Effects of intra-articular administration of lidocaine, mepivacaine, and the preservative methyl parahydroxybenzoate on synovial fluid biomarkers of horses

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OBJECTIVE
To compare the extent of inflammation and catabolic collagen response in the middle carpal joints (MCJs) of healthy horses following intra-articular injection of 2% lidocaine, 2% mepivacaine, lactated Ringer solution (LRS), or 0.1% methyl parahydroxybenzoate.

ANIMALS
17 adult horses.

PROCEDURES
In the first of 2 experiments, the left middle carpal joint (MCJ) of each of 12 horses was injected with 10 mL of 2% lidocaine (n = 3), 2% mepivacaine (3), or LRS (control; 6). After a 4-week washout period, the right MCJ of the horses that received lidocaine or mepivacaine was injected with 10 mL of LRS, and the right MCJ of horses that received LRS was injected with 10 mL of 2% lidocaine (n = 3) or 2% mepivacaine (3). In experiment 2, the left MCJ of each of 5 horses was injected with 10 mL of 0.1% methyl parahydroxybenzoate. After a 48-hour washout period, the right MCJ of each horse was injected with 10 mL of LRS. Synovial fluid (SF) samples were aseptically collected before and at predetermined times after each injection. Synovial fluid WBC count, neutrophil percentage, and total protein, neutrophil myeloperoxidase, neutrophil elastase, and Coll2-1 concentrations were compared among treatments.

RESULTS
Both lidocaine and mepivacaine induced SF changes indicative of inflammation and a catabolic collagen response, but the magnitude of those changes was more pronounced for lidocaine. Methyl parahydroxybenzoate did not cause any SF changes indicative of inflammation.

CONCLUSIONS AND CLINICAL RELEVANCE
Results suggested that mepivacaine was safer than lidocaine for intra-articular injection in horses. (*Am J Vet Res* 2020;81:479–487)

In horses, lameness is one of the most common health problems, and joint disease is the leading cause of lameness.[1] Localization of the focus of pain is essential during lameness investigations, and intra-articular injection of local anesthetics is routinely used for that purpose.[1,2]

Results of previous studies[3–6] suggest that intra-articular injection of local anesthetics may harm the joint cavity of horses and humans. In human patients that undergo arthroscopy, prolonged intra-articular administration of local anesthetics via a pain pump is associated with the development of chondrolysis.[5,6]

ABBREVIATIONS
ELT	Neutrophil elastase
LRS	Lactated Ringer solution
MCJ	Middle carpal joint
MPO	Neutrophil myeloperoxidase
SF	Synovial fluid
TP	Total protein

In horses, intra-articular injection of lidocaine and mepivacaine is associated with joint inflammation that is characterized by an increase in the SF WBC count, neutrophil percentage, and TP concentration,[4] and intra-articular injection of bupivacaine and lidocaine causes an increase in the SF concentrations of 2 biomarkers of cartilage matrix synthesis, which is suggestive of an anabolic effect.[3] Results of that study[3] also indicate that intra-articular injection of bupivacaine causes a decrease in the SF concentrations of 2 collagen degradation biomarkers. The anabolic effect caused by intra-articular injection of bupivacaine and lidocaine in that study[3] was attributed to an undetected cartilage insult despite the decrease in the SF concentrations of 2 collagen degradation biomarkers.

Results of in vitro studies indicate that local anesthetics have cytotoxic effects on chondrocytes of equine[7] and human origins.[8] In an in vitro study[9] involving human chondrocytes, the preservative so-

dium metabisulfite was associated with an increase in cell death, whereas the preservative methyl parahydroxybenzoate, which is commonly present in commercial lidocaine solutions, was not associated with any cytotoxic effects. To our knowledge, a study to determine whether the same happens in vivo or in equine chondrocytes has yet to be performed.

The extent of joint inflammation is routinely assessed by quantification of the TP concentration and total and differential WBC counts in SF.[10,11] For research purposes, SF concentrations of MPO and ELT are occasionally measured to evaluate intra-articular inflammation. Myeloperoxidase is an antibacterial heme enzyme, which is released from the azurophilic granules of neutrophils that have been exposed to inflammatory stimuli.[12,13] The MPO concentration is abnormally increased in the SF of dogs[13] and humans[14] with degenerative joint disease and horses with septic arthritis.[15] Elastase is a serine protease, which is released from the azurophilic granules of activated neutrophils during inflammatory processes.[16] An increase in the SF ELT concentration has been reported in human patients with arthritis[16] and horses with septic arthritis.[17]

Coll2-1 is a peptide located in the α1 and α3 chains of the triple helix of cartilage-specific collagen types II and XI. In horses and humans, the presence of Coll2-1 is indicative of articular collagen degradation, and SF Coll2-1 concentrations increase in individuals with osteochondral lesions,[18,19] osteoarthritis, and rheumatoid arthritis.[20]

The aim of the study reported here was to compare the extent of inflammation and catabolic collagen response in the joints of horses induced by intra-articular injection of 10 mL of a 2% lidocaine solution, 2% mepivacaine solution, 0.1% methyl parahydroxybenzoate solution, or LRS (control). Variables measured for comparison purposes included SF WBC count, neutrophil percentage, and TP, MPO, ELT, and Coll2-1 concentrations. We hypothesized that intra-articular injections of lidocaine and mepivacaine would cause joint inflammation and catabolism of the collagen network of cartilage and that adverse joint effects induced by lidocaine would be more pronounced than those induced by mepivacaine.

