Preliminary Evaluation of Sustained-release Compared with Conventional Formulations of Meloxicam in Sheep (*Ovis aries*)

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Sustained-release (SR) drugs refine current analgesic regimens by alleviating the need for multiple sessions of handling and restraint and by reducing the local tissue irritation that can occur due to repeated injections. Although a variety of SR drugs are already used in lab animal medicine, no studies exist that evaluate the suitability of an SR NSAID in sheep. This study used HPLC–MS to measure the plasma concentrations of 2 formulations of meloxicam—conventional and SRM—after subcutaneous administration in 6 adult ewes. Blood was collected at 0, 4, 12, 24, 36, 48, 60, 72, 84, 96, 120, 144, and 168 h after injection. In addition, physical exams, urinalysis, and biochemical analysis were performed at 0, 24, 48, and 120 h after dosage. Peak plasma concentrations were 1057 ± 433 ng/mL at 4 ± 0 h for conventional meloxicam and 3238 ± 1480 ng/mL at 6.7 ± 4.1 h for SR meloxicam (SRM). Elimination half-lives were 12.1 ± 4.2 for CM and 15.2 ± 2.4 h for SRM. One sheep had an episode of acute renal azotemia starting 24 h after SRM administration; the episode resolved over time, and the definitive relationship to SRM administration was not determined. Plasma levels of SRM were higher than CM throughout the initial 24 h, remained variably elevated until 60 h after injection, but failed to sustain presumed therapeutic levels of 400 ng/mL for the full 72 h across all animals in this study. Further investigation is warranted to determine the safety and clinical efficacy of SRM in sheep. Currently, when SRM is used in sheep, we recommend the combination of a preemptive and multimodal analgesia regimen with clinical assessments throughout the postoperative period.

Abbreviations: CM, conventional meloxicam; C_{max} , maximal plasma concentration; SRM, sustained-release meloxicam; T_{max} , time to maximal concentration

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Sheep are commonly used for soft tissue and orthopedic studies, because their anatomy and physiology render them suitable as models of human disease.^{9,15,25,30,36} For major survival surgeries in ruminants, multimodal perioperative analgesia¹ that lasts at least until the 3rd postoperative day is currently recommended.^{11,23} Although recurrent administration of analgesics is imperative during this period, repeated physical restraint together with aversive stimuli (that is, the physical separation of herd animals and needle sticks) have the potential to affect animal welfare by causing handling-associated stress.^{2,32} These stressors might confound study results by influencing hemodynamic parameters and the endocrine response.^{2,5,12} However, when combined with adaptation to gentle handling techniques, the use of sustained-release (SR) products may serve as valuable refinements to lessen the effects of stress and simultaneously ensure adequate analgesia. An SR formulation that is effective in maintaining a stable, therapeutic level over an extended period of time would prove beneficial in limiting the need for repeated restraint, separation from counterparts, and numerous injection events in a given animal.

With several SR drug classes on the market (for example, opioids, local analgesics and anesthetics, NSAID), multimodal analgesia using these formulations are being further explored, because they may effectually improve animal welfare in a variety of species.^{3,6,13,19,21,32,39} Previous studies in biomedical sheep have demonstrated that SR buprenorphine reaches steady plasma concentrations and provides continuous analgesia against thermal nociception for at least 72 h.³⁹ In addition, liposomal bupivacaine has been evaluated as a SR local analgesic product for the prevention of transduction and transmission of acute and perioperative pain lasting 4 d in a rat model¹⁹ and as long as 72 h in dogs.²² The NSAID meloxicam belongs to the enolic acid class and has antiinflammatory, analgesic, and antipyretic properties.³³ Meloxicam has been formulated as a SR product in a fully biodegradable liquid polymer matrix that—according to the manufacturer—releases and provides therapeutic blood levels for as long as 72 h after a single subcutaneous injection in mice and rats.⁴⁰ Studies evaluating SR meloxicam (SRM) in several species have yielded variable results. At the recommended rodent dose (4 mg/kg SC once), mechanical hypersensitivity—but not thermal hypersensitivity—was attenuated for a maximum of 48 h in a rat incisional pain model,³² and in mice, plasma levels of SRM were sustained for at least 24 h, but therapeutic levels were maintained for only 12 h.²¹ In amazon parrots, SRM showed highly variable results, with maintenance of target plasma concentrations for 12 to 96 h,¹³ yet in cynomolgus macaques, SRM achieved adequate steady-state plasma concentrations for 48 to 72 h, demonstrating more

