Received: 28 July 2020 | Accepted: 19 November 2020

DOI: 10.1111/evj.13396

GENERAL ARTICLE

Equine Veterinary Journal WILEY

Treatment effects of intra-articular triamcinolone acetonide in an equine model of recurrent joint inflammation

Clodagh M. Kearney[1] | Nicoline M. Korthagen[2] | Saskia G. M. Plomp[2] | Margot C. Labberté[1] | Janny C. de Grauw[2] | P. R. van Weeren[2] | Pieter A. J. Brama[1]

[1]UCD School of Veterinary Medicine, University College Dublin, Dublin, Ireland

[2]Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Correspondence
Clodagh M. Kearney, UCD School of Veterinary Medicine, University College Dublin, Dublin, Ireland.
Email: clodagh.kearney@ucd.ie

Funding information
This study was partly funded by the UCD WELLCOME Institutional Strategic Support Fund Clinical Primer Scheme, UCD Foundation, and the Dutch Arthritis Association (LLP-22)

Abstract

Background: Intra-articular triamcinolone acetonide is a widely used treatment for joint inflammation despite limited scientific evidence of its efficacy.

Objectives: To investigate if intra-articular triamcinolone acetonide has sustained anti-inflammatory effects using an equine model of repeated joint inflammation.

Study design: Randomised controlled experimental study.

Method: For three consecutive cycles 2 weeks apart, inflammation was induced in both middle carpal joints of eight horses by injecting 0.25 ng lipopolysaccharide (LPS). After the first LPS injection only, treatment with 12 mg triamcinolone acetonide (TA) followed in one randomly assigned joint, while the contralateral joint was treated with sterile saline (control). Clinical parameters (composite welfare scores, joint effusion, joint circumference) were recorded and synovial fluid samples were analysed for various biomarkers (total protein, WBCC; PGE_2; CCL2; TNFα; MMP; GAGs; C2C; CPII) at fixed timepoints (post injection hours 0, 8, 24, 72 and 168). The effects of time and treatment on clinical and synovial fluid parameters and the presence of time–treatment interactions were tested using a linear mixed model for repeated measures with horse as a random effect, and time and treatment as fixed effects.

Results: The TA treated joints showed significantly higher peak synovial GAG concentrations (Difference in means 283.1875 μg/mL, 95% CI 179.8, 386.6, $P < 0.000$), and PGE_2 levels (Difference in means 77.8025 pg/mL, 95% CI 21.2, 134.4, $P < 0.007$) after the first inflammation induction. Significantly lower TP levels were seen with TA treatment after the second induction (Difference in means −7.5 g/L, 95% CI −14.8, −0.20, $P < 0.04$) . Significantly lower WBCC levels were noted with TA treatment after the first (Difference in means -23.7125×10^9 cells/L, 95% CI −46.7, −0.7, $P < 0.04$) and second (Difference in means -35.95×10^9 cells/L, 95% CI −59.0, −12.9, $P < 0.002$) inflammation inductions. Significantly lower general MMP activity was also seen with TA treatment after the second inflammation inductions (Difference in means −51.65 RFU/s, 95% CI −92.4, −10.9, $P < 0.01$).

Main limitations: This experimental study cannot fully reflect natural joint disease.

Conclusions: In this model, intra-articular TA seems to have some anti-inflammatory activity (demonstrated by reductions in TP, WBCC and general MMP activity) up to

The abstract is available in French in the Supporting Information section of the online version of this article.

2 weeks post treatment but not at 4 weeks. This anti-inflammatory effect appeared to outlast a shorter-lived, potentially detrimental effect illustrated by increased synovial GAG and PGE_2 levels after the first induction.

KEYWORDS

horse, triamcinolone acetonide, joint, inflammation, lipopolysaccharide

1 | INTRODUCTION

Intra-articular administration of corticosteroids is a common intervention for the treatment of osteoarthritis (OA) in humans and horses.[1] While this practice is well-established, to date there is minimal or conflicting evidence regarding its efficacy.[1-3] Systematic reviews of the existing data have shown that while intra-articular corticosteroids may cause short-term relief of some clinical signs, the overall quality of evidence for short-term relief of clinical signs is low.[1,4] Basic scientific studies have not to date provided concrete answers regarding its efficacy; empirically prolonged effects and side effects are assumed, but discrepancies have been noted, particularly between in vitro and in vivo studies.[5] For example, a study by Dechant et al.[6] showed that corticosteroids (methylprednisolone and triamcinolone) did not protect from the inflammatory effects of IL-8 conditioning and supressed cartilage metabolism (as illustrated by GAG metabolism), whereas a different study by Bolt et al.[7] concluded that triamcinolone protected chondrocytes from the harmful inflammatory effects of lipopolysaccharide (LPS). In vivo models have shown similarly mixed findings. A study using the osteochondral fragment model reported favourable effects of TA on lameness and on some synovial fluid, synovial membrane and articular cartilage morphological parameters.[8] Another study investigating the effects of repeated injections of TA on normal joints suggested deleterious effects on cartilage metabolism.[9] There is a need for more information to allow us to make evidence-based conclusions about the effects of corticosteroids on cartilage and inflammation.[10]

Synovial fluid biomarkers in horses have been extensively studied[11] and changes have been used as outcome measures in studies investigating the effects of various interventions and therapeutics.[12,13] Intra-articular low dose lipopolysaccharide (LPS) injection is a well-established model in horses for induction of inflammation, which has now been widely used to study the anti-inflammatory potential of various therapeutics.[13-15]

While the intra-articular LPS model stimulates a reliable intra-articular inflammation, clinical symptoms are generally transient, typically resolving within 48 hours and the self-limiting nature of this inflammation could be regarded as a limitation, as it is not reflective of natural disease where recurrent episodes of inflammation play a crucial role in development and progression of OA.[16,17] Recently, we have refined and expanded the equine LPS model by introducing repeated low dose LPS inductions every 2 weeks within the same joint[18] which we believe make the model more suitable for investigation of longer term effects of therapeutics and provide a better translational model for clinical conditions such as OA.

In this study we use repeated inductions of LPS in an equine bilateral middle carpal joint model to investigate direct and potential prolonged effects of the most commonly used corticosteroid, triamcinolone acetonide, on various clinical and synovial parameters. We hypothesised that a single clinically relevant dose of triamcinolone acetonide would significantly ameliorate the repeated inflammatory effects of LPS over a prolonged period of time in the horse.