Materials and Methods

Animals

All study procedures were reviewed and approved by the ethical committee of the University of Copenhagen Large Animal Teaching Hospital and the Danish Animal Experiments Inspectorate (license No. 2015-15-0201-00608). The Danish Animal Experiments Inspectorate works under European Directive 2010/63/EU. All procedures were conducted in accordance with the Danish Animal Testing Act, and care of the horses was in accordance with institutional guidelines. All horses were owned by and maintained at the University of Copenhagen for teaching and research purposes.

The study consisted of 2 experiments. Experiment 1 involved 12 adult mares with a mean ± SD age of 11 ± 3.9 years, body weight of 553 ± 38.5 kg, and height of 160.6 ± 5.2 cm. Experiment 2 consisted of 5 adult geldings with a mean ± SD age of 6.8 ± 2.7 years, body weight of 485 ± 29.1 kg, and height of 157.2 ± 4.8 cm. The horses of each experiment were determined to be healthy, without clinical evidence of disease in the carpal joints, and free of lameness on the basis of results of physical and lameness examinations that were performed immediately before initiation of each experiment. For each horse, lameness was subjectively assessed by each of 2 experienced veterinarians (one of whom was certified by the European College of Veterinary Surgery as a specialist in equine surgery) on 2 separate occasions. Each horse was evaluated while walking and trotting in a straight line on hard and soft surfaces and while traveling in a circle (lunged) in both directions at a walk, trot, and canter on a soft surface and at a walk and trot on a hard surface.

All horses were housed individually in stalls (3 × 3 m) on the days when the assigned treatment was injected and SF samples were collected. At all other times, the horses were maintained in separate stalls with free access to a paddock (3 × 6 m). Horses were fed 100 g of concentrate[a]/100 kg body weight and had ad libitum access to hay and water.

Experiment 1

Experiment 1 had a randomized crossover design with 2 phases and a 4-week washout period between phases. All assigned treatments were injected intra-articularly in the left MCJ during phase 1 and the right MCJ during phase 2. In phase 1, each of 12 mares was randomly assigned to 1 of 2 groups (groups 1 and 2; 6 horses/group) by means of picking numbers from a bowl. Within group 1, horses were randomly assigned by means of picking numbers from a bowl to receive 10 mL of a 2% mepivacaine solution[b] (n = 3) or LRS (control; 3). The same method was used to randomly assign the horses of group 2 to receive 10 mL of a 2% lidocaine hydrochloride solution[b] (n = 3) or LRS (3). During phase 2, the horses of group 1 that received mepivacaine in phase 1 were administered 10 mL of LRS, and those that received LRS in phase 1 received 10 mL of a 2% lidocaine solution. Similarly, the horses of group 2 that received lidocaine in phase 1 were administered 10 mL of LRS, and those that received LRS in phase 1 received 10 mL of a 2% mepivacaine solution. Thus, for experiment 1, 6 MCJs (3 right and 3 left) were injected with lidocaine, 6 MCJs (3 right and 3 left) were injected with mepivacaine, and 12 MCJs (6 right and 6 left) were injected with LRS (control joints).

Experiment 2

Results of experiment 1 revealed significant differences between SF variables of lidocaine-treated and mepivacaine-treated joints. The mepivacaine so-

lution did not contain any additives, whereas the lidocaine solution contained 0.1% methyl parahydroxybenzoate as a preservative. Therefore, experiment 2 was conducted to determine whether any of the differences observed between treatments in experiment 1 could be attributed to the effects of methyl parahydroxybenzoate.

In experiment 2, each of 5 geldings received an intra-articular injection of 10 mL of a 0.1% methyl parahydroxybenzoate solution in the left MCJ. Forty-eight hours later, each horse received an intra-articular injection of 10 mL of LRS (control) in the right MCJ.

Experimental procedures

In both phases of experiment 1, each horse was clinically evaluated immediately before (0 hours; baseline) and every 2 hours for 24 hours after injection of the assigned treatment (ie, at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 hours) and then every 24 hours thereafter for 14 days. In experiment 2, each horse was clinically evaluated at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, and 48 hours after injection of the assigned treatment. Each evaluation included assessment of the horse's general appearance, heart rate, respiratory rate, and rectal temperature. Lameness was also assessed with the horse walking on a hard surface.

During both phases of experiment 1, the circumference of and range of motion for the injected joint and the skin temperature of the injected and contralateral joints were determined at 0, 6, 12, 24, and 48 hours and 7 and 14 days after injection of the assigned treatment. The hair from a small area at the palmer aspect of the injected joint was clipped to create a landmark to ensure that all joint circumference measurements were obtained at the same location. A tape measure was used to measure joint circumference with the dorsalmost edge of the tape measure positioned over the joint space of the injected MCJ.

