.

Observational Assessments of Lesions. A single person who was blind to treatments performed all daily physical examinations, beginning 1 d after treatment. General health, injection site, and surrounding tissues were examined in every animal. Health assessment included mentation, general activity level, hydration (via skin tenting), coat quality, body condition score, presence and characteristics of any lesions, and any appreciable health concerns. Mice were gently restrained by the examiner, who held the base of the tail and palpated by gently running 2 fingers over the dorsal length of the body and extending down the limbs and lateral body wall. We developed and implemented 5-point scoring systems to assess the presence of erythema and mass at the injection site (Figure 1). The researcher's gloves, benchtop, wire cage-top, and pen were each disinfected by using 70% isopropyl alcohol between cages of animals.

Postmortem assessment of lesions. Mice were submitted for gross necropsy and histopathology (Comparative Pathology Laboratory, University of California–Davis). Mice were euthanized by CO_2 asphyxiation and cardiac exsanguination. The injection site and internal organs were evaluated for gross abnormalities. Representative tissue samples were collected and immersion-fixed in 10% neutral buffered formalin. Tissues were processed routinely for histopathology, embedded in paraffin, sectioned at 4 to 5 µm thickness, and stained with hematoxylin and eosin. Histopathology was evaluated by a board-certified comparative anatomic pathologist (DMI), who was blind to treatment group. Inflammation was scored according to severity (inflammation severity score): 0, no inflammation; 1, minimal inflammation; 2, mild inflammation; 3, moderate inflammation; and 4, marked inflammation. Stage of resolution was scored according to the character of the inflammatory response (resolution score): 0, no abnormality; 1, fibrous scar; 2, histiocytic, pyogranulomatous, or granulomatous inflammation; and 3, necrotizing inflammation (active tissue destruction and inflammation).

Statistical analysis of erythema and mass data. Erythema and mass observation data and assessments of associations with histology score were analyzed by using SAS (version 9.4, SAS Institute, Cary, NC). The proportion of mice in each injection treatment group that developed lesion signs at any point during follow-up was calculated, with 95% CI and, among animals with lesions, the proportion of animals in which lesion burden decreased. Proportions were compared across injection treatment groups by using the Fisher exact test. Lesion proportions were compared according to strain and sex by using the χ^2 test. Logistic regressions examined the simultaneous effects of these variables on the presence of lesions at any point during follow-up.

Time to first reported lesion was characterized by using a life-table approach, as illustrated by a Kaplan–Meier plot. Estimated median time to the first lesion and 95% confidence interval were calculated and compared between SC and active treatment (that is, MSR and MEL) by log-rank testing.

Detailed examination of the active-treatment group modeled the individual mice's trajectories on the ordinal scale. We fitted mixed models to allow for repeated measures. We considered generalized linear models with a logistic link and both linear and quadratic terms in time and generalized additive mixed models, to allow for more complicated patterns with time. The model with the best fit to observed data was illustrated graphically, with 95% CI.

Score	Erythema	Mass
0	No visible reaction	No palpable thickening or mass
1	Mild alopecia or erythema	Palpable thickening, borders undefined, cannot measure
2	Moderate alopecia or erythema overlying skin intact	Palpable mass, borders defined, measurable, overlying skin intact
3	Moderate alopecia or erythema with partial thickness ulceration	Palpable mass, borders defined, measurable, and partial thickness ulceration
4	Moderate to severe alopecia or erythema with full thickness ulceration	Palpable mass, borders defined, measurable with full thickness ulceration

Figure 1. Erythema and mass scoring system.

Statistical methods for histologic semiquantitative data. Statistics for histologic data were performed in Prism (version 7.03, GraphPad Software, La Jolla, CA). Nonparametric data were analyzed by using the Kruskal–Wallis test for variance, with the Dunn posthoc multiple-comparisons test and Mann–Whitney test for significance.

Results

Visual appearance of MSR lesions. Lesion appearance and ease of identification varied widely. Some lesions were not visible and were discernible only through palpation. In contrast, others created a visible effect (Figure 2 A) that resulted in alopecia (Figure 2 B) or cutaneous ulcerations with crusts (Figure 2 C). Several lesions were apparent on the side of an animal's body, distant from the actual injection site near the thoracic limbs (Figure 2).