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consistent levels than other formulations that required daily dosing.³ An ideal SR NSAID would maintain therapeutic efficacy for at least 72 h, with at most minimal disruption to renal and hepatic function. According to a compilation of several prior studies across multiple species, 390 to 911 ng/mL has been considered as a reference range for the therapeutic plasma concentration of conventional meloxicam (CM).^{3,16,20,21,26,33} In one study evaluating stride length and lameness in horses, effective concentrations of meloxicam were as low as 130 ng/mL and 195 ng/mL, respectively.³⁷ To date, no studies evaluating the pharmacokinetics or efficacy of SRM in sheep are available.

In this study, we aimed to determine the suitability of using SRM in sheep. For utility, we conservatively interpreted a plasma concentration of 400 ng/mL or greater as a presumed therapeutic level for SRM in sheep, in light of CM therapeutic levels established in several species, including sheep, and in consultation with the manufacturer. Our aim was to evaluate the efficacy of SRM as a potential alternative to CM for perioperative pain management in sheep. We hypothesized that SRM could be administered as a one-time dose that would reach and maintain assumed therapeutic plasma concentrations of at least 400 ng/mL over 72 h, thus avoiding the time-dependent fluctuations in drug concentration that occur with once-daily dosing of CM for 3 d.

Materials and Methods

Animals. Healthy, experimentally naive, Dorset ewes ($n = 6$; age, 9 to 12 mo; weight, 60 to 80 kg) were acquired from a university-approved vendor. On arrival, sheep were identified by using USDA ear tags, randomly assigned individual identification numbers of 1 through 6, and housed in AAALAC-accredited facilities at the University of Minnesota. Room conditions were set to a 12:12-h light:dark cycle, temperature was controlled at 18 to 22 °C, and animals were pair-housed on Tenderfoot flooring (Tandem Products, Minneapolis, MN) in pens with floor space measuring 96 to 80 ft², thus exceeding the minimal space recommended by the *Guide for the Care and Use of Laboratory Animals*.¹⁷ Sheep were provided a daily mixed pelleted ration (7060 Teklad Ruminant Diet, Envigo, Indianapolis, IN) with sweet feed (oats, corn, and molasses; Bombay, Kenyon, MN) and fed free-choice mixed alfalfa loose hay from a local source. This study was conducted in accordance with the University of Minnesota Animal Use Policies and was approved by the IACUC. At the conclusion of this study, ewes were transferred for use on another approved protocol.

Drug handling and administration. According to the manufacturer's instructions, SRM (10 mg/mL, Zoopharm, Fort Collins, CO) was stored in the refrigerator at 4 °C and was allowed to reach room temperature (approximately 20 to 22 °C) prior to injection. CM (5 mg/mL, Loxicom, Norbrook, Overland, KS) was stored at room temperature. Drugs were administered subcutaneously in the axillary or inguinal skin fold of sheep by using sterile syringes with 18-gauge needles for SRM and 20-gauge needles for CM.

Pilot procedure and additional study animals. A single pilot sheep (no. 1) was used to verify the dosing strategy and sampling intervals for the planned study. To facilitate daily visualization of the region where the drug was administered, the hair overlying the injection site was shaved by using electric clippers and outlined by using a permanent marker. CM was given at 0.5 mg/kg SC daily for 3 consecutive days, followed by a 14-d washout period. The one-time dose for SRM use in sheep was calculated and based on the summation of 3 doses