For each horse, a goniometer[c] was used to measure the angle of the injected MCJ when the carpus was flexed to the maximum extent allowed by the subject. This angle was used as a proxy for the range of motion for the injected joint. Briefly, the injected carpus was gently flexed until the horse resisted further flexion or full carpal flexion was achieved. The injected joint was recorded as having full range of motion whenever full carpal flexion was achieved without resistance.

Infrared thermography[d] was used to measure the skin temperature at the medial and lateral aspects of both the injected and contralateral MCJs. At each assessment time, the mean skin temperature was calculated for each joint and used for analysis purposes. All skin temperature measurements were obtained in the same windowless room with all doors closed. The mean skin temperature of the contralateral joint served as a control for each assessment time to account for variation in the environmental temperature and potential diurnal variations in skin temperature.

Arthrocentesis of the injected MCJ was aseptically performed at 0, 6, 12, 24, and 48 hours and 7 and 14 days after injection of the assigned treatment for the horses of experiment 1 and at 0, 6, 12, 24, and 48 hours after injection of the assigned treatment for the horses of experiment 2. When deemed necessary, horses were sedated with xylazine[e] (200 to 300 mg, IV) prior to arthrocentesis. During each arthrocentesis procedure, 3 mL of SF was obtained and immediately divided into two 1.5-mL aliquots. One aliquot was placed into a sterile blood collection tube without any additives for determination of TP concentration. The other aliquot was placed into a blood collection tube containing EDTA (EDTA tube) for determination of total and differential WBC counts and MPO, ELT, and Coll2-1 concentrations. The TP concentration and WBC counts were determined within 12 hours after sample collection. The remainder of the SF sample in the EDTA tube was centrifuged at 2,500 × *g* and 4°C for 10 minutes. The supernatant was decanted, placed in a cryovial, and stored at −80°C until quantification of MPO, ELT, and Coll2-1 concentrations, which was performed within 6 months after SF sample collection.

Laboratory analyses

For each SF sample, the TP concentration was determined by use of a refractometer,[f] and the WBC count was determined manually by use of a hemacytometer.[g] Differentiated WBC counts were performed manually by microscopic evaluation of cytospin slides stained with hemacolor stain,[g] and then the neutrophil percentage was calculated. Synovial fluid concentrations of MPO, ELT, and Coll2-1 were determined by use of equine-specific ELISAs[h] as described[21,22] in accordance with the manufacturer's instructions. For each SF sample, the MPO, ELT, and Coll2-1 concentrations were determined in duplicate, and the mean was used for analysis purposes.

Statistical analysis

Descriptive data were derived by use of a commercially available statistical software program.[i] For each joint, the mean skin temperature difference was calculated as the difference in the mean skin temperature for the joint between 6 and 48 hours after injection of the assigned treatment. Paired *t* tests were used to compare the mean skin temperature difference between the injected joint and the untreated contralateral joint during each phase of experiment 1. Then, for each lidocaine- and mepivacaine-treated joint, the mean skin temperature of the untreated contralateral joint was subtracted from the mean skin temperature of the injected joint. An unpaired *t* test was used to compare the resulting differences between lidocaine-treated and mepivacaine-treated joints.

Outcome variables of interest were SF WBC count, neutrophil percentage, and TP, MPO, ELT, and Coll2-1 concentrations for experiment 1 and SF WBC