2 | MATERIALS AND METHODS

2.1 | Experimental animals

Eight horses (16 joints) were selected to participate in a randomised controlled experiment. Horses were of mixed breed (6 mares and 2 geldings; mean ± SD age 14.6 ± 2.4 years, bodyweight 370.4 ± 27.6 kg) from a research herd, with no known history of forelimb orthopaedic disease, free from forelimb lameness and with clinically and radiographically normal carpal joints. Horses were stabled individually in single boxes (4 m × 4 m) on wood shavings and were familiar with their environment. Horses received concentrates once daily, with regular hay and water provided ad libitum. After each induction with LPS and the subsequent week of sampling and measurements, the horses had a week of turn out to pasture in a familiar group. During these weeks, the horses were inspected to ensure they were healthy once daily.

2.2 | Experimental protocol

2.2.1 | Induction of inflammation

At post induction hour (PIH) timepoint (*t*) 0, each carpus of each horse was clipped and prepared for dorsal arthrocentesis. Lipopolysaccharide from Escherichia coli O55:B5 (catalogue number L5418; Sigma-Aldrich Ireland Ltd., Arklow, Co.) was diluted to a final concentration of 0.25 ng/mL in sterile lactated Ringer's solution. Horses were sedated with xylazine (0.2–0.5 mg/kg intravenously, Chanazine 10%®) and butorphanol (0.01–0.02 mg/kg intravenously; Alvegesic vet 10®, ALVETRA u. WERFFT GmbH). Arthrocentesis was performed in each limb with a 20 G × 40 mm needle and 1 mL LPS solution (0.25 ng LPS) was delivered aseptically into each middle carpal joint after withdrawal of the PIH t0 synovial fluid (SF) sample.

LPS was again injected bilaterally 2 weeks after the first induction and again a further 2 weeks after that, resulting in a total of three inflammation episodes (PIH_1, PIH_2 and PIH_3). The timeline of the experiment is illustrated in Figure 1.

2.2.2 | Treatment

Two hours following the first induction of inflammation with LPS (PIH_1t2), one randomly assigned middle carpal joint of each horse was treated with 12 mg of triamcinolone acetonide (TA) (Adcortyl Intra-articular/Intradermal Injection 10 mg/mL, Bristol-Myers Squibb Pharmaceuticals uc) diluted with sterile saline to a total volume of 2 mL, and the opposite middle carpal joint was injected with 2 mL of sterile saline alone so each horse acted as its own control (within animal controlled experiment). Treatment limbs were randomised and all investigators were unaware of the treatment assignment with the exception of the first author.

2.3 | Clinical evaluations

2.3.1 | Welfare monitoring

Before arthrocentesis and induction of inflammation and consecutively every 2 hours until PIH t8, and thereafter daily until PIH t168, a Composite Welfare Score (CWS) was assigned. The CWS is the sum of scores (scale 0–4) for each of the following categories: food and water intake; clinical parameters (temperature, pulse, and respiratory rate); natural behaviour; and provoked behaviour. This scoring system has been designed by our group for this bilateral equine LPS model to monitor welfare and to fulfil institutional and national ethical regulatory requirements (scoresheet available in Table S1).

2.3.2 | Clinical measurements

In each induction, before arthrocentesis at PIH t0, every 2 hours until PIH t8, and thereafter daily until PIH t168, middle carpal joint effusion was graded on a subjective scale as previously described.[19] Briefly, the joints were palpated and assigned a score ranging from 0 to 4; a score of 1, 2 or 3 denoting mild, moderate or severe intercarpal joint effusion, respectively, and 4 indicating severe swelling of the entire carpal region. Carpal circumference was measured at a fixed anatomical landmark at the level of the accessory carpal bone with a tape measure. All clinical measurements were performed by the first author and therefore cannot be considered to be blinded.

2.4 | Synovial fluid analysis

At fixed timepoints (PIH t0, t8, t24, t72 and t168), arthrocentesis of each middle carpal joint was performed under sedation as described above. A portion of the synovial fluid was separated for evaluation of manual white blood cell count (WBC) and total protein (TP) measurement (refractometer). The remainder was immediately centrifuged in plain tubes for 15 minutes at 4°C at 10,000 rpm and then aliquoted and stored at − 80°C until further analysis.

2.4.1 | Synovial fluid biomarker analysis

A total of seven assays were performed on each SF sample. Prostaglandin E_2 (PGE_2) concentrations were determined by high-performance liquid chromatography (HPLC)–tandem mass spectrometry (MS/MS) analysis as described previously.[20] C–C motif chemokine ligand 2 (CCL2) and tumour necrosis factor-α (TNF-α)

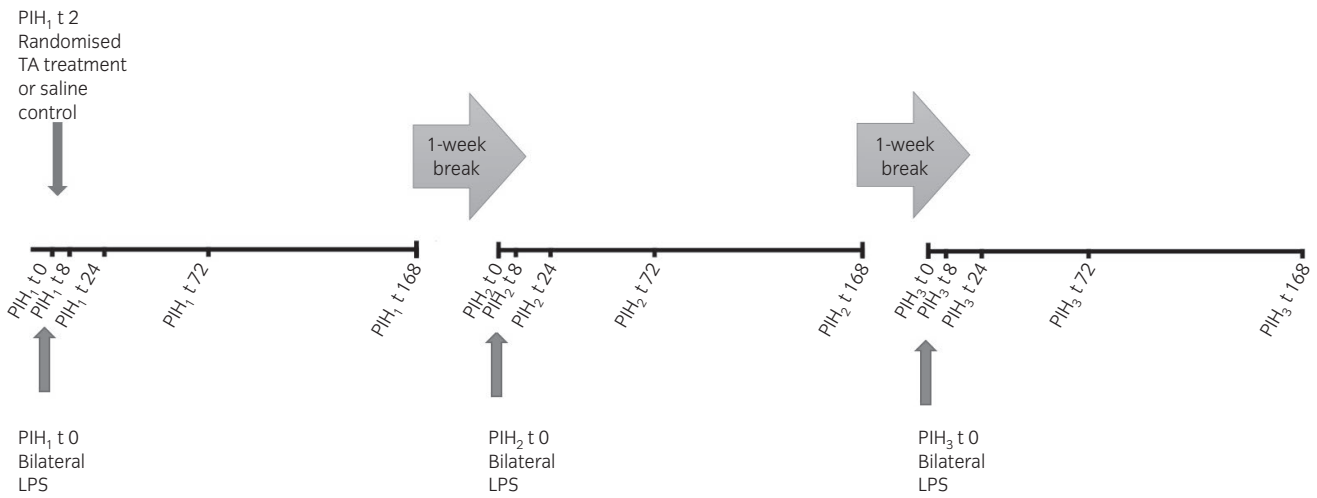

FIGURE 1 Experimental Timeline. Timeline of experimental period, representing 5 wk in total. PIH (Post Induction Hour) indicates *t* (time) in hours after induction of inflammation with intra-articular injections of 0.25 ng of lipopolysaccharide (LPS) in each middle carpal joint (MCJ) of eight horses. At PIH_1 t2, one randomly selected MCJ of each horse was injected with intra-articular triamcinolone acetonide (TA) and the contralateral joint injected with sterile saline (control). Following both PIH_1 t168 and PIH_2 t168 horses had a 1-week rest in the field prior to the next induction of inflammation. *indicates times of synovial fluid sampling