of CM to cover a 72-h period ($0.5 \text{ mg/kg} \times 3 = 1.5 \text{ mg/kg}$)²⁸ and after discussion with the manufacturer.^{3,40} on review of an unpublished study of the same compound in dogs. For the pilot study, an increased dose that was twice the recommended dose of SRM ($1.5 \text{ mg/kg} \times 2 = 3 \text{ mg/kg}$) was also evaluated, after an additional 14-d washout period. This increased dose was evaluated because of an evaluation of a SR opioid, manufactured by the same company, where twice the recommended dose yielded therapeutic plasma concentrations in sheep.³⁹ Samples from the pilot animal were processed prior to proceeding with additional animals. The remaining 5 sheep (nos. 2 through 6) were evaluated in the same way as the pilot ewe, by using a dose of 1.5 mg/kg of SRM instead of 3 mg/kg due to concerns regarding the excessive plasma levels of the drug observed in the pilot animal (see Results and Discussion sections).

Physical exam and time points. Baseline weight and standard physical exam were performed prior to the start of the study, and additional physical exams were performed at each sample collection point. Exams included evaluation of the overall condition of the animal and monitoring for signs of local tissue reaction at the drug injection site; these reactions had been noted with the previous SRM formulation.³ A final weight was collected at the end of the study.

Time points for the pilot animal were 0, 0.5, 1, 4, 12, 24, 48, 72, 96, 120, 144, and 168 h after initial drug administration. In light of the results of the pilot study, time points for sheep 2 through 6 were 0, 4, 12, 24, 36, 48, 60, 72, 84, 96, 120, 144, and 168 h. During the washout period, blood was collected once daily to ensure that no residual drug was detected before the next drug administration.

Sample collection. For both formulations, blood for CBC and serum chemistry and urine for urinalysis were collected at 4 time points after injection (0, 24, 48, and 120 h). For blood collection, the neck was shaved at the baseline collection; the skin overlying the jugular vein was swabbed with alcohol at each time point, and collection was alternated between the 2 jugular veins. Blood was collected directly into sterile blood tubes (Monoject blood collection tubes, Covidien, VWR, Radnor, PA) by using a 21-gauge, 1.5-in. vacuum phlebotomy tube needle (Multi-Sample Blood Collection Needle, EXEL International, St Petersburg, FL). After venipuncture, pressure was applied to the site to prevent hematoma formation. Indwelling catheters were not used given the increased maintenance, handling, and risks associated with chronic catheterization in group-housed sheep.³¹ For CBC and plasma evaluation, purple-top (EDTA) tubes were used; red-top (no anticoagulant) and blue-top (sodium citrate) tubes were used for ovine serum chemistry. Blood was processed inhouse within 1 h of collection and shipped on ice to the University of Minnesota, College of Veterinary Medicine Diagnostic Lab within 4 h. Plasma was stored in a -18 °C freezer until shipped overnight on dry ice for analysis of meloxicam levels (Protea Biosciences, Morgantown, WV).

Urine was collected through free catch prior to the physical exam or within 1 h of the sample collection time. When a ewe did not urinate spontaneously, its nostrils were gently held for 15 to 30 s to induce urination,¹⁴ and the procedure was aborted on any indication of distress or failure to produce urination. The animal was allowed to rest and attempts to free-catch urine were performed at a later time during or after the physical exam. An in house urine reagent strip (catalog no. 2161 Multistix 10 SG Reagent Strips, Siemens Healthcare, Erlangen, Germany) was used and urine specific gravity by using a spectrometer was performed immediately after collection. Remaining urine was refrigerated and transported on ice with the blood samples. All

samples were evaluated by a board-certified veterinary clinical pathologist.

Pharmacokinetic evaluation. Samples were shipped overnight on dry ice, and plasma levels of meloxicam were evaluated by using MS (Protea Biosciences). Preparation of samples included protein precipitation and HPLC. These commercial quantitative assays were performed according to standard operating procedures under an established quality system for human and animal samples. Briefly, for protein precipitation, acetonitrile was added to 100 μ L of spiked K_2 EDTA plasma, 100 μ L of internal standard diluent (50:50 water:methanol), and 100 μ L of working internal standard (250 ng/mL meloxicam-d3). The samples were vortexed for 3 min at maximal speed and then centrifuged at 2000 rpm for 10 min at room temperature. Supernatant (100 μ L) was removed from each tube and added to 500 μ L of water for dilution; 100 μ L of the diluted sample was added to a well of a 96-well plate, the plate vortexed for 1 min, and then centrifuged at 2000 rpm for 2 min at room temperature.

