Published in final edited form as:

Equine Vet J. 2014 January ; 46(1): . doi:10.1111/evj.12095.

Efficacy of cyclooxygenase inhibition by two commercially available firocoxib products in horses

M.H. Barton, E. Paske, N. Norton, D. King, S. Giguère, and S. Budsberg*

*Departments of Large Animal Medicine and Small Animal Medicine and Surgery, University of Georgia, Athens, GA 30602, USA

Summary

Reasons for performing study—Two firocoxib preparations for oral use are approved for use in animals in many countries: a chewable canine tablet and an equine paste. Cost savings have led many veterinarians to use the canine product in horses even though this is an off-label use of the preparation.

Objective—To determine the relative efficacy of 2 commercially available firocoxib products to inhibit prostaglandin E₂ (PGE₂) synthesis after oral dosing in horses.

Design—The study was a cross over design using 8 adult horses (n = 4 for each preparation during each treatment period; body weight range 532 to 614 kg).

Methods—Horses received 57 mg of the assigned firocoxib preparation orally once daily for 7 days, with a 14 day wash out period between drug cross over. Ten healthy adult light breed horses were used as no treatment controls. During each treatment period, blood was taken before dosing on Days 0 and 7, and on Day 7 one hour after dosing for *ex vivo* lipopolysaccharide (LPS) stimulation to induce (PGE₂) synthesis. Heparinised plasma was also collected on Day 7 immediately prior to and one hour after dosing to determine plasma firocoxib concentrations.

Results—In the control group, there was no significant change in LPS-induced PGE₂ over time. In contrast, immediately prior to and one hour after treatment on Day 7, the mean LPS-induced PGE₂ concentration decreased significantly compared to Day 0 values in all treated horses. There was no difference in PGE₂ or plasma firocoxib concentrations between firocoxib treatment groups.

Conclusion—In this model, the canine chewable preparation of firocoxib was as effective as the equine paste formulation at reducing LPS-induced PGE₂ synthesis. The canine chewable preparation of firocoxib may be a suitable alternative to the paste formulation in horses for situations where extra-label drug use can be legally justified.

Corresponding Author: Michelle Henry Barton, tel: 706-542-6326; bartonmh@uga.edu.

Conflicts of Interest

No competing interests have been declared.

Ethical Animal Research

The University's Institutional Animal Use and Care Committee approved the use of horses for this study (A2012 04-008-Y1-A0) and horses were cared for according to the guidelines stated in an Animal Use Protocol determined and approved by the University's Department of Animal Resources.

Authorship

Michelle Barton and Elyse Paske were involved in all phases of the manuscript. Natalie Norton and Deanne King were involved in data collection and study execution and preparation of the manuscript. Steeve Giguere and Steve Budsberg were involved in the study design, data analysis and interpretation, and manuscript preparation.

Keywords

horse; nonsteroidal anti-inflammatory drugs; COX1 sparing; prostaglandin E₂; Equioxx; Previcox

Introduction

Once released from the cell membrane by phospholipase A₂, arachidonic acid is metabolised by cyclooxygenase to form prostaglandins G₂ and H₂, the later of which is subsequently converted to form various prostanoids [1]. Cyclooxygenase (COX) enzymes exist in at least 2 isoforms. COX1 is constitutively expressed in nearly all tissues and is responsible for controlling physiologic processes such as platelet aggregation, vasodilation in the kidneys, and the production of the protective mucus coating in the gastrointestinal tract [2]. In contrast, COX2 is induced by pro-inflammatory molecules, such as lipopolysaccharide (LPS). The prostanoids produced by COX2 are primarily involved in the pathologic responses of inflammation, such as fever and nerve ending sensitisation to pain.[2]