concentrations were quantified using commercial equine-specific ELISA kits (DIY0694E-003 Kingfisher Biotech, Minnesota USA and #ESS0017, Thermo Fisher Scientific) using an adapted protocol. Coating buffer consisted of carbonate/bicarbonate buffer (pH 9.6) and blocking/dilution buffer was PBS with 1% w/w bovine serum albumin (BSA; Sigma Aldrich). Samples were diluted 1:1 in PBS/1% BSA/0.1% (v/v) Tween-20, and results were calculated to a standard curve plotted on four parameter logistic curve fit. Values equal to, or below the blank were set to zero. General matrix metalloproteinase (MMP) activity was measured using cleavage of fluorogenic substrate FS-6 (Calbiochem) as previously described.[21] Synovial fluid samples were evaluated for glycosaminoglycan (GAG) concentrations using a modified 1,9-dimethylmethyleneblue assay adapted for use in microtitre plates, as previously described.[13] Commercial ELISA kits were used to determine concentrations of collagen-cleavage neoepitope of type II collagen (C2C), and carboxypropeptide of type II collagen epitope (CPII) (IBEX Technologies) in accordance with the manufacturer's recommendations.

2.5 | Data analysis

An a priori power analysis was performed. The power calculation was based on previous similar studies using the LPS model with described differences in synovial fluid biomarkers.[13,15,21] The power calculation suggested that eight horses would give a power of 0.8 and an alpha error rate of 0.05.

Data are presented as the mean ± standard deviation (SD).

A linear mixed effects model for repeated measures with horse as a random effect, was fitted, with time, treatment, LPS induction number and their respective two- and three-way interactions as fixed effects. Independent variance-covariance structure was used in the

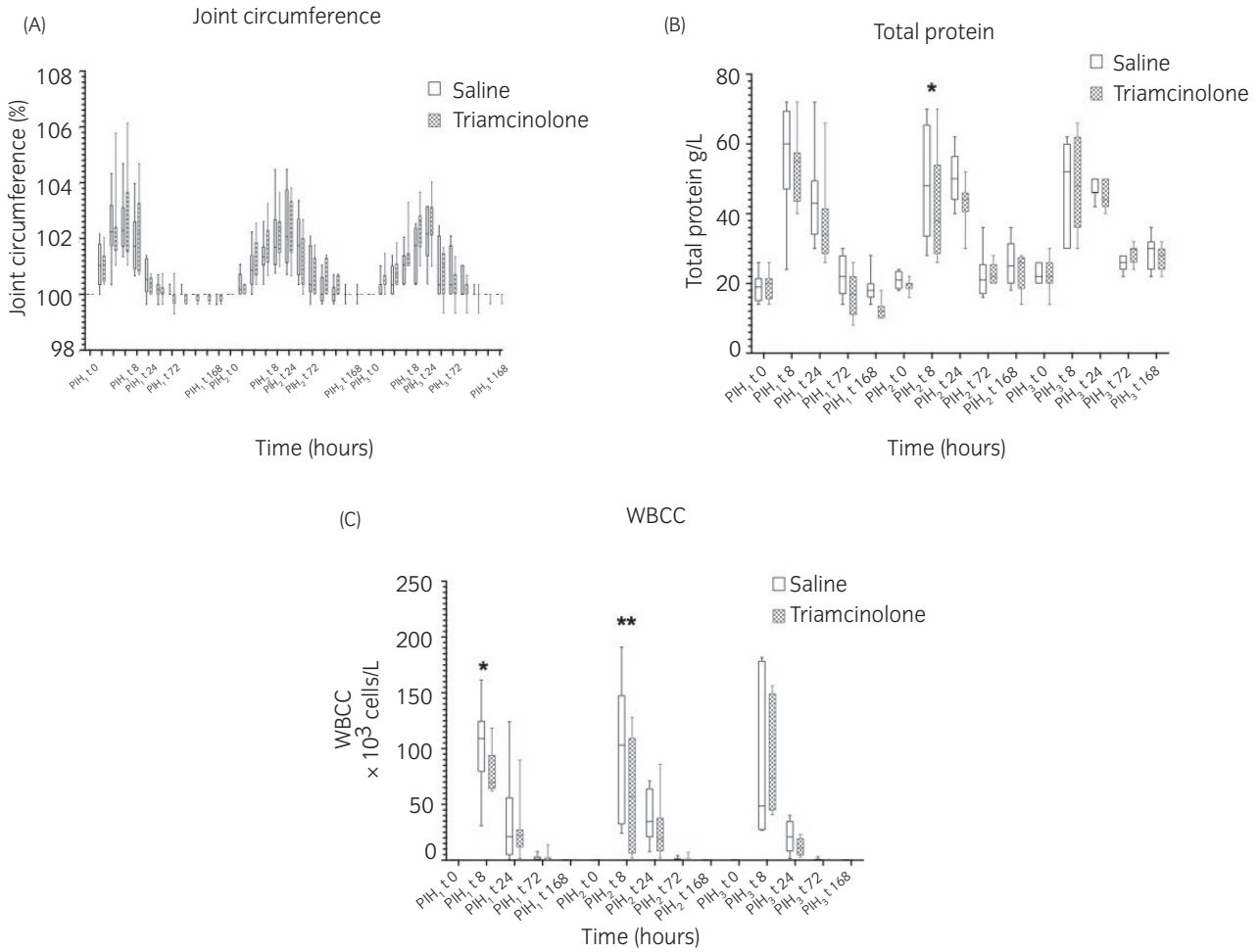

FIGURE 2 Joint circumference, synovial fluid total protein and white blood cell counts. (A) Joint circumference, (B) Synovial fluid total protein and (C) Synovial white blood cell count over time following repeated inductions of inflammation with intra-articular injections of 0.25 ng of LPS in the middle carpal joint of horses at PIH_1 t0, PIH_2 t0 and PIH_3 t0. (n = 8 horses, for all except the third induction where n = 7). Joints were treated with either 12 mg of triamcinolone acetonide (TA) or a similar volume of sterile saline (control) at PIH_1 t2. Boxes depict median and interquartile range; whiskers denote minimum and maximum values. *$p < 0.05$, **$p < 0.01$ indicating timepoints where there are significant treatment effects

model. Planned univariate contrasts (Wald tests) between marker concentrations in TA- and saline-treated joints at specific timepoints following observation of an overall significant effect of treatment used Bonferroni's correction for multiple comparisons. Normality was assessed by visual inspection of plots of standardised residuals. Suitability of the mixed effects model over a linear model without the random effect was assessed by AIC, BIC and Likelihood Ratio Test. Computer software was used (*Stata Statistical Software: Release 15*. StataCorp LLC) and the level of significance was set at $P < 0.05$ for all statistical analyses ($P < 0.0125$ with Bonferroni correction).