Injection-site reactions. In the MSR group, 33 of 60 mice (55%; 95% CI, 42% to 68%) developed erythematous lesions with a score greater than or equal to 1, compared with 9 of the 24 mice in the MEL group (38%; 95% CI, 19% to 59%) and 2 of the 24 mice in the SC group (8%; 95% CI, 1% to 27%; $P < 0.001$, Fisher exact test). In the MSR group, 49 of 60 mice (82%; 95% CI, 70% to 90%) developed mass lesions with a score greater than or equal to 1, compared with 5 of 24 mice in the MEL group (21%; 95% CI, 7.1% to 42%) and 1 of the 24 SC mice (<5%; $P < 0.001$, Fisher exact test). Daily average erythema and mass scores showed that MSR mice had significantly (both $P < 0.001$) higher scores than MEL and SC animals, regardless of strain or treatment day (Figure 3).

Erythema severity scores over time. The median time to first erythematous lesion score of greater than or equal to 1 in the MSR treatment group was 3 d (95% CI, 2 to 3 d), showing a consistent, highly significant difference from the SC group ($P < 0.001$, log-rank test). In contrast to SC animals, mice treated with MSR uniformly developed observable erythematous lesions. Rapid progression continued thereafter, with most mice at a score of 2 toward the end of the first week. The average trajectory across animals showed a 7-fold increase (95% CI, 2- to 25-fold; $P = 0.003$) daily.

Erythematous lesions stably persisted after the rapid progression, with only a few mice reaching stage 3 and with very few reversions. No mouse developed lesions with erythema scores of 4. Mice treated with MEL had an intermediate pattern, with fewer lesions than the MSR group but more than the SC group. Neither the MEL nor SC group reached a 50% incidence of erythematous lesions over the duration of the study (Figure 4 A).

Mass severity scores over time. The median time to the first mass lesion in MSR treatment group was 2 d (95% CI, 2 to 3 d) and differed significantly from the control group ($P < 0.001$, log-rank test; Figure 4 B). The trajectories of mass lesion severity in the active-treatment groups (MSR and MEL, $n = 84$ total) showed rapid progression from onset at day 2 to 3 at stage 1 to stage 2 by day 5 or 6 for almost all animals. Because a substantial number of mice experienced regression from their peak level, a logistic-linear fit was inadequate.

Among all the 55 mice (49 MSR, 5 MEL, 2 SC) with mass lesions of greater than or equal to 1, 36 (65%; 95% CI, 51% to 78%) experienced a reduction in observation score over time. Of these 36, more than half ($n = 20$; 56%; 95% CI, 38% to 72%) reverted to 0 at some point prior to the completion of the study. The MEL and SC groups did not reach a 50% incidence of mass lesions over the 7- or 14-d periods (Figure 4 B).

Postmortem assessment of inflammation and injection-site resolution. Reflection of the skin revealed that the subcutaneous lesions identified in MSR-treated mice varied in appearance from discrete nodules to clear cavitations to diffuse discoloration (yellow-tan to brown) and thickening of the subcutaneous fat (Figure 5) with hemorrhage. Histologic findings in all treated mice was consistent with injection-site panniculitis (Figure 6), ranging from chronic necrotizing to histiocytic or pyogranulomatous or granulomatous inflammation.

Severity of inflammation at 7 d was significantly greater in the MSR group, regardless of strain or sex, than in the MEL ($P < 0.0001$) or SC ($P < 0.001$) groups (Figure 7 A). Furthermore, inflammation at 14 d was significantly greater in the MSR group, regardless of strain or sex, than in the MEL ($P < 0.0001$) or SC ($P = 0.0002$) groups (Figure 7 B).

No statistical difference in the character of the inflammation between 7 and 14 d was observed in any treatment group ($P > 0.99$; Figure 8).