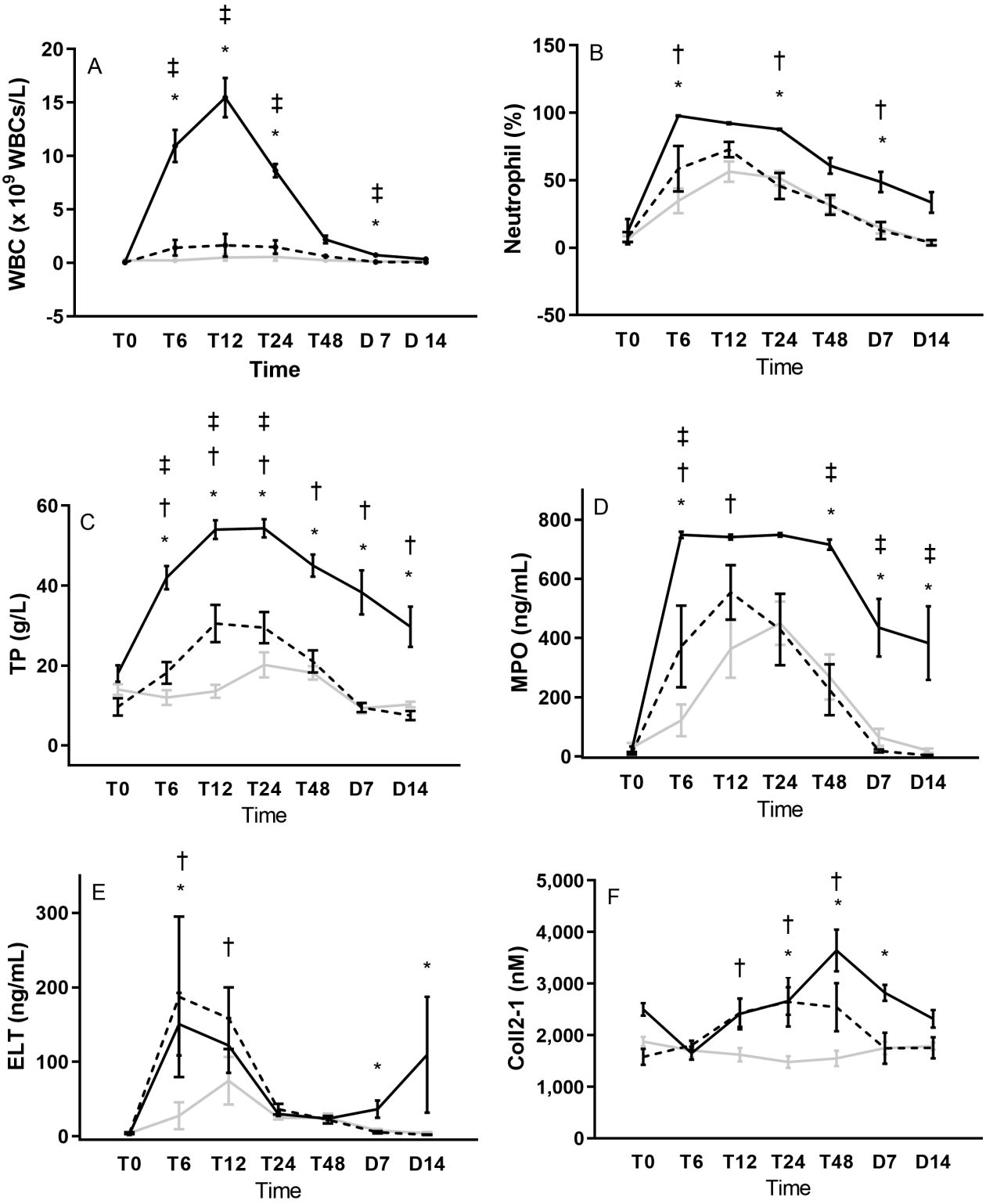

Figure 1—Mean ± SEM SF WBC count (A), neutrophil percentage (B), and TP (C), MPO (D), ELT (E), and Coll2-1 (F) concentrations for MCJs of 12 healthy adult horses before (T0) and at 6 (T6), 12 (T12), 24 (T24), and 48 (T48) hours and 7 (D7) and 14 (D14) days after intra-articular injection of 10 mL of a 2% lidocaine solution (black line; n = 6), a 2% mepivacaine solution (dashed line; 6), or LRS (gray line; 12 [experiment 1]). For each of the 12 horses, the left MCJ was randomly assigned to be injected with 10 mL of 2% lidocaine (n = 3), 2% mepivacaine (3), or LRS (control; 6). After a 4-week washout period, the right MCJ of the horses that previously received lidocaine or mepivacaine was injected with 10 mL of LRS, and the right MCJ of horses that previously received LRS was injected with 10 mL of 2% lidocaine (n = 3) or 2% mepivacaine (3). *Within a specific time, mean differs significantly ($P < 0.05$) between lidocaine-injected and LRS-injected joints. †Within a specific time, mean differs significantly ($P < 0.05$) between mepivacaine-injected and LRS-injected joints. ‡Within a specific time, mean differs significantly ($P < 0.05$) between lidocaine-injected and mepivacaine-injected joints.