3 | RESULTS

3.1 | Horses

One horse sustained an injury to a hindlimb during the turnout phase at pasture after the second induction sampling period and was excluded from the final third phase of the study.

3.2 | Welfare monitoring

There were no statistically significant time-effects found for the increases in CWS observed across the entire period of the experiment. For those horses that had slight CWS increases, their scores had returned to 0 (normal) by 24 hours post induction (*Data not shown*).

3.3 | Clinical monitoring

No significant treatment effects were observed for joint circumference measurements. Comparable peaks in joint circumference were observed in control and corticosteroid treated joints after each induction of LPS (Figure 2a). As joint effusion scores were on an ordinal scale, after consideration of the repeated measures design, in particular in conjunction with the small sample size (n = 8), formal statistical methods such as ordinal logistic regression were considered inappropriate. No appreciable differences were apparent from simple observation between treatment groups. Results are summarised in Table S2.

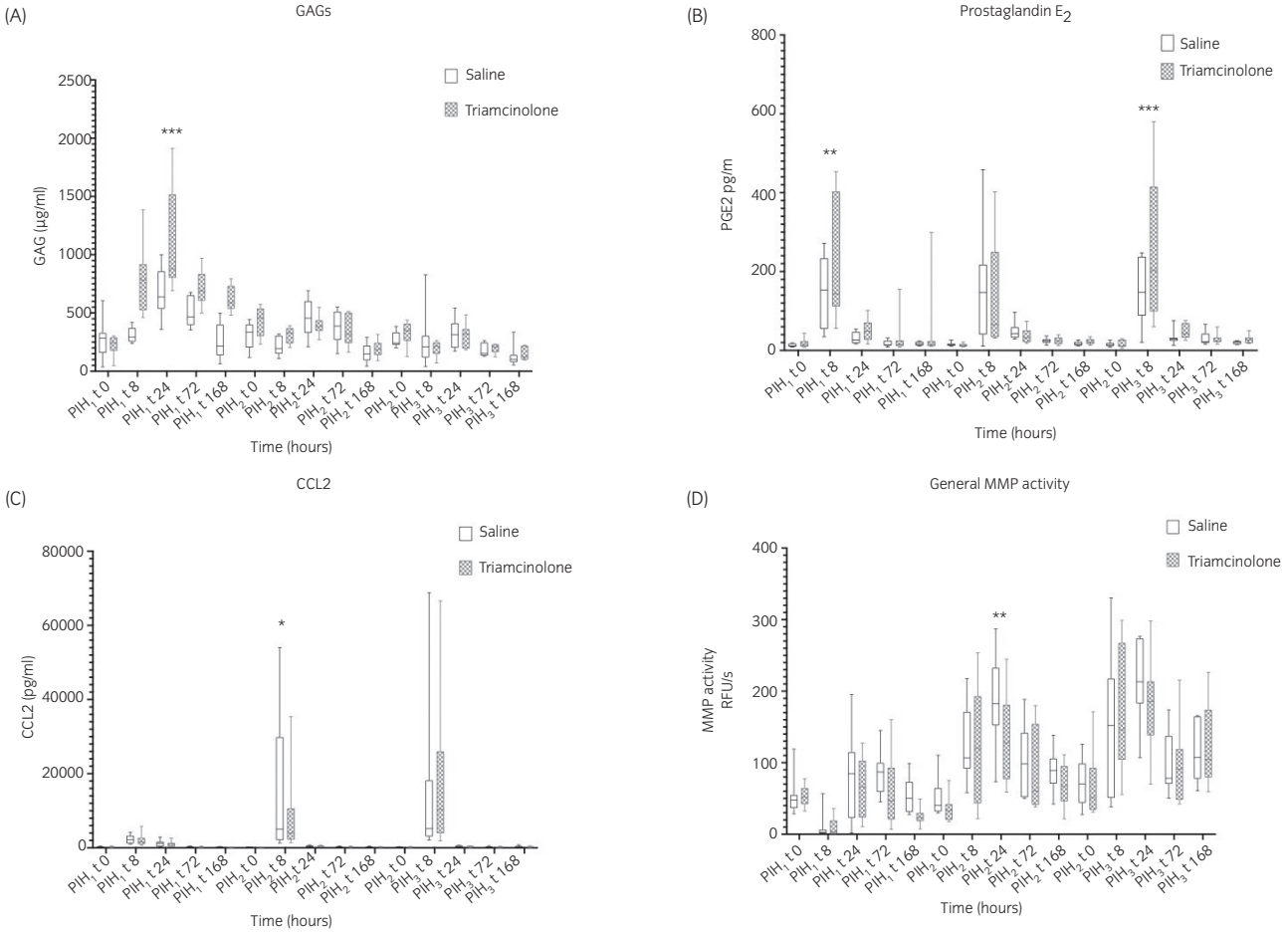

FIGURE 3 Synovial fluid glycosaminoglycans, prostaglandin E2, C–C motif chemokine ligand 2 and general matrix metalloproteinase activity. (A) Glycosaminoglycans, (B) Prostaglandin E_2 (C) C–C motif chemokine ligand 2(CCL2) and (D) general matrix metalloproteinase (MMP) activity over time following repeated inductions of inflammation with intra-articular injections of 0.25 ng of LPS in the middle carpal joint of horses at PIH_1 t0, PIH_2 t0 and PIH_3 t0. (n = 8 horses, for all except the third induction where n = 7). Joints were treated with either 12 mg of triamcinolone acetonide (TA) or a similar volume of sterile saline (control) at PIH_1 t2. Boxes depict median and interquartile range; whiskers denote minimum and maximum values. *p < 0.05, **p < 0.01, ***p < 0.001, indicating timepoints during significant treatment effects

3.4 | Synovial fluid biomarker monitoring

Regarding the effects of intra-articular administration of TA on synovial concentrations of biomarkers, the most notable of these are mentioned below. The results for all synovial fluid parameters are summarised in Table S3.