Effect of sex on injection-site reactions. Evaluation of the pooled strain data revealed no significant differences in the occurrence of erythema lesions between males (48%) and females (33%; $P = 0.12$) or of mass lesions between males (52%) and females (50%; $P > 0.99$), with no significant correlation between time to first mass or erythematous lesion and sex ($P = 0.61$). The severity of inflammation at 7 d, which was scored postmortem according to histology, was not significantly different between sexes of mice when all strains were pooled. The severity of inflammation at 14 d was significantly greater in MSR-treated females ($P = 0.0013$) than males (Figure 6 C) when all strains were pooled. When resolved to the strain level, the statistical difference between sexes (Figure 7 C) was due to more severe inflammation in MSR-treated Crl:CD1(ICR) females than in Crl:CD1(ICR) males ($P = 0.048$).

Effect of strain on injection-site reactions. The incidence of erythematous lesions was significantly lower in BALB/cJ (22%) than in Crl:CD1(ICR) (53%) or C57BL/6J (47%) mice ($P = 0.024$, χ^2 test), when data from all treatment groups were pooled. When assessed for erythema, BALB/cJ mice had a longer lesion-free period. The pattern persisted for MSR-treated animals: BALB/cJ, 30%; Crl:CD1(ICR), 75%; and C57BL/6J, 60% ($P = 0.01$, χ^2 test; Figure 3 C). No significant differences were found in occurrence of mass lesions between strains: BALB/cJ, 53%; Crl:CD1(ICR), 53%; and C57BL/6J, 47% ($P = 0.91$; Figure 3 D) No significant correlation was found between time to first mass lesion and mouse strain ($P = 0.62$). No statistically significant difference was found between strains of mice treated with MSR in histopathologic inflammatory severity scores at 7 d ($P = 0.13$) and 14 d ($P = 0.84$).

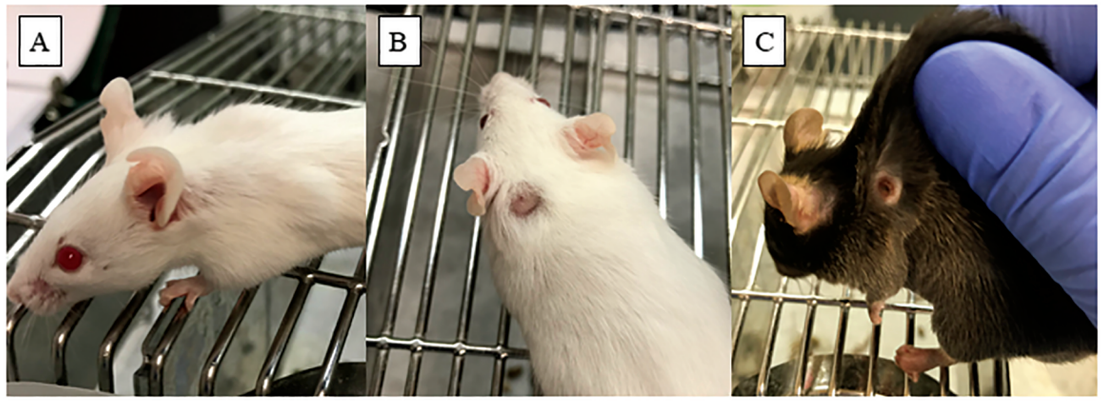

Figure 2. Examples of variation in lesion appearance. (A) Nonulcerative mass caudal to the shoulder joint. (B) Alopecic mass with pinpoint ulceration lateral to midline in the cervical (scruff) region. (C) Slightly thickened, alopecic ulceration caudodorsal to the shoulder.