Although nonselective non-steroidal anti-inflammatory drugs (NSAIDs) are highly effective, undesirable side effects of kidney damage, gastric ulcers, and colitis are reported [3,4]. Selective COX2 inhibiting drugs offer the advantage of reducing inflammatory induced prostanoid production while sparing the effects of physiologic regulation by COX1 [2]. The net result is a potent anti-inflammatory drug with reduced chance of adverse side effects. Firocoxib is a COX1 sparing NSAID approved for use in horses [5–7]. Using a relatively simple *in vitro* model measuring the release of thromboxane (COX1) from clotted blood relative to LPS-induced COX2 production of prostaglandin E₂ (PGE₂) in heparinised whole blood in the horse, firocoxib is approximately 265 times more selective in inhibiting COX2, relative to COX1 [4,8]. Pharmacokinetic studies in horses indicate that when oral firocoxib paste for horses is given daily, steady state plasma concentrations are achieved by the seventh day and plasma concentrations of the drug peak approximately one hour after the seventh dose [7]. Firocoxib has been shown to be a highly effective analgesic in horses with osteoarthritis [3]. Furthermore, interest has recently been sparked in the gastrointestinal protective effects of firocoxib, relative to nonselective NSAIDs, on recovery of mucosal barrier function following ischemia [4]. These findings, combined with the convenience of once-a-day dosing, have boosted the popularity of firocoxib in horses.

Firocoxib is also marketed as either a 57 mg or 220 mg chewable tablet for use in dogs with osteoarthritis. Interestingly, the daily recommended dose in dogs is 50 times greater on a mg/kg body weight basis than that used in horses [9]. Considering that a 57 mg chewable canine tablet can provide the recommended daily dose for a 570 kg horse at considerable cost savings, many veterinarians would like to prescribe the canine product in horses. However this is an off-label use of the preparation and, in the United States, Great Britain and many other countries, cost is not a legitimate reason to use a drug extra-label. It is only when an approved product has been determined by the attending veterinarian to be clinically ineffective for the labeled use that another animal-approved drug may be used in an extra-label manner [10]. Even if legitimate extra-label use of the canine product could be justified in an individual horse, the dose is not known and it is uncertain whether the canine formulation is absorbed and results in appropriate cyclooxygenase inhibition in horses. Thus, the main purpose of the study herein was to determine the pharmacodynamic efficacy of the canine chewable preparation of firocoxib to inhibit LPS-induced PGE₂, relative to the equine paste formulation, in an *ex vivo* model in horses. The main hypothesis was that compared to the equine paste formulation of firocoxib, the canine chewable preparation would be equally effective at inhibiting LPS-induced PGE₂ production in an *ex vivo* model

in horses. Furthermore, it was hypothesised that there would be no difference in steady state plasma concentrations of firocoxib between the 2 preparations.

Materials and Methods

Animals

The efficacy of each firocoxib preparation was tested in 8 healthy adult light breed horses with a mean weight of 575 kg (range of 532 to 614 kg). Ten healthy adult light breed horses were used as no treatment controls. Horses did not receive any additional medication during the entire study period. The University's Institutional Animal Use and Care Committee approved the use of horses for this study (A2012 04-008-Y1-A0) and horses were cared for according to the guidelines stated in an Animal Use Protocol determined and approved by the University's Department of Animal Resources.

Dosing protocol

The study was a cross over design using 8 adult horses (n = 4 for each preparation during each treatment period), with a 14 day wash out period between drug cross over. With both firocoxib preparations, horses were dosed with the amount of medication a 570 kg horse would receive on a daily basis at the recommended dose of 0.1 mg/kg body weight: one whole tube of the equine paste formulation (Equioxx®)^a or one 57 mg tablet of the canine chewable tablet (Previcox®)^a. The tablet was dissolved in 5 ml of water with an additional 5 ml of corn syrup added for palatability. Horses were maintained on pasture during the treatment period and feed (i.e. pasture) was not withheld prior to dosing. The drugs were administered daily for 7 days. The 10 control horses did not receive any medication during each 7 day treatment period.