For TP, slightly lower levels were seen in the TA-treated joints compared with the control treated joints in the peak after the first LPS induction(PIH_1 t8), though these were not statistically significant (Difference in means −3.0 g/L, 95% CI −10.3, 4.3, $P < 0.4$,). A statistically significant reduction in TP in the corticosteroid treated group was noted at PIH_2 t8 (Difference in means −7.5 g/L, 95% CI −14.8, −0.20, $P < 0.04$; Figure 2b). After the first and second induction, lower WBCC was observed in the corticosteroid-treated group at PIH_1 t8 (Difference in means -23.7125×10^9 cells/L, 95% CI −46.7, −0.7, $P < 0.04$) and PIH_2 t8 (Difference in means -35.95×10^9 cells/L, 95% CI −59.0, −12.9, $P < 0.002$; Figure 2c).

For synovial fluid GAG concentrations an overall treatment effect was found. For the specific contrasts tested, significantly greater levels were observed in the TA-treated joints after the first LPS induction from PIH1 t24 (Difference in means 283.1875 μg/mL, 95% CI 179.8, 386.6, $P < 0.000$), but not after the subsequent 2 inductions (Figure 3a). Significantly greater PGE_2 levels were noted in TA-treated joints at PIH_1 t8 (Difference in means 77.8025 pg/mL, 95% CI 21.2, 134.4, $P < 0.007$) and PIH_3 t8 (Difference in means 100.42 pg/mL, 95% CI 39.9, 160.9, $P < 0.001$; Figure 3b). Reductions in CCL2 measurements in TA-treated joints were observed at PIH_2 t8 (Difference in means −6747.75 pg/mL, 95% CI −13137.3, −358.2, $P < 0.04$; Figure 3c).

Treatment effects on General MMP activity were only noted after the second induction where significantly smaller increases were seen in TA treated joints at PIH_2 t24 (Difference in means −51.65 RFU/s, 95% CI −92.4, −10.9, $p < 0.01$; Figure 3d). Finally, no treatment effects were noted for either C2C or CPII.

4 | DISCUSSION

In this study we investigated the effects of a once off treatment of intra-articular triamcinolone acetonide (TA) in a model of repeated inflammation in equine joints. Joint inflammation is well established as an inherent component of all joint diseases, including rheumatoid arthritis and OA.[16] As joint inflammation is known to be a cause of many of the clinical symptoms of joint disease, in addition to playing a pivotal role in the propagation of disease, intra-articular inflammatory pathways are often targeted in the development of novel therapeutics.[17] It is the ability of corticosteroids to block the arachidonic acid cascade, limit capillary dilation and inhibit the release of several soluble mediators[22] that have supported their frequent use in the management of OA in both horses and humans. In spite of these known desirable traits and their widespread use in clinical practice based on empiricism, scientific reports on the clinical efficacy of intra-articular corticosteroids often report disappointing results.[2,23] In the human clinic, concerns are increasingly being expressed over potential detrimental effects, on articular cartilage in particular.[2]

Triamcinolone acetonide, a corticosteroid considered to have medium duration of action, is the most commonly used intra-articular corticosteroid in equine practice[24] and is equally widely used in human medicine. For this study we used a dose of 12 mg per joint, to allow comparison with previous experimental studies.[8,9] The study by Frisbie et al.[8] is often quoted in support of the use of intra-articular TA. This study found that two treatments of 12 mg TA had beneficial effects on the carpal osteochondral chip model, leading to reductions in histopathological markers of cartilage damage compared with controls. In clinical practice, 12 mg could, however, be considered at the high end of the dosing range. The relationship of corticosteroid dose to potential effects needs be considered when interpreting the results of our study as many chondrocyte studies have shown dose-dependent chondrotoxic effects of triamcinolone acetonide (TA).[25,26] Trahan et al. recently investigated the effects of different concentrations of various corticosteroids on equine articular chondrocyte cocultures, and their study suggests that a comparable dose to what was used in the presented study should be beneficial without having deleterious effects on cartilage.[27]

While no experimental model will exactly replicate naturally occurring disease, our group has focused on the equine intra-articular LPS synovitis model, which has now been widely used for testing potential therapeutics.[13,21] This model moves away from the focal (carpal chip) or extensive (models of joint instability) damage to cartilage seen with surgical models, and focuses more on the 'whole-joint' processes seen in naturally occurring inflammatory joint diseases such as OA. In the horse sub-nano doses of LPS have been shown to elicit marked and reliable yet transient effects on certain synovial fluid inflammatory biomarkers, MMP activity and some markers of cartilage turnover.[28] Additionally, a large number of synovial biomarkers have been investigated as measures of joint health or disease, giving us a panel of measurements that can be used, not only as outcome measures, but through serial sampling also to track the influence of any therapeutic over a period of time.[13,21] While the transient and self-limiting nature of the inflammation described is seen as one of the benefits of this model, it is also frequently cited as one of the main limitations of the model in terms of its ability to mimic natural disease.[13,14,29] To overcome this, we have recently refined the model by introducing repeated injections of LPS at 2-week intervals. We found that a consistent, reliable, repeated intra-articular inflammatory response across a panel of biomarkers can be produced with repeated injections of 0.25 ng LPS. While previous studies have used more frequent injections of LPS to model persistent inflammation—for example Kay et al.[30] who repeated injections of a higher dose of LPS every 5 days—from our previous work using 0.5ng of LPS in a unilateral model we know that while the clinical signs of synovitis—lameness, joint effusion—are expected to resolve within 48 hours of LPS injection, increases in markers

of collagen II turnover can persist for at least 7 days.[28] In moving to this bilateral model with a lower dose of LPS we wanted to have a less severe synovitis induced, and we also wanted to model low grade inflammatory flares[18] so we felt repeated inductions of the LPS inflammatory cascade within the same joints every 2 weeks were appropriate to avoid any potential cumulative effects of the LPS inductions. This repeated LPS model allows for the effects of interventions or novel therapeutics to be investigated in a longer term model of recurrent joint inflammation.[18] We believe that this model is more reflective of natural disease, where ongoing bouts or flare-ups of inflammation incite both clinical signs and propagation of the disease.[16,17]

While overall our findings suggest that in this model the efficacy of intra-articular TA on LPS-induced inflammation is minimal, we report a number of interesting effects that reflect both what has been described in previous in vivo and in vitro models and may give insight into many of the empirical observations from its use in clinical practice.