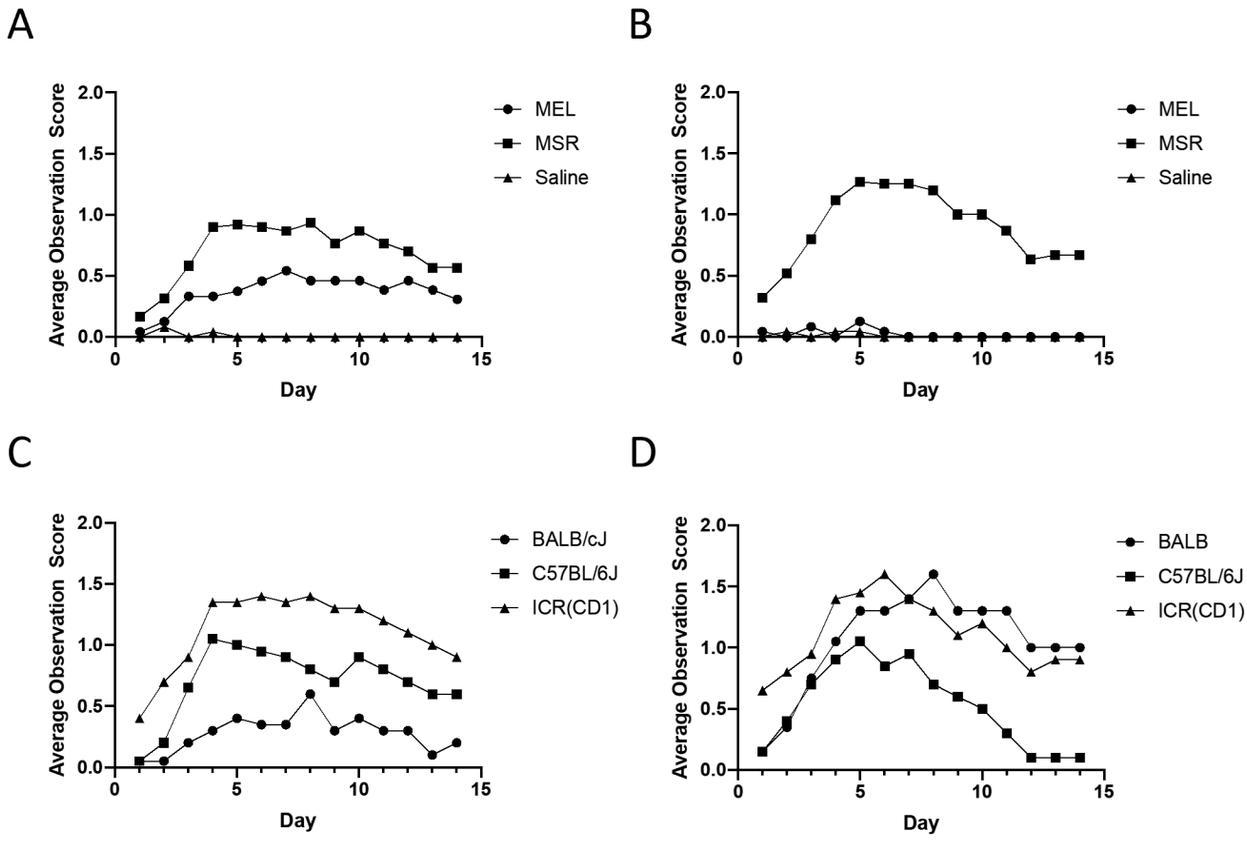

Figure 3. Average scores for (A and C) erythema or (B and D) mass according to (A and B) treatment group or (C and D) strain.

Discussion

This study demonstrates that MSR leads to the development of erythematous and mass lesions more frequently than MEL and saline in 3 commonly used strains of mice: BALB/cJ, C57BL/6J, and Crl:CD1(ICR). This finding raises concern for the welfare of mice treated with MSR and could represent a potential confounding factor in research in which inflammation could affect data and study outcomes. In some circumstances, the benefits of less handling could outweigh the risks of MSR injection-site reactions. For example, postsurgical treatment with MSR, especially in mice at risk for injury from handling, may have enough recovery time to allow lesion resolution. However, our results indicate that resolution could take over 14 d, given that the severity of inflammation was not significantly lower at 14 d than at 7 d nor was the character of the inflammatory response (stage of resolution) significantly more mature (macrophage-rich) at 14 d compared with 7 d. These results underscore the importance of considering alternative drugs, in light of the relatively high potential for MSR-associated drug reactions in the form of necrotizing to pyogranulomatous panniculitis in BALB/cJ, C57BL/6J, and Crl:CD1(ICR) mice.

