Equine Veterinary Education

BEVA AAEP

e558

EQUINE VETERINARY EDUCATION
Equine vet. Educ. (2022) **34** (12) e558-e562
doi: 10.1111/eve.13588

Original Article

Oral prednisolone achieves measurable concentrations in equine synovial fluid within 3 hours of administration: Preliminary observations

***D. W. Ramey*[†,*] *and H. K. Knych*[‡]**

[†]*Ramey Equine, Chatsworth, California; and* [‡]*K.L. Maddy Equine Analytical Chemistry Laboratory, Davis, California, USA*
**Corresponding author email: ponydoc@pacbell.net*

Keywords: horse; corticosteroid; prednisolone; joint; arthritis

Summary

Background: Clinical and anecdotal evidence exists for the use of orally administered prednisolone in the treatment of osteoarthritis in human patients; however, prednisolone is not commonly prescribed by this route of administration in horses for the treatment of osteoarthritis.

Objectives: This preliminary study tested the hypothesis that orally administered prednisolone could be detected in equine synovial fluid 3 h after administration.

Study design: Pre/post study.

Methods: Six horses were used in this study. Blood was drawn pre-prednisolone administration to assure that all horses were prednisolone free. Three hours after being given 400 mg prednisolone per os, an additional blood sample was taken, as well as samples from the radiocarpal (RC) and tibiotarsal (TT) joints. Samples were frozen and sent to the laboratory for the determination of the presence of prednisolone using liquid chromatography tandem mass spectrometry.

Results: Prednisolone appeared in measurable concentrations in the blood (Mean = 92.655 ng/mL; SD = 54.95 ng/mL) and synovial fluid (RC Mean = 28.44 ng/mL; SD = 20.58 ng/mL and TT Mean = 20.33; SD = 15.67 ng/mL) of all horses when tested 3 h after administration.

Main limitations: This study did not test for any possible therapeutic effects of oral prednisolone in osteoarthritis. It did not attempt to establish a therapeutic dosage for the medication, nor did it attempt to establish the pharmacokinetics of the medication.

Conclusions: Orally administered prednisolone appears in equine blood and synovial fluid at 3 h post-administration. Since it appears in joint fluid at concentrations that have been shown to be therapeutic for other corticosteroids, orally administered prednisolone may have potential value as a therapeutic agent in the treatment of equine osteoarthritis.

Clinical relevance

- Orally administered prednisolone can be detected in equine synovial fluid 3 h after administration.
- Orally administered prednisolone has been shown to be an effective treatment in human osteoarthritis.
- Orally administered prednisolone deserves further investigation as a therapeutic option for equine osteoarthritis.

Introduction

Intra-articular corticosteroids have been a mainstay of therapy for equine joints for at least the past 50 years. Intra-articular injections of one of three corticosteroid suspensions—betamethasone sodium phosphate and betamethasone acetate (BM), triamcinolone acetonide (TCA) and methylprednisolone acetate (MPA)—are likely among the most commonly performed procedures in equine medicine (McIlwraith, 2016).

The most common method of administration of corticosteroids for horses with osteoarthritis is intra-articular (IA). While the practice is common, several questions and concerns persist. There is no consensus on the 'best' corticosteroid for use in joint therapy. There is no known minimum effective dose for anti-inflammatory effects; therefore, dosing is empirical. Both beneficial and deleterious effects of intra-articular BM, MPA and TCA have been defined for the horse. Beneficial effects include the reduction of inflammation and chondroprotection (McIlwraith & Latterman, 2019).

However, intra-articular administration of any medication is associated with a number of risks, albeit small, including joint infection and joint flare. In addition, there is a risk to the veterinarian from the procedure of administering IA medication to horses, especially into a joint where the animal can easily strike out while the veterinarian is in a vulnerable position, for example, the tarsometatarsal joint. Thus, an alternate method of administration of medication that does not involve injecting a joint might offer some advantages for both horses and equine practitioners.