The HPLC used a Poroshell 120 EC-C18 column (50 $\times$ 3.0 mm, 2.7 μ m; Agilent, Blacksburg, VA) with a column temperature of 45 $^{\circ}$ C and autosampler temperature of 4 $^{\circ}$ C. The HPLC gradient was 6 min, with an injection volume of 2 μ L. The LC-MS method for meloxicam yielded a quadratic curve with an R^2 value of 0.9975. All of the calibration curve points were within the specification of less than 15% error. The quantitation range for meloxicam was 10.0 to 4000 ng/mL.

Statistical analysis. PKSolver 2.0 (a free add-in program for Microsoft Excel that is written in Visual Basic for Applications, <http://dx.doi.org/10.1016/j.cmpb.2010.01.007>) was used to determine pharmacokinetic parameters (C_{max} , T_{max} , AUC_{0-t} , mean residence time, and half-life) after subcutaneous injection and to generate noncompartmental models. Demographic data are expressed as mean $\pm$ 1 SD. The program Prism (GraphPad Software, San Diego, CA) was used to compare between measured CM and SRM concentrations.

To assess differences in the plasma level of the drug between treatments at each time point, a mixed model was fit, with plasma level as the response; time, treatment, and their interaction as fixed effects; and animal as a random effect. Least-squares means and 95% CI were calculated for each treatment at each time point and back-transformed from the log scale, as were SRM:CM ratios with 95% CI. These ratios were tested for equality with 1 (that is, no difference), with P values corrected for multiple comparisons by using Bonferroni-Holm correction. A P value less than 0.05 was considered significant. Models were fit and analyzed by using R version 3.43.²⁹

Results

Physical exam findings. All sheep remained healthy according to physical exam throughout the study and either maintained or gained weight. The administered volume of 1.5 mg/kg SRM ranged from 10 to 11 mL and produced a firm round, swelling (1 cm $\times$ 3 cm) at the site of injection, due to the large injection volume. This swelling flattened out as the drug was absorbed into the surrounding tissue over the initial 24 h. As the study progressed, the swelling became a small, residual nodule that remained palpable. At no time—even during administration of the 3-mg/kg dose in the pilot sheep—did lesions occur, in contrast to the results from a previous formulation of this drug in other species.³ Skin appearance remained otherwise normal, with no ulcerations or inflammation; no sheep exhibited signs of injection-site irritation or pain.

Pharmacokinetic findings. Plasma concentration values are summarized for both formulations of meloxicam. In this study, CM (Table 1) and SRM (Table 2) had elimination half-lives of 12.1 ± 4.2 and 15.2 ± 2.4 h, respectively. The C_{max} of CM was 1057 ± 433 ng/mL and of SRM was 3238 ± 1480 ng/mL, where CM consistently reached peak levels (T_{max}) at 4 h in all animals, whereas T_{max} for SRM ranged between 4 to 12 h, with a mean of 6.7 ± 4.1 h. The mean residence time, representing the average amount of time the drug remains in the system, was 41.5 ± 3.8 h for CM and 20.5 ± 4.4 h for SRM. During the initial dosing trial, the pilot ewe received an additional dose of SRM at 3 mg/kg, which produced peak levels of 10,100 ng/mL at 4 h post injection, close to 3 times the mean peak of the 1.5 mg/kg dosage for all sheep ($n = 6$).