Given the high frequency of lesion development in our study, one might ask why reports of adverse reactions in the literature are somewhat scant. Our study results reveal several possible

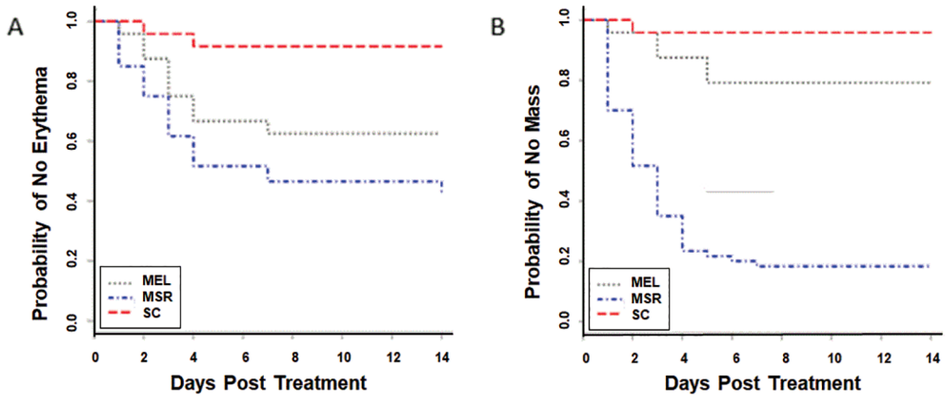

Figure 4. Time to first (A) erythematous lesion and (B) mass lesion according to treatment group.

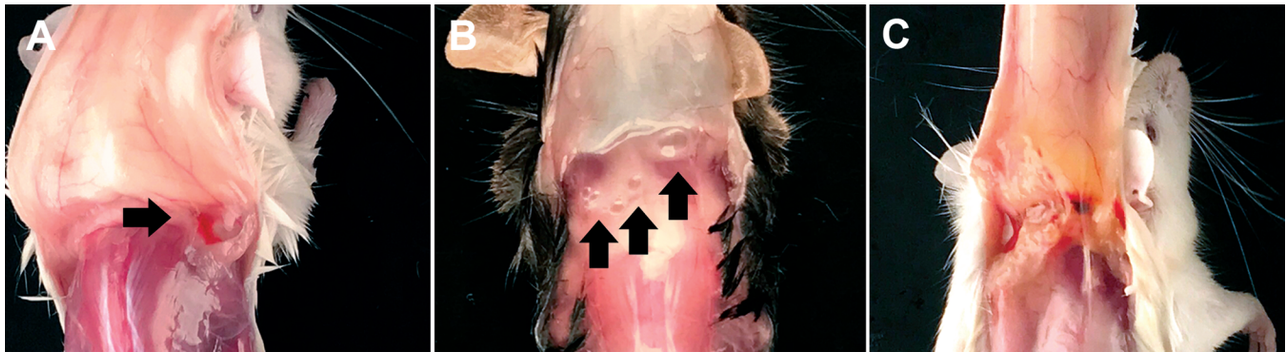

Figure 5. Reflection of the skin revealed that the gross appearance of the MSR-associated injection site reaction in the scapular subcutis varied from (A) a discrete cavitated nodule to (B) multiple clear discrete cavitations to (C) poorly demarcated yellow thickening of the intrascapular fat with edema and hemorrhage. Image area, 187 × 50 mm (96 × 96 DPI).

reasons why lesions could be missed. The amount of time between the injection to the initial emergence of a lesion provides one potential explanation. The median presentation time was 2 d for palpable mass lesions and 3 d for erythema, so lesions may not be present or discernible in a typical 72-h safety-and-efficacy study window. Another explanation uncovered in our study was that many of the lesions were not visually apparent and were revealed only through thorough palpation and careful assessment of the skin underlying haired areas. That level of follow-up assessment may not typically be provided in research settings, may be impractical or impossible for animals in wildlife or zoo contexts, and would eliminate the benefit of sustained-release drugs to reduce handling. A third explanation is that the lesions identified in this study often occurred on the side of an animal's body, distant from the actual injection site near the thoracic limbs, where the drug could have pooled in the subcutaneous tissue. Those lesions could be missed even with reasonably close monitoring and would be especially difficult to notice when they occur with no or minimal alopecia or ulceration. A final consideration is that lesions might be attributed inaccurately to other etiologies, such as fight wounds, barbering, or dermatitis. Alopecic and ulcerative lesions in the C57BL/6J strain could be mistaken for ulcerative dermatitis, which is a common condition of the strain.[8,14] Because this strain developed lesions more frequently than the other inbred strain, the predisposition for ulcerative dermatitis may be related but is outside of the scope of this study.