Recently, prednisolone per os has been shown to be beneficial in the treatment of osteoarthritis of the human hand. In this placebo-controlled, randomised study, treatment with 10 mg prednisolone for 6 weeks was shown to be safe and efficacious for the treatment of human patients with painful hand osteoarthritis and signs of inflammation (Kroon et al., 2019).

Oral prednisolone was administered because of its chemical similarity to MPA. MPA differs from prednisolone in the addition of a methyl group ($-CH_3$), which increases its potency relative to prednisolone. While theoretically, it would take less of the more potent drug to elicit a therapeutic effect, both prednisolone and methylprednisolone have the same mechanism of action.

The lead author has used per os prednisolone in practice for several years in horses with confirmed osteoarthritis, especially in aged horses with multiple joint involvement, based on the hypothesis that since corticosteroids

administered into the joint can rapidly be detected in the systemic circulation (Machin et al., 2019), orally administered corticosteroids might be able to be detected in synovial fluid. In horses, prednisolone has been shown to have excellent bioavailability when administered orally (Peroni et al., 2002); however, it is not known if orally administered prednisolone can be detected in equine joint fluid after administration.

In the lead author's experience, clients have typically been enthusiastic in their horse's response to medication; however, anecdotal reports have limited evidentiary value. Thus, this study was undertaken to investigate whether prednisolone administered at a dose of 400 mg/horse (approximately 0.8 mg/kg in the investigated horses) could be detected in equine synovial fluid, in order to determine whether it might be possible for per os prednisolone to have a direct effect at the articular level.

Materials and methods

Six horses were used in this study in a pre/post design. A pre–post study measures the occurrence of an outcome before and again after a particular intervention is implemented. In such studies, each individual serves as its own control and the time relationship between samples strongly suggests that the outcome is impacted by the intervention (Thiese, 2014).

Horses were all adult geldings, from 16 to 24 years of age, of similar weight (450–500 kg) based on weight tape estimates (no scales were available for accurate weights). All horses were fed a diet consisting only of alfalfa hay. Breeds represented were two American Paint Horses, two Thoroughbreds, one Quarter Horse and one American Saddlebred. All horses were riding school horses and free of apparent lameness when trotted in hand (no other lameness tests were performed). The horses were clinically normal on physical examination and used with the written consent of the single owner. No horse was receiving concurrent medication for any condition.

After an initial baseline blood sample, horses were fed 400 mg prednisolone that had been dissolved in water and mixed in ground alfalfa and molasses, which is a common method of administration of such medication to horses by horse owners. Since the study was only to determine if prednisolone appeared in synovial fluid after administration, since all horses were approximately the same size and weight, since pharmacokinetics were not being studied and since effective therapeutic doses are as yet unknown, each horse was given the same amount of prednisolone. Horses readily and rapidly ate all of the medication.

At 3 h post-prednisolone administration, an additional blood sample was taken. Prior to collection of synovial fluid, the area over the left radiocarpal and tibiotarsal joints was scrubbed with povidone-iodine solution and rinsed three times. Samples from the left radiocarpal and tibiotarsal joints were collected by arthrocentesis with a gloved hand using an 18 gauge, 1½ inch needle (Moyer, 2011) into a plastic blood collection tube containing no additives. No chemical or physical restraint was required and samples were obtained without difficulty. Horses were monitored by the owner for adverse effects after sampling.

Blood samples were centrifuged and serum was separated. Samples were then frozen at 0°F and sent to the laboratory for analysis. The laboratory was blinded as to which samples were pre- and post-administration of prednisolone.

The prednisolone working solutions were prepared by dilution of the 1 mg/mL stock solution (Sigma Aldrich, St. Louis, MO) with methanol to concentrations of 0.001, 0.01, 0.1, 1 and 10 ng/μL. Plasma calibrators were prepared by dilution of the working standard solutions with drug-free equine plasma or synovial fluid to concentrations ranging from 0.1 to 150 ng/mL. Calibration curves and negative control samples were prepared fresh for each quantitative assay. In addition, quality control samples (drug-free equine matrix fortified with analyte at three concentrations within the standard curve) were included with each sample set as an additional check of accuracy.