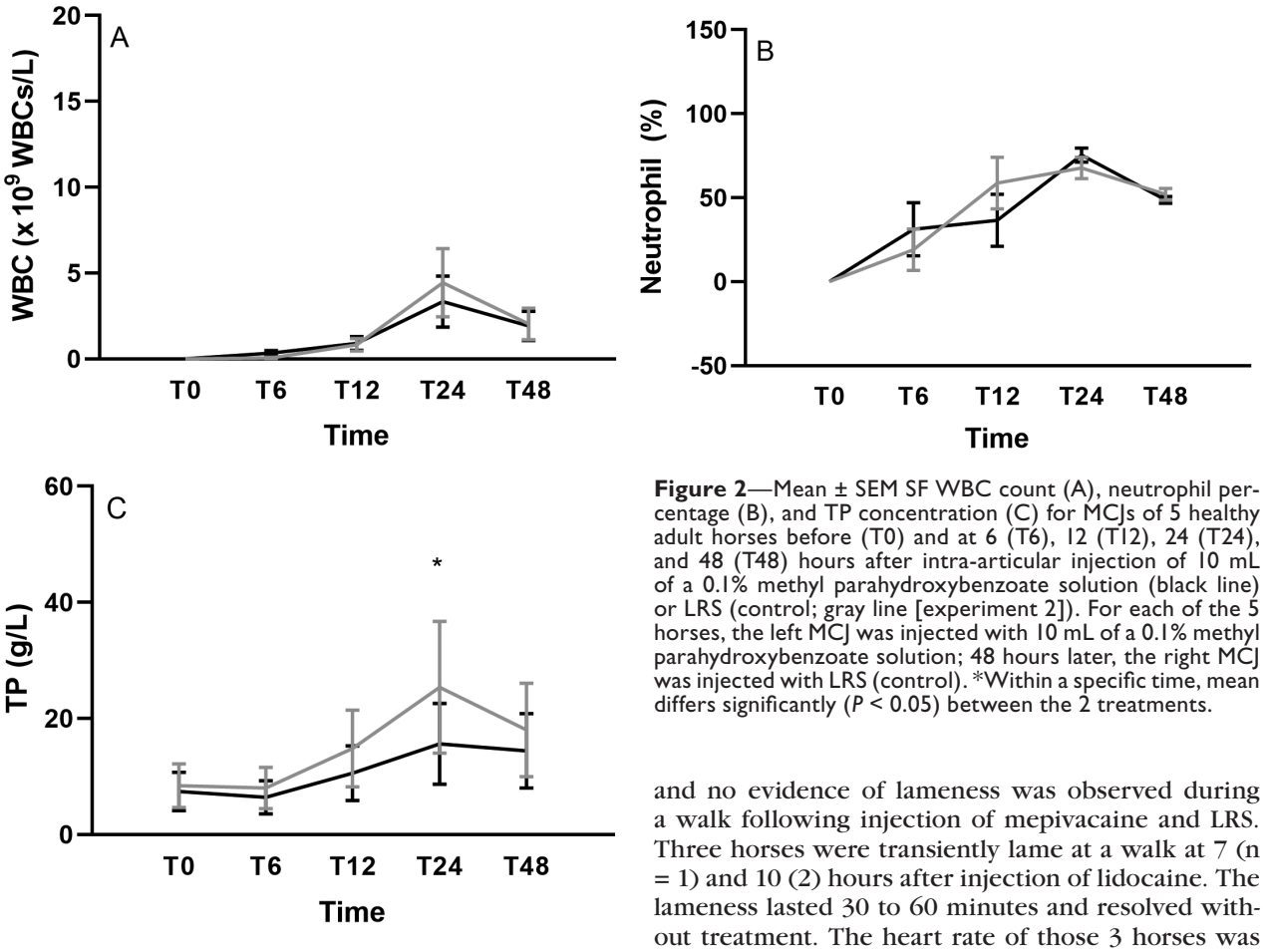

Figure 2—Mean ± SEM SF WBC count (A), neutrophil percentage (B), and TP concentration (C) for MCJs of 5 healthy adult horses before (T0) and at 6 (T6), 12 (T12), 24 (T24), and 48 (T48) hours after intra-articular injection of 10 mL of a 0.1% methyl parahydroxybenzoate solution (black line) or LRS (control; gray line [experiment 2]). For each of the 5 horses, the left MCJ was injected with 10 mL of a 0.1% methyl parahydroxybenzoate solution; 48 hours later, the right MCJ was injected with LRS (control). *Within a specific time, mean differs significantly ($P < 0.05$) between the 2 treatments.

count, neutrophil percentage, and TP concentration for experiment 2. For each experiment, linear mixed models were used to assess the effects of treatment (lidocaine, mepivacaine, or LRS for experiment 1 and methyl parahydroxybenzoate or LRS for experiment 2), SF sample acquisition time (time), and the interaction between treatment and time on each outcome variable of interest. Each model included a random effect to account for repeated measures within each horse. The Bonferroni correction was used for post hoc pairwise comparisons when necessary. For each fitted model, residuals were visually assessed for normality. A logarithmic transformation was applied to the SF WBC count and ELT concentration data for experiment 1 and to the SF WBC count data for experiment 2 to normalize the distributions of the respective residuals. A statistical software program[j] was used to fit each model[k] and for post hoc pairwise comparisons of least squares means[23] as described. Values of $P < 0.05$ were considered significant.

Results

Experiment 1

For the 12 horses of experiment 1, rectal temperature (range, 37.2°C to 38.5°C), heart rate (range, 32 to 44 beats/min), and respiratory rate (range, 12 to 20 breaths/min) remained within reference limits, and no evidence of lameness was observed during a walk following injection of mepivacaine and LRS. Three horses were transiently lame at a walk at 7 (n = 1) and 10 (2) hours after injection of lidocaine. The lameness lasted 30 to 60 minutes and resolved without treatment. The heart rate of those 3 horses was increased from baseline and ranged from 52 to 80 beats/min for 30 to 240 minutes after lidocaine injection. The other 3 horses that received the lidocaine treatment did not become noticeably lame and did not have an increased heart rate.

The circumference of the injected joint did not change significantly following administration of the assigned treatment in any horse. The range of motion (ie, joint angle at maximum allowed flexion) for the injected joint changed substantially following administration of the assigned treatment for only 1 of the 12 horses. For that horse, the joint angle for the MCJ was 29° prior to (baseline) injection of lidocaine and 60° at 14 days after injection of lidocaine.