The lack of significant treatment effect on clinical signs is not unexpected here. In other studies, lameness is the main clinical parameter shown to be affected by corticosteroid treatment.[8,30,31] In the presented study, the lower 0.25 ng dose of LPS is injected bilaterally. While still producing reliable intra-articular inflammation, it is known that doses <0.5 ng LPS can give variable and inconsistent levels of lameness.[32] The bilateral model gives tighter control of individual variations—which is a particular concern in large animal studies, where the animals are generally not specifically bred for research purposes, and allows for reduction of animal usage.[33] However, the bilateral model also invalidates lameness as a reliable outcome measure. No currently described lameness grading systems can be satisfactorily applied to bilateral lameness, and assigning grades in bilateral lameness is even considered by some experts to be potentially misleading.[34] Hence lameness levels, while monitored as part of the overall composite welfare scores, were not analysed as a distinct outcomes measure.

The minimal effects on joint effusion and circumference are similar to the findings of Kay et al. and Ekstrand et al., where both groups used LPS models to investigate the effects of intra-articular corticosteroids.[30,31] This lack of significant clinical treatment effect could be due to the short time between the induction of LPS and injection of TA, or it could be due to the minimal and variable clinical signs elicited by this low dose of LPS. It is of interest that in human literature, the success of intra-articular injections is most commonly determined by patient-based outcomes, which are essentially the patient's perception of improvement in clinical signs. This can lead to significant bias and in a recent Cochrane review,[1] only one randomised clinical trial was identified as to having taken sufficient measures to reduce this bias.[35] Neither that study, which looked at effects of a single treatment, nor a more recent extensive clinical trial looking at repeated treatments,[2] reported clear clinical benefits of corticosteroid treatment.

The increases in synovial GAG concentrations with TA treatment throughout the first induction of LPS are, again, not surprising. In vitro studies have demonstrated some conflicting findings with some showing reduced[6] or no[7] effect on GAG concentrations, and others showing dose-dependent increases in GAG concentrations, with corticosteroid treatments.[27,36] In vivo findings have been more consistent with Celeste et al.[9] demonstrating significant increases in specific aggrecans with TA treatment. In the carpal chip model Frisbie et al. also found higher GAG concentrations in TA treated joints—with greater increases seen in control joints compared with operated joints which led the authors to speculate that TA administration in abnormal joints did not alter GAG levels as much as in normal joints.[8] While different researchers have diverged on whether increased GAG release is a positive[8] or negative[9] finding, it has been well-established that GAG levels in synovial fluid are an indicator of proteoglycan breakdown or release which could be the result of cartilage insult. In the recent study by McAlindon et al.,[2] joints subjected to repeated treatments of TA were associated with significantly greater loss of cartilage volume compared with saline treated joints. Interestingly, in our study the increase in GAG concentration was only noted for the first induction of LPS and did not extend into the second or third phases of the study. It may be inferred that this effect, while potentially detrimental, only lasts for a relatively short period of time.

Perhaps the most confusing finding in this study are the increases seen in Prostaglandin E_2. Prostaglandin E_2 is an important inflammatory and pain-related mediator released into synovial fluid by inflamed synovial membrane and, to a lesser extent, cartilage.[20] As a sensitive marker of joint inflammation and also a direct target of many forms of anti-inflammatory therapy it is considered to be a particularly useful surrogate outcome measure when trialling novel therapies.[12,21,37-39] Based on the findings in the in vitro study by Trahan et al.[27] where all doses of the corticosteroids investigated gave reductions in culture medium Prostaglandin E_2, we would expect the Prostaglandin E_2 levels in our treated joints to be reduced rather than increased. While prostaglandin levels are not reported in many in vivo studies, in Frisbie et al.'s 1998 investigation of the effects of MPA, the corticosteroid treated joints had lower Prostaglandin E_2 concentrations[40] and in an LPS model, Neuenschwander et al.[41] found that TA treatment reduced Prostaglandin E_2 levels compared with treatment with hyaluronic acid, although control joints were not investigated. There is no clear explanation for the unexpected finding in this study, although there are some other reports with similar findings. In a study by Mangal et al.[42] looking at effects of TA on concentrations of bioactive eicosanoids in equine plasma, the overall results indicated that TA suppressed biosynthesis of bioactive eicosanoids. However, from 48 to 168 hours post treatment sustained increases were noted. Glucocorticoids have elsewhere also been indicated to have pro-inflammatory effects, in the central nervous system,[43] and in certain murine cells such as rat gastric mucosa, they have been shown to stimulate prostaglandin production.[44] Overall, we believe further investigation of the effects of TA and LPS on prostaglandin production in equine joints is warranted.

Triamcinolone acetonide treatment reduced general MMP activity across the first and second LPS induction. The persistence of these

reductions in the second induction suggests a potential sustained anti-inflammatory effect of TA. General MMP activity has been reported in LPS studies investigating the effects of meloxicam and phenylbutazone. In the meloxicam study, reductions in general MMP activity were noted, and considered to be beneficial in protection against MMP incited cartilage damage.[21] The phenylbutazone study did not demonstrate a treatment effect on general MMP activity, which the authors inferred could limit the effectiveness of phenylbutazone for treatment of certain severe inflammatory conditions.[13] The findings of this study would therefore suggest that intra-articular TA should have some protective anti-inflammatory effect for at least 2 weeks post treatment. While there is not a lot of evidence regarding persistence of TA in equine joints, previous studies in normal[45] joints and in joints with LPS-induced synovitis[30] have demonstrated the presence of TA for up to 21 and 10 days respectively following intra-articular administration. While differences in joints studied, drug doses and laboratory quantification methods make it difficult to directly correlate these findings to our results, it could be inferred that some levels of TA persist in our joints at least through the second induction of LPS synovitis. Further work quantifying the levels of TA across the different timepoints in this model is warranted and could provide useful information on the mechanism of action of TA intra-articularly.

5 | LIMITATIONS

As with any experimental set up, there are a number of limitations to this study. While the repeated inductions of LPS led to inflammation being detectable over a prolonged time period, this inflammation was apparent as 'peaks' and 'troughs' and may still be disparate from natural disease states. However, any animal model has its limitations and the used model certainly has proven a step closer to modelling recurrent inflammation.

Another limitation is that we only investigated markers of cartilage metabolism and did not directly evaluate cartilage either before or after so we cannot compare the findings in our biomarker panel to any changes in the cartilage or synovium. While several studies that used single LPS injections at higher dosages did not demonstrate any effect on cartilage morphology[13,46,47] and LPS therefore is assumed to not cause gross cartilage damage, it is not certain that this is still the case with our model of repeated inflammatory episodes. However, we felt that a study design with the addition of general anaesthesia and arthroscopic procedures, and/or an a priori determined euthanasia of the horses was not justified from a welfare perspective.