Prior to analysis, 0.5 mL of plasma or synovial fluid was diluted with 100 μL of water containing beclomethasone internal standard (Sigma Aldrich, St. Louis, MO) at 0.5 ng/μL, the samples vortexed briefly to mix, 5 mL of methyl tert-butyl ether added to each plasma sample and the samples were mixed by rotation for 20 min at 40 revolutions per minute (rpm). After rotation, samples were centrifuged at 3300 rpm (2260 ***g***) for 5 min at 4°C. The top organic layer was transferred to a glass tube. Samples were dried under nitrogen, dissolved in 100 μL of 5% acetonitrile (ACN) in water with 0.2% formic acid and 40 μL were injected into the liquid chromatography tandem mass spectrometry (LC-MS/MS) system.

The concentration of prednisolone was measured in plasma by LC-MS/MS in positive mode (LC-MS/MS (+)). Quantitative analysis was performed on a TSQ Altis triple quadrupole mass spectrometer coupled with a Vanquish liquid chromatography system (Thermo Scientific, San Jose, California, USA). The spray voltage was 3500V, and the sheath and auxiliary gas were 50 and 10 respectively (arbitrary units). Product masses and collision energies of each analyte were optimised by infusing the analytes into the mass spectrometer. Chromatography employed an ACE 3 C18 10 cm × 2.1 mm 3 μm column (Mac-Mod Analytical, Chadds Ford, Pennsylvania, USA) and a linear gradient of ACN in water with a constant 0.2% formic acid at a flow rate of 0.35 mL/min. The initial ACN concentration was held at 5% for 0.3 min, ramped to 75% over 11.7 min and then to 90% over 0.2 min, before re-equilibrating for 3.8 min at initial conditions.

Detection and quantification were conducted using selective reaction monitoring (SRM) of initial precursor ion of prednisolone (mass to charge ratio *(m/z)* 361) and beclomethasone (*(m/z)* 409). The response for the product ions for prednisolone (*m/z* 147, 171, 173, 289) and beclomethasone (*m/z* 147, 279, 391) was plotted and peaks at the proper retention time integrated using Quanbrowser software (Thermo Scientific). Quanbrowser software was used to generate calibration curves and quantitate prednisolone in all samples by linear regression. A weighting factor of 1/X was used for all calibration curves.

The response was linear and gave correlation coefficients of 0.99 or better. Accuracy was reported as percent nominal concentration and precision was reported as percent relative standard deviation. For prednisolone, accuracy and precision were within 15% of the nominal concentration. The technique was optimised to provide a limit of quantitation of 0.1 ng/mL and a limit of detection of approximately 0.05 ng/mL for prednisolone in both matrices.

Results

Prednisolone was found in the blood and synovial fluid of all horses, in both joints tested, at 3 h post-administration (**Table 1**). Concentrations of prednisolone in the blood and synovial fluid varied between horses. Since the drug was found in all the six horses tested, it is possible to calculate the likelihood of this occurrence using a simple baseline assumption. Assigning a small probability, say 0.01, of this finding happening at random (perhaps due to an assay false positive), then the likelihood of this occurring in six consecutive horses is approximately 9.99×10^{-13}.

Discussion

Whether corticosteroids appear in joints after other routes of administration appears to relate to the steroid studied. For example, while corticosteroids such as TCA and MPA administered into the joint can be rapidly found in plasma, intramuscular TCA could not be detected in equine synovial fluid after administration (Knych, Vidal, et al., 2013). Whether MPA given by non-IA routes of administration can be detected in the synovial fluid appears not to have been studied.