BALB/cJ, an inbred strain, developed lesions least frequently, and the only outbred strain in our study, Crl:CD1(ICR), developed lesions most frequently, but only slightly more than the inbred C57BL/6J strain. This pattern of results does not allow us to form a predictive assessment for how other strains may react. Differences in reaction frequency may be due to underlying differences in strain immune responses. The BALB/cJ strain is known to exhibit a bias toward a Th2-type immune reaction, which induces M2 macrophages that are associated with wound healing and tissue repair.[11,15] In contrast, the C57BL/6J strain exhibits predominantly a Th1-type immune reaction, which induces M1 macrophages that are associated with phagocytosis of bacteria and viruses, involving more destructive processes.[11,15] Histologic finding of panniculitis was a common feature of MSR reactions, and most of these injection-site reactions included granulomatous, pyogranulomatous, or histiocytic elements. Given that macrophages are defining features of granulomatous responses, strain-associated differences in immune-cell activities, and cytokine responses may underlie the strain-associated differences in reactions to MSR.

Neither BALB/cJ nor C57BL/6J mice showed sex-associated differences in reactions to MSR, MEL, or SC or in lesion severity assessed by histology. The only sex-associated difference found was that female Crl:CD1(ICR) mice had greater inflammation severity than males at 14 d. Sex-associated differences in inflammatory cytokines have been found in a group of C57BL/6J mice with melanoma induced by subcutaneous inoculation with melanoma stem cells.[17] However, despite the potential for variations in cytokine responses to drive differences, we did not find sex differences in this strain. Potential causes for the sex-associated difference we saw are beyond the scope of this study.