6 | CONCLUSIONS

As determined by the effects on MMP, and WBCC seen in the second induction, it could be concluded that in this model of repeated equine joint inflammation a single dose of TA had some anti-inflammatory activity up to 2 weeks but not at 4 weeks post treatment. This anti-inflammatory effect would seem to outlast a shorter lived, potentially detrimental effect on cartilage as illustrated by increases in synovial GAG concentrations. While further studies are clearly warranted, particularly to further investigate the unexpected findings regarding Prostaglandin E_2, we believe that there is some evidence for judicial use of TA in carefully selected cases to dampen inflammation.

ACKNOWLEDGEMENTS

We express our sincere gratitude to the staff and students at UCDVH and UCD Lyons Research farm for help with data collection.

INFORMED CONSENT

Not applicable.

CONFLICT OF INTERESTS

No competing interests have been declared.

ETHICAL ANIMAL RESEARCH

All experimental procedures and protocols were pre-approved by the University College Dublin Animal Research Ethical Committee (AREC-16-29-Brama) and the Irish Health Products Regulatory Authority (AE18982-P105), in compliance with Irish legislation on experimental animal use.

AUTHOR CONTRIBUTIONS

C. Kearney participated in the study design, carried out the experimental procedures, performed the statistical analysis and drafted the manuscript. N. Korthagen and S. Plomp provided technical support with the synovial fluid analyses, performed the mediator and marker assays and assisted in manuscript preparation. M. Labberté assisted with the experimental procedures, provided technical support with the synovial fluid processing and analyses, and assisted in manuscript preparation. P. Brama, J. de Grauw and R. van Weeren conceived the study, participated in its design and coordination and helped draft the manuscript. All authors read and approved the final manuscript.

DATA ACCESSIBILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/evj.13396.

ORCID

Clodagh M. Kearney https://orcid.org/0000-0003-1518-5151
Janny C. de Grauw https://orcid.org/0000-0003-3715-150X

REFERENCES

1. Jüni P, Hari R, Rutjes AW, Fischer R, Silletta MG, Reichenbach S, et al. Intra-articular corticosteroid for knee osteoarthritis. Cochrane Database Syst Rev. 2015;28:496.

2. McAlindon TE, LaValley MP, Harvey WF, Price LL, Driban JB, Zhang M, et al. Effect of intra-articular triamcinolone vs saline on knee cartilage volume and pain in patients with knee osteoarthritis: A randomized clinical trial. JAMA. 2017;317:1967–75.
3. Orchard JW. Is there a place for intra-articular corticosteroid injections in the treatment of knee osteoarthritis? BMJ. 2020;368:l6923.
4. Bellamy N, Campbell J, Welch V, Gee TL, Bourne R, Wells GA. Intraarticular corticosteroid for treatment of osteoarthritis of the knee. Cochrane Database Syst Rev. 2006;21:429.
5. Wernecke C, Braun HJ, Dragoo JL. The effect of intra-articular corticosteroids on articular cartilage: a systematic review. Orthop J Sports Med. 2015;3:232596711558116.
6. Dechant JE, Baxter GM, Frisbie DD, Trotter GW, McIlwraith CW. Effects of dosage titration of methylprednisolone acetate and triamcinolone acetonide on interleukin-1-conditioned equine articular cartilage explants in vitro. Equine Vet J.. 2003;35:444–50.
7. Bolt DM, Ishihara A, Weisbrode SE, Bertone AL. Effects of triamcinolone acetonide, sodium hyaluronate, amikacin sulfate, and mepivacaine hydrochloride, alone and in combination, on morphology and matrix composition of lipopolysaccharide-challenged and unchallenged equine articular cartilage explants. Am J Vet Res. 2008;69:861–7.
8. Frisbie DD, Kawcak CE, Trotter GW, Powers BE, Walton RM, McIlwraith CW. Effects of triamcinolone acetonide on an in vivo equine osteochondral fragment exercise model. Equine Vet J. 1997;29:349–59.
9. Celeste C, Ionescu M, Robin Poole A, Laverty S. Repeated intraarticular injections of triamcinolone acetonide alter cartilage matrix metabolism measured by biomarkers in synovial fluid. J Orthop Res. 2005;23:602–10.
10. Vandeweerd J-M, Zhao Y, Nisolle J-F, Zhang W, Liu Z, Clegg P, et al. Effect of corticosteroids on articular cartilage: have animal studies said everything? Fundam Clin Pharmacol. 2015;29:427–38.
11. McIlwraith CW. Use of synovial fluid and serum biomarkers in equine bone and joint disease: a review. Equine Vet J. 2005;37:473–82.
12. Van den Boom R, van de Lest CHA, Bull S, Brama RAJ, Van Weeren PR, Barneveld A. Influence of repeated arthrocentesis and exercise on synovial fluid concentrations of nitric oxide, prostaglandin E2 and glycosaminoglycans in healthy equine joints. Equine Vet J. 2005;37:250–6.
13. de Grauw JC, van Loon JPAM, van de Lest CHA, Brunott A, Van Weeren PR. In vivo effects of phenylbutazone on inflammation and cartilage-derived biomarkers in equine joints with acute synovitis. Vet J. 2014;201:51–6.
14. Williams LB, Koenig JB, Black B, Gibson TWG, Sharif S, Koch TG. Equine allogeneic umbilical cord blood derived mesenchymal stromal cells reduce synovial fluid nucleated cell count and induce mild self-limiting inflammation when evaluated in an LPS induced synovitis model. Equine Vet J. 2015;48:619–25.
15. Sladek S, Kearney C, Crean D, Brama PAJ, Tajber L, Fawcett K, et al. Intra-articular delivery of a nanocomplex comprising salmon calcitonin, hyaluronic acid, and chitosan using an equine model of joint inflammation. Drug Deliv and Transl Res. 2018;8:1421–35.
16. Sellam J, Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. Nat Rev Rheumatol. 2010;6:625–35.
17. Punzi L, Galozzi P, Luisetto R, Favero M, Ramonda R, Oliviero F, et al. Post-traumatic arthritis: overview on pathogenic mechanisms and role of inflammation. RMD Open. 2016;2:e000279.
18. Cokelaere SM, Plomp SGM, de Boef E, de Leeuw M, Bool S, van de Lest CHA, et al. Sustained intra-articular release of celecoxib in an equine repeated LPS synovitis model. Eur J Pharm Biopharm. 2018;128:327–36.
19. Owens JG, Kamerling SG, Stanton SR, Keowen ML, Prescott-Mathews JS. Effects of pretreatment with ketoprofen and phenylbutazone on experimentally induced synovitis in horses. Am J Vet Res. 1996;57:866–74.
20. de Grauw JC, van de Lest CHA, van Weeren PR. A targeted lipidomics approach to the study of eicosanoid release in synovial joints. Arthritis Res Ther. 2011;13:R123.
21. Grauw JC, Lest CHA, Brama P, Rambags BPB, Weeren PR. In vivo effects of meloxicam on inflammatory mediators, MMP activity and cartilage biomarkers in equine joints with acute synovitis. Equine Vet J. 2009;41:693–9.
22. McIlwraith CW, Lattermann C. Intra-articular corticosteroids for knee pain-what have we learned from the equine athlete and current best practice. J Knee Surg. 2019;32:9–25.
23. Labens R, Voute LC, Mellor DJ. Retrospective study of the effect of intra-articular treatment of osteoarthritis of the distal tarsal joints in 51 horses. Veterinary Record. 2007;161:611–6.
24. Ferris DJ, Frisbie DD, McIlwraith CW, Kawcak CE. Current joint therapy usage in equine practice: a survey of veterinarians 2009. Equine Vet J. 2011;43:530–5.
25. Syed HM, Green L, Bianski B, Jobe CM, Wongworawat MD. Bupivacaine and triamcinolone may be toxic to human chondrocytes: A pilot study. Clin Orthop Relat Res. 2011;469:2941–7.
26. Dragoo JL, Danial CM, Braun HJ, Pouliot MA, Kim HJ. The chondrotoxicity of single-dose corticosteroids. Knee Surg Sports Traumatol Arthrosc. 2012;20:1809–14.
27. Trahan RA, Byron CR, Dahlgren LA, Pleasant RS, Werre SR. In vitro effects of three equimolar concentrations of methylprednisolone acetate, triamcinolone acetonide, and isoflupredone acetate on equine articular tissue cocultures in an inflammatory environment. Am J Vet Res. 2018;79:933–40.
28. de Grauw JC, Van de Lest CH. Inflammatory mediators and cartilage biomarkers in synovial fluid after a single inflammatory insult: a longitudinal experimental study. Arthritis Res Ther. 2009;11:R35.
29. Palmer JL, Bertone AL. Experimentally-induced synovitis as a model for acute synovitis in the horse. Equine Vet J. 1994;26:492–5.
30. Kay AT, Bolt DM, Ishihara A, Rajala-Schultz PJ, Bertone AL. Anti-inflammatory and analgesic effects of intra-articular injection of triamcinolone acetonide, mepivacaine hydrochloride, or both on lipopolysaccharide-induced lameness in horses. Am J Vet Res. 2008;69:1646–54.
31. Ekstrand C, Bondesson U, Giving E, Hedeland M, Ingvast-Larsson C, Jacobsen S, et al. Disposition and effect of intra-articularly administered dexamethasone on lipopolysaccharide induced equine synovitis. Acta Vet Scand. 2019;61:1–17.
32. Palmer JL, Bertone AL, Malemud CJ, Mansour J. Biochemical and biomechanical alterations in equine articular cartilage following an experimentally-induced synovitis. Osteoarthritis Cartilage. 1996;4:127–37.
33. Orth P, Zurakowski D, Alini M, Cucchiarini M, Madry H. Reduction of sample size requirements by bilateral versus unilateral research designs in animal models for cartilage. Tissue Eng. 2013;19:885–91. https://home.liebertpub.com/tec
34. Dyson S. Can lameness be graded reliably? Equine Vet J. 2011;43:379–82.
35. Henriksen M, Christensen R, Klokker L, Bartholdy C, Bandak E, Ellegaard K, et al. Evaluation of the benefit of corticosteroid injection before exercise therapy in patients with osteoarthritis of the knee: a randomized clinical trial. JAMA Intern Med. 2015;175:923–30.
36. Garvican ER, Vaughan-Thomas A, Redmond C, Gabriel N, Clegg PD. MMP-mediated collagen breakdown induced by activated protein C in equine cartilage is reduced by corticosteroids. J Orthop Res. 2010;28:370–8.
37. Frisbie DD, Al-Sobayil F, Billinghurst RC, Kawcak CE, McIlwraith CW. Changes in synovial fluid and serum biomarkers with exercise and early osteoarthritis in horses. Osteoarthritis Cartilage. 2008;16:1196–204.