The mean skin temperature difference between the injected joint and untreated contralateral joint was significant for all 3 treatments and was greatest for the mepivacaine treatment (2.8°C; $P = 0.034$) followed by the LRS (1.8°C; $P = 0.011$) and lidocaine (1.3°C; $P = 0.004$) treatments. After controlling for the skin temperature of the untreated contralateral joint (ie, effect of environmental temperature and diurnal variation), the skin temperature for mepivacaine-injected joints was significantly ($P = 0.033$) greater than that for lidocaine-injected joints by a mean of 1.6°C.

The mean SF WBC count, neutrophil percentage, and TP, MPO, ELT, and Coll2-1 concentrations for the lidocaine-injected joints were significantly ($P < 0.001$

for all comparisons) greater than the corresponding mean values for the LRS-injected joints **(Figure 1)**. The mean SF TP, MPO, ELT, and Coll2-1 concentrations for the mepivacaine-injected joints were significantly ($P < 0.001$ for all comparisons) greater than the corresponding mean values for the LRS-injected joints; however, the mean SF WBC count ($P = 0.010$ [after Bonferroni correction, for which values of $P < 0.008$ were considered significant]) and neutrophil percentage ($P = 0.053$) did not differ significantly between the mepivacaine-injected and LRS-injected joints.

Lidocaine-injected joints had significantly greater mean SF WBC count ($P < 0.001$), neutrophil percentage ($P = 0.002$), and TP ($P < 0.001$) and MPO ($P = 0.001$) concentrations, compared with mepivacaine-injected joints (Figure 1). However, the mean SF ELT ($P = 0.07$) and Coll2-1 ($P = 0.112$) concentrations did not differ significantly between lidocaine-injected and mepivacaine-injected joints.

Experiment 2

Owing to the unexpected severe inflammatory response induced by the lidocaine treatment in experiment 1, we decided to investigate whether methyl parahydroxybenzoate, the preservative in the lidocaine solution, contributed to that response. For all 5 horses of experiment 2, rectal temperature, heart rate, and respiratory rate remained within reference limits, and no evidence of lameness was observed following injection of methyl parahydroxybenzoate. The mean SF TP concentration for the methyl parahydroxybenzoate–injected joints was significantly ($P = 0.003$) less than that for the LRS-injected joints **(Figure 2)**. However, the mean SF WBC count ($P = 0.905$) and neutrophil percentage ($P = 0.839$) did not differ between the methyl parahydroxybenzoate–injected and LRS-injected joints.

Discussion

In the present study, a single intra-articular injection of lidocaine or mepivacaine into the MCJ of nonlame horses resulted in joint inflammation and a catabolic collagen response. The inflammatory responses were more severe in lidocaine-injected joints than in mepivacaine-injected joints. In fact, 3 of the 6 horses that received the lidocaine treatment became transiently lame following the intra-articular injection, whereas none of the horses that received the mepivacaine treatment became lame. Joint inflammation following intra-articular injection of lidocaine and mepivacaine was demonstrated in horses of another study.[4] However, to our knowledge, the present study was the first to evaluate biomarkers of collagen catabolism in conjunction with inflammation following intra-articular injection of lidocaine or mepivacaine in horses. In the present study, SF concentrations of biomarkers associated with inflammation peaked within 24 hours after injection of the local anesthetic. Synovial fluid concentrations of Coll2-1, a biomarker of collagen degradation, peaked at 24 hours after injection of mepivacaine and at 48 hours after injection of lidocaine. The increase in SF Coll2-1 concentration was much more pronounced following intra-articular injection of lidocaine than after intra-articular injection of mepivacaine. The delayed and more pronounced peak in SF Coll2-1 concentration for lidocaine-injected joints relative to mepivacaine-injected joints was likely a reflection of the ongoing and more severe inflammation induced by lidocaine.

Other researchers have investigated synovial inflammatory responses induced following intra-articular injection of local anesthetics in horses[4] and rabbits.[24] In horses, an intra-articular injection of 5 mL of a 2% mepivacaine solution or 2% lidocaine solution in an MCJ resulted in an increase in the SF WBC count, neutrophil percentage, and TP concentration throughout the 48-hour postinjection observation period.[4] In rabbits, an intra-articular injection of bupivacaine in the stifle joint caused signs of inflammation in the articular cartilage and synovium (as determined on postmortem evaluation) for 10 days following injection.[24] The increases in the mean SF TP and MPO concentrations observed for the horses of experiment 1 of the present study suggested that joint inflammation may persist for > 14 days after intra-articular injection of lidocaine.