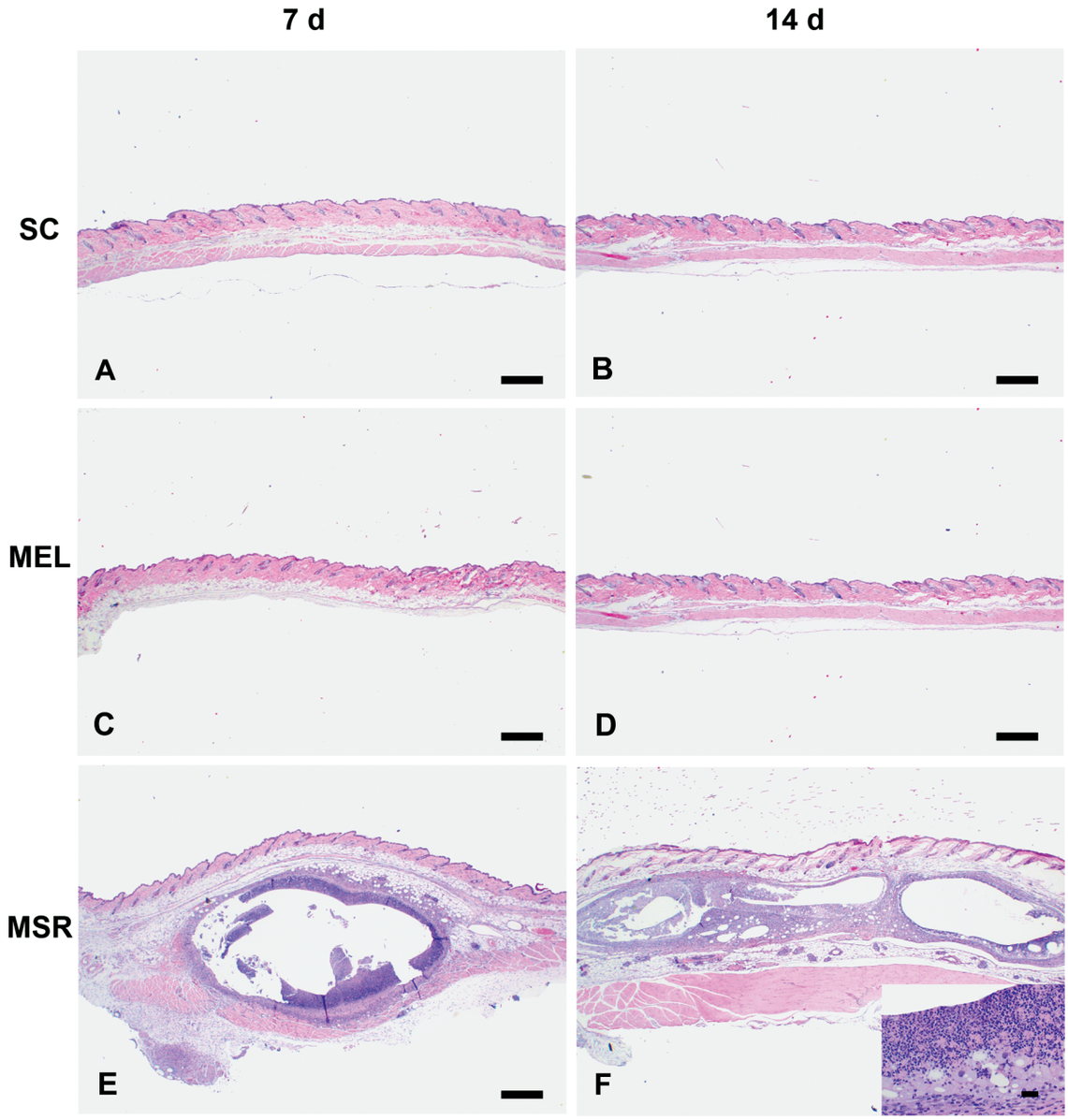

Figure 6. Injection-site panniculitis was identified in the MRS-treated groups only and persisted for at least 14 d after injection. Representative sections of skin from mice injected with SC, MEL, and MSR. SC injection sites at (A) 7 d and (B) 14 d exhibit no signs of inflammation. MEL injection sites at (C) 7 d and (D) 14 d exhibited no signs of inflammation. (E) MSR-injected sites at 7 d exhibited marked chronic necrotizing panniculitis with peripheral fibroplasia. (F) MSR-injected sites at 14 d demonstrated a shift from predominantly necrotizing to more granulomatous inflammation. The increased numbers of large peripheral macrophages (*) represent the start of resolution. Hematoxylin and eosin stain; magnification, 2× (inset, 40×).

Our study found no predictable association of inflammation severity with observation scores or onset and severity. Thus, observational characteristics cannot fully predict the expected histologic outcomes. Mice with erythema scores of greater than or equal to 1 often had inflammation scores of 0. Mice with mass scores of greater than or equal to 1 consistently had inflammation scores of greater than or equal to 1. Given that mass scores represent the gross observation of tissue changes due to inflammation or edema, these scores would logically correlate loosely. In addition, some animals developed only one of the 2 lesion types, indicating that the erythema and mass lesions were independent. Discernable resolution was not present in MSR-treated mice at 14 d. The lack of statistical difference in the character of inflammation in MEL and SC groups was due to the overall lack of inflammatory reaction in the 7-d group. The lack of difference in the MSR group was due to the persistence of inflammation, suggesting that the inflammatory reactions incited by MSR injections can persist for weeks and possibly longer. This information should carefully be considered in studies in which inflammation may influence outcomes, and when MSR is used, sufficient time should be provided to allow resolution.

Particular care should be used when administering MSR. We advise close monitoring of injection sites and surrounding tissues.