According to pharmacokinetic findings, the SRM profiles split into 3 groups: rapid, intermediate, and slow elimination (Figure 1). SRM plasma levels in the rapid-elimination sheep ($n = 2$) fell below 400 ng/mL by 36 h, whereas these levels fell below 400 ng/mL at 48 and 60 h in the intermediate-elimination group ($n = 3$) and at 72 h in the slow-elimination sheep. Similar patterns of clearance rate have been described for SRM in other species.^{13,21}

The ratio of SRM:CM plasma levels during the first 24 h was between 2.88 (95% CI, 1.95 to 4.25) at 4 h and 4.23 (95% CI, 2.86 to 6.25) at 24 h (Figure 2). There were no significant differences at 36, 48, and 120 h ($P = 0.16$, $P = 0.42$, and $P = 0.42$, respectively). All other time points showed significant ($P \leq 0.0001$) differences in plasma level between the 2 formulations.

During the first 4 h, SRM had notably higher plasma concentrations than CM, ranging from 1810 to 6090 ng/mL (Figure 3); the CM concentration at 4 h ranged from 692 to 1870 ng/mL. This difference was statistically significant ($P < 0.001$), with SRM being 2.88 times greater (95% CI, 1.95 to 4.25 ng/mL) than CM. Evaluation of the geometric mean at 4 h showed that SRM had a mean of 2866 ng/mL (95% CI, 1334 to 6157 ng/mL), and CM had a mean of 996 ng/mL (95% CI, 464 to 2140 ng/mL). However, by 60 h, the values reversed, with CM having a higher mean (760; 95% CI, 352 to 1639 ng/mL) than SRM (mean, 121 ng/mL; 95% CI: 56.28 to 261.80), with CM being 6.26 times greater (95% CI, 4.08 to 9.60).

Clinical parameters. The pilot sheep showed transient acute renal azotemia at 24 h after SRM administration; her condition was slightly worse at 48 h but showed recovery at 120 h (Table 3). Findings were consistent with an episode of acute kidney injury, the cause of which was not definitively identified. The damage appeared to be somewhat—if not completely—reversible, because the sheep's creatinine concentration returned to baseline during administration of the higher dose (that is, 3 mg/kg) of SRM and remained clinically normal thereafter (Table 4). The CBC and biochemistry data from the other 5 sheep were all within the normal reference ranges. Parameters indicative of renal insult were of particular interest, and all of the remaining sheep maintained values within acceptable ranges for BUN (7 to 23 mg/dL), creatinine (0.5 to 1.0 mg/dL), and urine specific gravity (1.015 to 1.045), according to our lab's reference intervals.

Discussion

The aim of this study was to compare the plasma concentrations of SRM and CM, with the ultimate goal of refining postoperative analgesia in sheep. We hypothesized that plasma levels of a one-time administration of SRM would remain more consistently at a concentration of 400 ng/mL or greater for 72 h compared with CM given once daily for 3 d, thus preventing fluctuations in plasma concentrations experienced at the end

Table 1. Pharmacokinetic parameters of CM (0.5 mg/kg daily for 3 d) according to noncompartmental analysis

	Sheep						Mean $\pm$ 1 SD
	1	2	3	4	5	6	
T_{max} (h)	4	4	4	4	4	4	4 $\pm$ 0
C_{max} (ng/mL)	1,870	692	938	728	1,160	954	1,057 $\pm$ 433
AUC_{0-168} (ng/mL) $\times$ h	96,210	26,890	48,538	24,937	59,864	39,831	49,378 $\pm$ 26,450
Mean residence time (h)	42.5	39.3	41.3	36.3	47.8	42	41.5 $\pm$ 3.8
Half-life (h)	9.6	9.3	13	8.7	19.9	12.3	12.1 $\pm$ 4.2

Table 2. Pharmacokinetic parameters of SRM (1.5 mg/kg single dose) according to noncompartmental analysis

Sheep	Sheep						Mean $\pm$ 1 SD
	1	2	3	4	5	6	
T_{max} (h)	4	4	12	4	12	4	6.7 $\pm$ 4.1
C_{max} (ng/mL)	6,090	2,470	2,870	1,810	3,180	3,010	3,238 $\pm$ 1,480
AUC_{0-168} (ng/mL) $\times$ h	187,887	38,569	88,104	30,605	93,918	65,534	84,103 $\pm$ 56,857
Mean residence time (h)	24.8	13.9	24.2	16.3	22.7	21.2	20.5 $\pm$ 4.4
Half-life (h)	16.6	11.1	17.4	17	15.5	13.6	15.2 $\pm$ 2.4