Prednisolone per os would appear to have the potential for relatively few adverse effects compared with IA medication. While oral prednisolone does suppress endogenous cortisol production (Peroni, 2002), it does not appear to increase the incidence of acute laminitis (Jordan et al., 2017). The effects of corticosteroids given IA are also not necessarily limited to the joint. For example, IA MPA improves pulmonary function in asthmatic horses (Millares-Ramirez et al., 2021). This is because IA MPA is absorbed systemically; in fact, plasma concentrations can last as long as 6 days (Machin et al., 2019). It is possible that some of the clinical effects seen in horses treated with prednisolone are not directly related to its effects on joint cartilage.

Medications such as MPA, TCA and BM are insoluble crystalloid suspensions. Because the active corticosteroid is released gradually from their crystalline particles, the anti-inflammatory effects are delayed, but sustained. Soluble corticosteroids, such as prednisolone, are shorter acting, but have a relatively rapid onset (McMahon et al., 2016). In rats, cartilage destruction is more pronounced in the knees injected with crystalloid glucocorticoids when compared with water-soluble preparations (Rusanen et al., 1986). Cartilage degradation as a result of IA administration of some corticosteroids, such as MPA, also appears to be dose dependent (Wernecke et al., 2015). Orally administered prednisolone could theoretically reduce or obviate some of the adverse effects of injectable crystalloid products.

There was variability in the levels of prednisolone found in the blood and synovial fluid of the studied horses and this would be anticipated based on published research. It has also been shown that following oral administration in horses, prednisolone is readily absorbed. Bioavailability after oral administration is reported to be 50%–60%. With reported bioavailability of 50%–60%, it is expected that there will be inherent variability in the data. When bioavailability is very low, or very high (e.g. 90% or more) variability among animals is low. However, at 50%–60% bioavailability, there is high between-subject variability, as occurred in this study.

The overall time to reach highest blood concentration (Tmax) has been reported to be from 45 min to 2.5 ± 3.1 h. Thus, sampling for this study was done at 3 h post-administration on all horses, at a time when prednisolone might reasonably be able to be detected in the serum and synovial fluid.

The half-life of prednisolone (T½) is reported to be 3.1 ± 2.3 h. Tablets result in higher maximum serum concentrations of prednisolone than do liquid formulations, and tablets are readily available; thus, tablets were chosen for use in this study. Excretion of prednisolone has been reported to be complete within 3 days. Multiple dosing does not result in plasma accumulation of prednisolone (Equisolon, 2014; Peroni, 2002).

It has been repeatedly demonstrated that the exogenously administered corticosteroids can enter the synovial fluid from the blood (Knych, Harrison, et al., 2013; Knych, Vidal, et al., 2013). Furthermore, the levels of methylprednisolone of 30–40 ng/mL in the joint have been estimated to be therapeutically effective (Autefage, 1986; Lillich, 1996). In fact, there are no minimum corticosteroid levels established that do not produce therapeutic effects in horses. In particular, the minimum effective concentrations for treating inflammation in the joint have not been established (Knych, 2017). Effects may not necessarily only occur at the articular level; it is also possible that the drug may affect the synovial membrane. Therapeutic effects of corticosteroids can persist long after the concentrations in plasma or synovial fluid have declined to low, or undetectable levels. The drug's action(s) are intracellular, as a result of binding to a receptor, and do not occur in the plasma or synovial fluid (Cruz-Topete, 2018). Finally, if extrapolated at a 10x level from the doses of orally administered corticosteroids that have been shown to be effective for the treatment of arthritis in

TABLE 1: Prednisolone levels, Pre- and 3 h post-administration of 400 mg prednisolone per os*

	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6
Serum, pre	ND†	ND	ND	ND	ND	ND
Serum, post‡	19.44	36.95	99.51	155.96	101.35	142.72
Radiocarpal§	2.45	5.98	32.85	39.13	34.01	56.23
Tarsometatarsal¶	4.89	5.16	38.87	38.88	22.09	12.10

Concentration in ng/mL.
† ND = Not Detectable.
‡ Mean = 92.655; Standard Deviation = 54.95.
§ Mean = 28.44; Standard Deviation = 20.58.
¶ Mean = 20.33; Standard Deviation = 15.67.

human patients (Jannsens, 2008; Kroon, 2019), the dose used in these horses, 400 mg per horse, is consistent with those doses.