38. Kirker-Head CA, Chandna VK, Agarwal RK, Morris EA, Tidwell A, O'Callaghan MW, et al. Concentrations of substance P and prostaglandin E 2in synovial fluid of normal and abnormal joints of horses. Am J Vet Res. 2000;61:714–8.
39. Bertone AL, Palmer JL, Jones J. Synovial fluid cytokines and eicosanoids as markers of joint disease in horses. Vet Surg. 2001;30:528–38.
40. Frisbie DD, Kawcak CE, Baxter GM, Trotter GW, Powers BE, Lassen ED, et al. Effects of 6alpha-methylprednisolone acetate on an equine osteochondral fragment exercise model. Am J Vet Res. 1998;59:1619–28.
41. Neuenschwander HM, Moreira JJ, Vendruscolo CP, Fülber J, Seidel SRT, Michelacci YM, et al. Hyaluronic acid has chondroprotective and joint-preserving effects on LPS-induced synovitis in horses. J Vet Sci. 2019;20:e67.
42. Mangal D, Uboh CE, Soma LR, Liu Y. Inhibitory effect of triamcinolone acetonide on synthesis of inflammatory mediators in the equine. Eur J Pharmacol. 2014;736:1–9.
43. Ronchetti S, Migliorati G, Bruscoli S, Riccardi C. Defining the role of glucocorticoids in inflammation. Clin. Sci.. 2018;132:1529–43.
44. Avunduk C, Eastwood GL, Polakowski N, Burstein S. Hydrocortisone has a biphasic effect on rat gastric mucosal prostaglandin generation in vivo: Inhibition at low doses, stimulation at high doses. Prostaglandins Leukot Essent Fatty Acids. 1992;45:329–32.
45. Knych HK, Vidal MA, Casbeer HC, McKemie DS. Pharmacokinetics of triamcinolone acetonide following intramuscular and intra-articular administration to exercised Thoroughbred horses. Equine Vet J. 2013;45:715–20.
46. van Loon JPAM, de Grauw JC, Brunott A, Weerts EAWS, Van Weeren PR. Upregulation of articular synovial membrane μ-opioid-like receptors in an acute equine synovitis model. Vet J. 2013;196:40–6.
47. Todhunter RJ, Fubini SL, Vernier-Singer M, Wootton JA, Lust G, Freeman KP, et al. Acute synovitis and intra-articular methylprednisolone acetate in ponies. Osteoarthritis Cartilage. 1998;6:94–105.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Kearney CM, Korthagen NM, Plomp SGM, et al. Treatment effects of intra-articular triamcinolone acetonide in an equine model of recurrent joint inflammation. *Equine Vet J*. 2021;53:1277–1286. https://doi.org/10.1111/evj.13396