Concurrent evidence of joint inflammation and a catabolic collagen response after intra-articular injection of lidocaine or mepivacaine in the horses of the present study supported the theory that there is a close relationship between synovitis and cartilage catabolism.[25–27] Synovitis and inflammation of other synovial structures can have an adverse effect on articular cartilage and lead to its degradation. Proinflammatory cytokines enhance articular cartilage matrix enzyme production and activation,[28] and there is extensive interaction between articular cartilage and the synovium.[25] Additionally, synovitis may contribute to progression of chondropathy by accelerating the catabolism of articular cartilage.[25] The presence of neutrophils in the SF has an important role in cartilage catabolism because activated neutrophils facilitate the degradation of proteoglycans and collagen in intact articular cartilage[29] through the release of ELT[30] and MPO.[31,32] The serine protease ELT exerts its degradative effect by cleaving the proteoglycan core protein and collagen telopeptide of articular cartilage.[29] Myeloperoxidase exerts its degradative effect on articular cartilage through its product hypochlorous acid, which degrades *N*-acetylglucosamine, chondroitin sulfate, and hyaluronic acid in articular cartilage.[31] In human medicine, intra-articular inflammation is positively correlated with and predictive of the severity of chondropathy in arthritic joints,[25] and the extent of joint effusion and synovitis detected by MRI is predictive of future cartilage loss.[26]

Continuous intra-articular administration of bupivacaine has adverse effects on joint structures,

which has led to concerns regarding the safety of intra-articular administration of all local anesthetics.[6,33] However, bupivacaine is the most chondrotoxic among currently available local anesthetics,[7,8] and investigation of the effects of potentially less harmful and more commonly used local anesthetics, such as lidocaine and mepivacaine, following intra-articular administration to horses is warranted. Expert opinion suggests that, in horses, joint problems are more likely to develop following intra-articular injection of lidocaine than after intra-articular injection of mepivacaine.[2] Prior to the present study, intra-articular injection of lidocaine to horses had been evaluated in only 2 studies,[3,4] and a clinical response was not observed following intra-articular injection of lidocaine in either of those studies. The SF concentrations of inflammatory biomarkers observed in the horses of the present study indicated that lidocaine was more irritating and induced more inflammation within the joint than did mepivacaine. Other researchers[2,34] suggested that was the case, albeit without data to support the claim. These findings were corroborated by results of an in vitro study[7] that indicate lidocaine is more toxic than mepivacaine to equine chondrocytes.

Investigators of another study[4] reported that, in horses, indices of inflammation (SF WBC count, neutrophil percentage, and TP concentration) did not differ significantly between joints that were injected with lidocaine and those injected with mepivacaine. It is unclear why the results of that study[4] differ from the findings of the present study, but differences in the doses of the local anesthetics administered might have played a role. An MCJ was injected with 5 mL of a 2% lidocaine solution in the other study,[4] compared with 10 mL of a 2% lidocaine solution in the present study. Results of in vitro studies[35–37] indicate that local anesthetics have dose-dependent cytotoxic effects.

In experiment 1 of the present study, we were surprised by the transient lameness induced in 3 of 6 horses and pronounced joint inflammation induced in all 6 horses after intra-articular injection of a single dose of lidocaine, especially when compared with horses that received an intra-articular injection of mepivacaine. The lidocaine solution used contained methyl parahydroxybenzoate (1 mg/mL) as a preservative, whereas the mepivacaine solution did not. Experiment 2 was conducted to investigate whether the methyl parahydroxybenzoate might have contributed to the inflammation observed in the lidocaine-injected joints of experiment 1. None of the 5 horses became lame following intra-articular injection of a 0.1% methyl parahydroxybenzoate solution, and none of the methyl parahydroxybenzoate–injected joints developed SF changes consistent with severe inflammation. In fact, the mean SF TP concentration for methyl parahydroxybenzoate–injected joints was significantly lower than that for LRS-injected (control) joints, which suggested that methyl parahydroxybenzoate caused less joint inflammation than did the control treatment. To our knowledge, the present study was the first to evaluate the in vivo effects of methyl parahydroxybenzoate on inflammatory biomarkers in SF, although results of in vitro studies indicate that the preservative is not cytotoxic to human[9] or canine[38] articular chondrocytes.

Further research is necessary to determine whether the SF changes observed following intra-articular injection of a single dose of lidocaine in the present study translate into clinical problems. Such research should involve in vivo studies in which macroscopic and histologic changes in synovial tissues are evaluated following intra-articular injection of lidocaine. On the basis of currently available scientific literature and the results of the present study, we advise clinicians to use mepivacaine rather than lidocaine for intra-articular administration in horses.

Studies involving a larger number of horses than was evaluated in the present study are necessary to determine whether lidocaine can be administered intra-articularly safely without any long-term adverse effects. Results of the present study indicated that the mean SF Coll2-1 concentration increased after intra-articular injection of both lidocaine and mepivacaine, but it did not differ significantly between lidocaine-injected and mepivacaine-injected joints. This suggested that both drugs caused a catabolic collagen response following intra-articular injection. To our knowledge, the present study was the first to describe a catabolic collagen response for articular cartilage following a single intra-articular injection of a local anesthetic to horses. Investigators of another study[3] reported an anabolic effect on the articular cartilage of horses following intra-articular injection of local anesthetics; however, they speculated that the observed increases in anabolic biomarkers were the result of an undetected cartilage insult.