Based on anecdotal reports from clients to the lead author, oral prednisolone may have value as a therapeutic agent in horses with osteoarthritis or other inflammatory conditions of one or more equine joints. This study confirmed the hypothesis that orally administered prednisolone could be detected in equine synovial fluid 3 h after administration. Although previous research indicated that per os prednisolone was of no value in treating human hand OA (Wenham et al., 2012), more recent reports indicate that prednisolone per os is an effective therapeutic agent in OA of the human hand (Kroon et al., 2019). Oral prednisolone has also been shown to be useful in the treatment of gout-related arthritis (Jannsens et al., 2008).

From a cost standpoint, and although costs may vary between locations and practitioners, a single bottle of prednisolone is presumably much less expensive than a single intra-articular injection. As orally administered prednisolone does enter equine synovial fluid, because administration is easier and less invasive than intra-articular administration of corticosteroids, and because multiple joints might be treated with a single dose, including joints that are relatively inaccessible by injection, its potential value as a therapeutic agent in horses with OA of one or more joints deserves further study.

Acknowledgements

The authors would like to thank Ms Veronica Bastionon for her assistance in handling, feeding and sample collection.

Authors' declarations of interest

No conflicts of interest have been declared.

Funding information

No financial support was obtained from any organisation for the work performed in this study.

Ethical animal research

The authors of this paper have adhered to the Principles of Veterinary Medical Ethics of the AVMA. All animals were used with client consent and were maintained under a high standard of care.

Author contributions

D. Ramey monitored horses, administered medication, collected samples, shipped samples and was the primary author on the manuscript. H. Knych tested the samples and added information to the materials and methods. Both authors have approved this manuscript.

References

Autefage, A., Alvinerie, M. and Toutain, P. (1986) Synovial fluid and plasma kinetics of methylprednisolone and methylprednisolone acetate in horses following intra-articular administration of methylprednisolone acetate. *Equine Veterinary Journal* **18**(**3**), 193-198.

Cruz-Topete, D. and Cidlowski, J.A. (2018) Glucocorticoids: molecular mechanisms of action. In: C. Riccardi, F. Levi-Schaffer and E. Tiligada (eds.) *Immunopharmacology and Inflammation*. Cham, Switzerland: Springer International Publishing, pp. 249-266.

Equisolon, INN-Prednisolon. European Medicines Agency (2014). https://www.ema.europa.eu/en/documents/product-information/equisolon-epar-product-information_en.pdf [Accessed 7th July 2021].

Jannsens, H.J.E.M., Jannsen, M., van de Lisdonk, E.H., van Riel, P.L.C.M. and van Weel, C. (2008) Use of oral prednisolone or naproxen for the treatment of gout arthritis: a double-blind, randomised equivalence trial. *Lancet* **371**(**9627**), 1854-1860.

Jordan, V.J., Ireland, J.L. and Rendle, D.I. (2017) Does oral prednisolone treatment increase the incidence of acute laminitis? *Equine Veterinary Journal* **49**(**1**), 19-25.

Knych, H.K., Harrison, L.M., Casbeer, H.C. and McKemie, D.S. (2013) Disposition of methylprednisolone acetate in plasma, urine, and synovial fluid following intra-articular administration to exercised thoroughbred horses. *Journal of Veterinary Pharmacology and Therapeutics* **37**(**2**), 125-132. Available on-line at https://rmtcnet.com/wp-content/uploads/2015/07/Knych-MPA-jvp12070.pdf [Accessed 1st September 2021].

Knych, H.K., Stanley, S.D., Harrison, L.M. and McKemie, D.S. (2017) Pharmacokinetics of betamethasone in plasma, urine, and synovial fluid following intra-articular administration to exercised thoroughbred horses. *Drug Testing and Analysis* **9**(**9**), 1385-1391.