Figure 1. Plasma concentrations of (A) CM (0.5 mg/kg SC daily for 3 d) and (B) SRM (single dose of 1.5 mg/kg SC) formulations in sheep.

of each 24-h CM dosing interval (that is, time points of 24, 48, and 72 h). In this trial, among all sheep, both formulations of meloxicam reached plasma levels of 400 ng/mL or greater. The peak levels were much higher for SRM than CM and remained well above the therapeutic threshold for at least 24 h. These findings indicate that during the first 24 h of the acute phase, SRM may be a more effective analgesic than a single dose of CM. This result also highlights the fact that at the end of the CM dosing interval, where plasma levels trough, clinical assessment of sheep is necessary to ensure appropriate analgesia. Contrary to our hypothesis, the characteristics of SRM dosage were less consistent than CM over all sheep throughout the 72-h period. CM uniformly peaked among sheep at 4 h but fell below the therapeutic level within 12 to 24 h. As expected at the end of each 24-h dosing interval, CM plasma levels were at or below

the therapeutic level for almost all animals. In contrast, SRM plasma concentrations peaked at either 4 or 12 h after injection, depending on the animal, plasma levels varied over a wide range, and decreased below therapeutic levels between 24 to 72 h. A particular appeal of using an SR drug is the ability to prevent inconsistent or rapid declines in plasma levels within the acute postoperative phase, when pain is assumed to be most significant. However, according to our findings, the tested formulation of SRM did not behave reliably as an SR drug in all sheep, in that it failed to stably maintain plasma levels at or above 400 ng/mL for the entire 72-h period.

We performed a pilot dosing trial on a single sheep to determine the dose to use for the remaining animals in this study. In addition to the recommended dose of 1.5 mg/kg, we gave the pilot ewe a doubled dose (3 mg/kg,) in light of a prior study

Figure 2. Ratio of plasma levels of SRM:CM at each time point (gray region, 95% CI). Differences at 4, 12, 24, 60, 72, 84, and 96 h were significant ($P < 0.0001$).

Figure 3. Estimated means and 95% CI for each treatment at each time point (back-transformed from log scale). The solid and dotted lines indicate the estimated mean levels of CM and SRM, respectively, in the sheep in the current study, the horizontal dashed line represents the presumed therapeutic level (400 ng/mL), and the pink and blue areas show the respective 95% CI.

of SR buprenorphine that required this magnitude of dose to reach therapeutic levels.³⁹ Despite no observations of obvious side effects, according to physical exam, blood, and urine analysis, we chose not to repeat the 3-mg/kg dose in the rest of the sheep, because of the much higher than expected plasma levels. However, the 3-mg/kg dose of SRM successfully maintained therapeutic plasma levels for the full 72 h, whereas the 1.5-mg/kg dose did not. This preliminary information suggests that an initial dose of 3 mg/kg could safely be given for the 72-h duration; however, further investigation is needed to support this conclusion.

In light of the pharmacokinetic profiles we noted, we segregated the sheep results into 3 categories: rapid elimination, which showed a similar profile to CM and did not display a SR effect; slow elimination, with a profile similar to what we expect for a SR product and lasting until 72 h; and an intermediate profile between rapid and slow elimination. Similar separations of clearance rates of SRM have been described in other species.^{13,21} The variability of drug plasma concentrations we observed in our sheep could be due to a variety of reasons

Table 3. Creatinine and urine specific gravity in the pilot animal after 1.5 mg/kg SRM

Time (h)	Creatinine (mg/dL; reference interval, 0.5–1.0)	Urine specific gravity
0	1.1	1.005
24	3.0	1.008
48	3.7	1.009
120	1.5	1.011

Note the evidence of acute renal azotemia beginning at 24 h after administration, worsening at 48 h, and showing improvement of signs at 120 h.