Myeloperoxidase and ELT have been assessed in the SF of horses and dogs in previous studies[13,15,17,22] and determined to be biomarkers of joint infection.[15,17] Myeloperoxidase is also a biomarker for nonseptic joint inflammation,[13,15] but it does not appear to be useful for detection of osteochondrosis in the tarsocrural joints of horses.[22] Evaluation of both MPO and ELT concentrations in SF is useful in studies such as the present study in which joint inflammation and articular cartilage catabolism are investigated simultaneously because they serve as biomarkers of inflammation and are involved in cartilage catabolism.[39,40] The presence of MPO and ELT in SF might induce articular cartilage damage. It is possible that the increase in SF MPO and ELT concentrations may have contributed to the increase in SF Coll2-1 concentrations observed in both the lidocaine-injected and mepivacaine-injected joints evaluated in the present study. The evidence of concurrent inflammation and a catabolic collagen response observed in the joints of the present study corroborated the existence of a close relationship between those 2 processes.[25,26] However, cause-effect relationships were not evalu-

ated among the biomarkers assessed in the present study, and further research is necessary to elucidate the effects of MPO and ELT in the synovial cavities of horses.

The present study was not without limitations. The effects of intra-articular injection of local anesthetics were evaluated only in apparently healthy horses that were free of clinical joint disease. In clinical practice, intra-articular administration of local anesthetics is likely to be performed in joints with preexisting inflammation or osteoarthritis. Therefore, the effects of intra-articular injection of local anesthetics in diseased joints warrant investigation. Results of an in vitro study[8] involving human articular cartilage explants indicate that, following exposure to various local anesthetics, the death rate for chondrocytes obtained from osteoarthritic joints was greater than that for chondrocytes obtained from healthy joints. Only changes in SF were evaluated for the joints of the present study; gross pathological and histologic assessments of the articular cartilage and synovium were not performed. Such evaluations are necessary to more fully elucidate the clinical consequences of the observed alterations in the SF. Additionally, the effect of only a single clinically relevant dose of a local anesthetic on SF variables was assessed. Results of in vitro studies[8,35] indicate that the cytotoxic effects of local anesthetics are dose dependent. Therefore, in vivo assessment of the effects of intra-articular injection of different doses of local anesthetics is warranted. Finally, the intra-articular effects of local anesthetics were assessed in only the MCJs for the horses of the present study. Results of in vitro studies[41,42] involving cartilage explants from human knee and ankle joints suggest that the catabolism of articular cartilage differs among joints. Thus, the catabolic collagen response induced by local anesthetics in the MCJ may differ from that induced in other joints.

Results of the present study indicated that a single intra-articular injection of lidocaine or mepivacaine in the MCJ of horses induced an increase in SF concentrations of inflammatory biomarkers for at least 14 days and a transient increase in SF Coll2-1 concentration, which was suggestive of an articular collagen insult. The SF alterations were more pronounced for lidocaine-injected joints than for mepivacaine-injected joints. Intra-articular injection of 0.1% methyl parahydroxybenzoate, a preservative contained in most commercial lidocaine solutions, did not induce any changes in SF variables indicative of inflammation. Thus, the SF differences observed between lidocaine-injected and mepivacaine-injected joints were likely a function of differences in the properties of the active ingredients. Despite the absence of data regarding the long-term effects associated with intra-articular administration of local anesthetics, the findings of the present study suggested that mepivacaine is safer than lidocaine for intra-articular injection in horses.

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The authors declare that there were no conflicts of interest.

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Footnotes

a. Salvana, Klein Offenseth-Sparrieshoop, Germany.
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c. Saehan Instrument Co Ltd, Busan, Republic of Korea.
d. Raynger MX4, Raytek, Berlin, Germany.
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g. Paul Marienfeld GmbH and Co KG, Lauda-Königshofen, Germany.
h. BiopTis, Liège, Belgium.
i. Prism, version 8.00 for Windows, GraphPad Software, San Diego, Calif.
j. R, version 3.6.2, R Foundation for Statistical Computing, Vienna, Austria. Available at: www.R-project.org. Accessed Dec 23, 2019.
k. nlme: linear and nonlinear mixed effects models, R package version 3.1-137, Pinheiro J, Bates D , DebRoy S, et al. Available at: cran.r-project.org/web/packages/nlme/index.html. Accessed Jul 23, 2019.

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This article was updated on June 15, 2020 to reflect the following changes:

Throughout the report, the text was changed to indicate that 0.1% (not 1%) methyl parahydroxybenzoate was injected into the left middle carpal joint of the horses in experiment 2. In addition, a change was made in the Results section to indicate that values of $P < 0.008$ were considered significant for the Bonferroni correction used for post hoc pairwise comparisons in the linear mixed models.

The last sentence of the Experiment 1 subsection of the Materials and Methods section was changed to read, "Thus, for experiment 1, 6 MCJs (3 right and 3 left) were injected with lidocaine, 6 MCJs (3 right and 3 left) were injected with mepivacaine, and 12 MCJs (6 right and 6 left) were injected with LRS (control joints)." The second sentence of the first paragraph of the Discussion section was changed to read, "The inflammatory responses were more severe in lidocaine-injected joints than in mepivacaine-injected joints." Finally, the last sentence of the first paragraph of the Discussion section was changed to read, "The delayed and more pronounced peak in SF Coll2-1 concentration for lidocaine-injected joints relative to mepivacaine-injected joints was likely a reflection of the ongoing and more severe inflammation induced by lidocaine."