Knych, H.K., Vidal, M.A., Casbeer, H.C. and McKemie, D.S. (2013) Pharmacokinetics of triamcinolone acetonide following intramuscular and intra-articular administration to exercised Thoroughbred horses. *Equine Veterinary Journal* **45**, 715-720.

Kroon, F.P.B., Kortekaas, M.C., Boonen, A., Böhringer, S., Reijnierse, M., Rosendaal, F.R. et al. (2019) Results of a 6-week treatment with 10 mg prednisolone in patients with hand osteoarthritis (HOPE): a double-blind, randomised, placebo-controlled trial. *Lancet* **394** (**10213**), 1993-2001.

Lillich, J.D., Bertone, A.L., Schmall, L.M., Ruggles, A.J. and Sams, R.A. (1996) Plasma, urine, and synovial fluid disposition of methylprednisolone acetate and isoflupredone acetate after intra-articular administration in horses. *American Journal of Veterinary Research* **57**(**2**), 187-192.

Machin, J., Duer, W., Maylin, G., Fenger, C., Wilson, D., Ivey, M. et al. (2019) Variability in plasma concentrations of methylprednisolone 6 days after intrasynovial injection of methylprednisolone acetate in racing horses: A field study. *Equine Veterinary Journal* **51**(**3**), 343-348.

MacMahon, P.J., Huang, A.J. and Palmer, W.E. (2016) Spine injectables: what is the safest cocktail? *American Journal of Roentgenology* **207**(**3**), 526-533. Available on-line at https://pubmed.ncbi.nlm.nih.gov/27341350/ [Accessed 11th March 2021].

McIlwraith, C. (2016) Intra-articular corticosteroids. In: C.W. McIlwraith, D.D. Frisbie, C.E. Cawcak and P.R. van Weeren. (Eds.) *Joint Disease in the Horse*, 2nd edn. St. Louis, Missouri: Elsevier. pp. 202-214.

McIlwraith, C.W. and Latterman, C. (2019) Intra-articular corticosteroids for knee pain-what have we learned from the equine athlete and current best practice. *Journal of Knee Surgery* **32**(**1**), 9-25.

Millares-Ramirez, E.M., Picotte, K. and Lavoie, J.-P. (2021) Pulmonary response of severely asthmatic horses after intra-articular administration of methylprednisolone. *Equine Veterinary Journal* **53** (**1**), 78-84.

Moyer, W. (2011) *Equine Joint Injection and Regional Anesthesia*. Chads Ford, PA, USA: Academic Veterinary Solutions, LLC.

Peroni, D.L., Stanley, S., Kollias-Baker, C. and Robinson, N.E. (2002) Prednisone per os is likely to have limited efficacy in horses. *Equine Veterinary Journal* **34**(**3**), 283-287.

Rusanen, M., Gröblad, M. and Korkala, O. (1986) Scanning electron microscopical study of the effects of crystalloid and water-soluble glucocorticoids on articular cartilage. *Scandinavian Journal of Rheumatology* **15**(**1**), 47-51.

Thiese, M. (2014) Observational and interventional study design types; an overview. *Biochemia Medica* (Zagreb). **24**(**2**), 199-210. Available on-line at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4083571/ [Accessed 7th July 2021].

Wenham, C.Y.J., Hensor, E.M.A., Grainger, A.J., Hodgson, R., Balamooy, S., Doré, C.J. et al. (2012) A randomized, double-blind, placebo-controlled trial of low-dose oral prednisolone for treating painful hand osteoarthritis. *Rheumatology (Oxford)* **51**(**12**), 2286-2294.

Wernecke, C., Braun, H.J. and Dragoo, J.L. (2015) The effect of intra-articular corticosteroids on articular cartilage: a systematic review. *Orthopedic Journal of Sports Medicine* **3**(**5**): 2325967115581163. Available on-line at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4622344/#:~:text=and%20long%20terms.-,Summary,viability%2C%20and%20gross%20cartilage%20morphology [Accessed 10th March 2021].