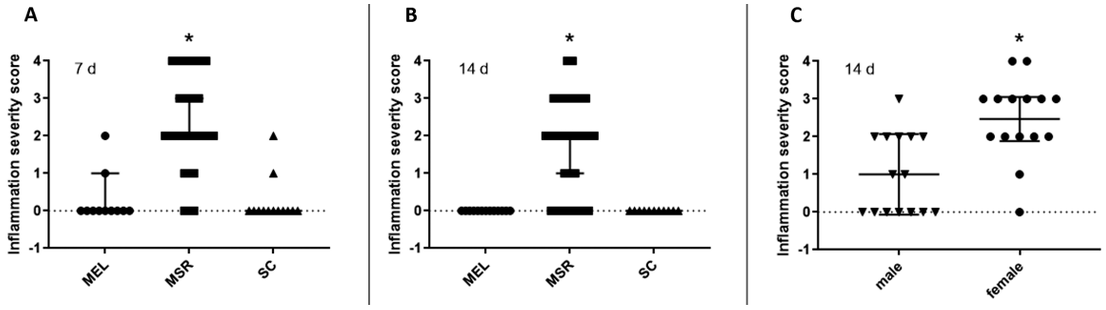

Figure 7. The severity (median with 95% CI) of the injection-site panniculitis was greater in the MSR-treated group than in the MEL- or SC-treated groups at (A) day 7 (*, $P < 0.0001$) and (B) day 14 (*, $P < 0.0001$, $P = 0.0002$). (C) More severe MSR-associated injection-site panniculitis was observed in female mice (*, $P = 0.0013$) compared with male mice, when sexes were grouped across strains.

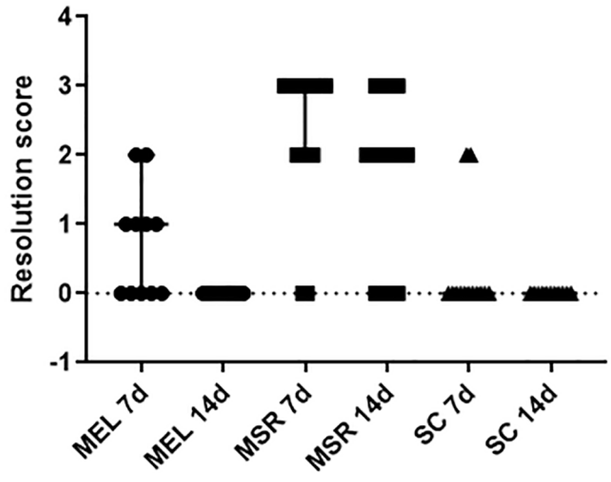

Figure 8. Injection-site panniculitis (median with 95% CI) persisted in MSR-treated mice at 14 d after injection. No significant differences in stage of resolution were observed between time points in any treatment group ($P > 0.9999$), but particularly in the MSR-treated group, where inflammation persisted.

The range of severity may require the treatment of some lesions, whereas others may resolve on their own. One option is to shave injection sites, but then lesions that would become alopecic could be missed, and shaving would not help identify mass lesions with only subtle thickening. Future studies to characterize reactions to the drug in other species or mouse strains should consider including palpating tissues in which the drug may pool for longer than 5 d after administration, because of time for lesion development. Finding loose fluid-filled pockets does not directly imply a reaction, because the sustained-release drug vehicle can be expected to dissipate as it slowly releases drug. Two mice receiving SC had erythema scores of 1, and one animal had a mass score of 1, perhaps indicating that the physical process of administering the injection could lead to mild tissue damage. However, lesions of greater severity, often seen in MSR groups, had defined borders that did not dissociate on palpation and that included other features such as erythema or alopecia and inflammatory cells on histology. Future studies could include a treatment group that receives only the compounder's proprietary vehicle, without the addition of meloxicam. Such data could inform the development of future formulations.

In conclusion, we present the first study to demonstrate severe injection-site reactions after MSR administration in mice. Given the current findings, careful consideration should be exercised when prescribing and administering MSR as a treatment option. While using a sustained released analgesic provides some benefits, the risk of potential inflammatory effects and lesion formation should be considered. The range of effects in different species, strains, and severity of lesions is wide and varied. Therefore, when possible, we recommend the palpation of injection sites and vigilant observation when administering MSR, even in repeated administrations. Furthermore, according to the manufacturer's guidelines, MSR cannot be diluted to mitigate such effects. MSR has been used in several species, some of which had little to no observed reactions (such as American flamingos and sheep[5,16]), although reactions may have been missed, whereas mice, macaques, and Hispaniolan parrots have all shown reactions.[1,7] Further research is needed to better understand the extent of adverse reactions across species and to gain additional pharmacokinetic–pharmacodynamic studies to fully characterize the efficacy and side effects of this analgesic.

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