Table 4. Creatinine and urine specific gravity in the pilot animal after a 14-d washout period and 3 mg/kg SRM

Time (h)	Creatinine (mg/dL; reference interval, 0.5–1.0)	Urine specific gravity
0	1.0	1.013
24	1.1	1.033
48	1.1	1.023
120	1.1	1.024

Note that the acute renal azotemia that occurred after 1.5 mg/kg SRM is not repeatable at the higher dose of SRM.

related to absorption and clearance. Meloxicam, similar to most NSAID, is highly protein bound (greater than 99%) and can be distributed into the extracellular space as well as penetrate other tissues³⁸ and may give rise to altered pharmacokinetics in situations where albumin concentration might vary (for example, surgical insult, disease states, perfusion changes, and inflammation). In the current study, we intentionally selected healthy sheep with similar demographics (that is, age, sex, and weight) to evaluate the pharmacokinetics of SRM in a relatively homogenous cohort. These animals likely lacked the disease states and inflammation in which an NSAID typically would be given and work most effectively. However, sheep were not screened beforehand for evidence of alterations in specific biomarkers of inflammation beyond what was available on hematology and chemistry panels. The results of our standard screening showed that enzymes of renal and hepatic origin as well as albumin levels were all within normal limits. Although not a part of this study, more specific blood tests could be used to further screen for inflammation in animals. Markers of inflammation including acute phase proteins, haptoglobin, serum amyloid A, and fibrinogen⁷ could all be measured by using standard lab tests or commercially available kits. In addition, although beyond the scope of this pilot study, genetic differences among animals, such as single-nucleotide polymorphisms in NSAID-metabolizing genes, could be explored. Alternatively, inconsistencies of the polymer matrix formulation pertaining to its release and absorption rates at the injection site might be present. Other reasons for pharmacokinetic differences might reflect characteristics of the individual animals, such as size, age, metabolism, and body composition,¹³ although we tried to acquire animals within similar ranges. As ruminants, sheep possess a complex gastric system that can affect drug distribution bioavailability.³⁴ In addition, differences may exist between the expression of cytochrome P450¹³ and biotransformation enzyme activity levels in the liver and other organs.^{33,35}

Meloxicam is considered a relatively safe NSAID in a variety of animal species because it selectively inhibits cyclooxygenase 2;¹⁰ yet caution should still be practiced when administering SR formulations due to differences in tissue exposure and depot

effect.¹³ Observation for possible side effects is recommended with any NSAID use, particularly gastric ulcers, gastrointestinal atony, and renal impairment,¹⁸ given that the primary mode of elimination of meloxicam is through the kidneys. As with all NSAID, meloxicam is contraindicated in animals with gastrointestinal disorders or impaired hepatic, cardiac, or renal function,⁴ and monitoring of blood work and urinalysis are highly recommended during therapy.³⁴ In our study, all clinical pathology results were reviewed by a board-certified veterinary clinical pathologist. The pilot animal during the 1.5-mg/kg SRM trial, had biochemistry and urinalysis data consistent with acute kidney injury starting at 24 h, worsening at 48 h, and resolving by 120 h. The animal did not exhibit any clinical signs associated with acute kidney injury, and the cause of the abnormality was not definitively determined. The sheep previously was housed on a farm but had no known exposure to any renal toxins or other nephrotoxic medications. In the absence of an inflammatory leukogram, fever, or any other clinical sign, pyelonephritis was ruled out. Perhaps the sheep had an idiosyncratic episode of meloxicam-induced nephrotoxicity, potentially precipitated by predisposing factors such as clinically unrecognized dehydration. However, the acute kidney injury was not reproducible, and renal parameters returned to baseline and within normal ranges prior to the subsequent SRM trial, when the doubled dose was used. Continued monitoring and biopsy with histopathology would be required for further characterization of the renal insult,⁸ but this option was not pursued, given that the animal was clinically healthy and subsequent bloodwork showed improvement. In addition, the remaining 5 sheep did not exhibit any biochemical evidence of acute kidney injury, thus suggesting that the overall risk of CM- or SRM-induced nephrotoxicity is low.

Studies evaluating meloxicam in ruminant species have been performed, but the reports refer only to pharmacokinetic data and do not include assessments of toxicity according to clinical signs, clinical pathology, or histopathology.^{20,33,34} Toxicologic overviews of meloxicam have been described for several other species,^{24,27} but to our knowledge, specific studies establishing toxic dose ranges for meloxicam in sheep are unavailable. In a published toxicology study of horses, oral administration of meloxicam at 3 to 5 times the recommended dose given daily over 2 wk was associated most commonly with gastrointestinal conditions and only one episode of renal damage.²⁷ Due to the fact that equine, rodent, and swine physiology differs from ruminants', we cannot definitively correlate the episode of transient acute renal injury in our sheep with toxic plasma drug levels in these other species. Given the lack of meloxicam-specific toxicologic data for sheep, we suggest that studies addressing toxicity analysis need to be performed to further characterize the safety profile of SRM in this species.

In this study, we identified several disadvantages associated with using SRM in sheep. The most important finding was the inability of SRM to maintain presumed therapeutic levels for the full 72 h as expected for an SR-formulated drug at a dose consistent with the manufacturer's recommendation. According to our pilot study, providing a higher dose (3 mg/kg) that maintained therapeutic plasma levels through 72 h would require twice the amount of drug. At 1.5 mg/kg, the volume needed for a typical sheep on our study (that is, 65 kg) was 9.8 mL, which was given subcutaneously; doubling this dose would require the injection of 19.6 mL. This dose also would incur a significant increase in cost, given that a single 5-mL, 10-mg/mL bottle was valued at US\$115 (US\$23 per mL) at the time of this study. Additional disadvantages of the drug formulation included its thick viscosity,

requiring the use of a large-bore needle (for example, 18 gauge); the need for adequate subcutaneous space in an animal to accommodate the large volume injection; and the manufacturer's storage requirement of refrigeration followed by 30 min to allow drug to reach room temperature prior to administration. For large species such as sheep, a higher concentration of SRM (for example, 20 mg/mL) would ease the preparation and administration by decreasing the amount needed to be delivered. Furthermore, reformulation of the polymer vehicle to enable the depot effect to extend to 72 h might make SRM a more suitable drug of choice for large-animal species.

We acknowledge limitations that potentially reduce the ability to generalize our current study results in the comprehensive sheep population. The major disadvantage is that the pharmacokinetic data were not evaluated in a clinical setting to determine the efficacy of the drug. A wide therapeutic range has been established for CM across several species,^{3,16,20,21,26,27,33} and whether these levels represent the true therapeutic threshold value for each individual animal is unknown. Although our study demonstrated that SRM reached higher peak plasma concentrations than CM, a blinded study comparing SRM with CM in a clinical surgery setting (for example, thoracotomy, sternotomy, orthopedic surgery) is required to establish a more accurate therapeutic range. Another drawback of the current study was the lack of a randomized cross-over design, as all sheep were provided the 2 drug formulations in the same order. Furthermore, our sample size was relatively small, potentially reducing the ability to detect a true effect in the population, and involved all female animals. We did not perform an in-depth analysis of drug safety and toxicity, with the inclusion of histologic analysis of injection sites. Additional evaluation of SRM is warranted to address these limitations.

In conclusion, in our study, SRM given once at 1.5 mg/kg did not maintain an assumed therapeutic level of 400 mg/mL over a 72-h period in sheep. Although SRM maintained presumed therapeutic levels for up to 48 to 60 h depending on the animal, it drastically declined from the blood prior to 72 h and did not behave consistently across animals. Based on our findings, we do not recommend the use of SRM at the dose of 1.5 mg/kg as a sole agent for analgesia in postoperative sheep until further investigation to characterize the safety profile and determine clinical efficacy is undertaken. If SRM is currently being used in this species, it is highly recommended to use a preemptive and multimodal analgesia regimen along with clinical assessments throughout the postoperative period.

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