Blood collection

On Days 0 and 7 of each treatment period, prior to dosing, horses were brought in from pasture and stalled for 24 h to facilitate blood collection. During stalling, horses were allowed free access to water and were fed coastal Bermuda hay 6 h after dosing. Blood was collected by direct jugular venipuncture after sterile preparation of the site. On Days 0 and 7 of each treatment period, prior to dosing and one hour after dosing blood for *ex vivo* PGE₂ analysis was collected into sterile tubes containing heparin. On Days 0 and 7 of each treatment period, prior to dosing, blood was also placed into glass clot tubes containing no anticoagulant for creatinine, total protein, and albumin concentration determinations. On Day 7 of each treatment period, prior to dosing and one hour after dosing, heparinised blood was collected from the study horses for plasma firocoxib concentration determination. Horses in the control group were maintained on pasture during the 7 day period and blood was collected on Days 0 and 7 for *ex vivo* PGE₂ analysis. Four control horses on the same pasture were included during the first treatment period. In addition to the same 4 control horses used during the first treatment period, 6 additional healthy adult horses on the same pasture were enrolled for the second treatment period.

Prostaglandin E₂ measurement

Blood samples were transported on ice to the lab within one hour. The samples were aliquoted into sterile polypropylene tubes and were incubated at 37°C on an orbital roller in the absence (control) or presence of O55:B5 LPS^b at a final concentration of 100 µg/ml. After 6 h, the samples were centrifuged, 100 µL of the plasma was combined with 900 µL of

^aMerial, Duluth, Georgia, USA.

^bList Biological Laboratories, Campbell, California, USA.

methanol and dried by evaporation. Samples were stored at -80°C until analysis. Prostaglandin E_2 was determined using an enzyme inhibition assay (PGE_2 Express EIA Kit)^c that has been previously validated in horses [11]. Samples were diluted with assay buffer 1:4 prior to analysis.

Plasma firocoxib concentrations

Heparinised plasma was collected on Day 7 immediately prior to dosing and one hour after dosing and stored at -80°C until analysed for firocoxib concentrations as previously described by high-pressure liquid chromatography (HPLC) with ultraviolet detection at 254 nm (Agilent 1100 series)^d at North Carolina State University [12,13].

Serum creatinine, total protein, and albumin measurement

Serum samples collected on Day 0 and Day 7 of each study period were submitted to The University of Georgia Clinical Pathology Laboratory for measurement of creatinine, total protein, and albumin concentrations, using a chemistry analyser (Hitachi P Module 800)^e.

Data analysis

For PGE_2 data, the unstimulated control PGE_2 value was subtracted from the LPS-induced value. Normality of the data and equality of variances were assessed using the Shapiro-Wilk and Levene's tests, respectively. Prostaglandin E_2 data were log-transformed to meet the assumptions of the ANOVA. In preliminary analyses, a two-way ANOVA with repeated measures was used to ensure that there was no significant effect of study cross over period or day within a treatment period (Day 0 versus Day 7) on PGE_2 concentrations in untreated control horses. Similar models were used to assess the effect of treatment group and time relative to treatment (Day 0, Day 7) on PGE_2 , serum creatinine, total protein, albumin, or firocoxib concentrations. Multiple pairwise comparisons were done using the Holm-Sidak test. Data are reported as the mean $\pm$ SE. For all analyses, $P < 0.05$ was considered significant.

Results

Prostaglandin E_2 concentrations

For control horses, there was no significant difference between treatment period ($P = 0.63$), day of treatment ($P = 0.84$), or period $\times$ day interaction ($P = 0.823$) (Fig 1). For horses receiving firocoxib, there was no significant difference between firocoxib preparations ($P = 0.7$), or firocoxib preparation $\times$ time of treatment interaction ($P = 0.27$), but there was a significant effect of time of treatment ($P < 0.001$). Prostaglandin E_2 values were significantly lower on Day 7 immediately prior to dosing and one hour after dosing for horses receiving either preparation, as compared to Day 0 values (Fig 2). Furthermore, for both firocoxib preparations, PGE_2 values at one hour post dosing on Day 7 were significantly lower than Day 7 immediately before dosing. Relative to Day 0 values, PGE_2 concentrations decreased by $58 \pm 11\%$ and $69 \pm 5\%$ immediately prior to dosing and one hour after dosing by $71 \pm 7\%$ and $79 \pm 8\%$ for horses receiving the canine chewable tablet and the equine paste, respectively.

^cCayman Chemical, Ann Arbor, Michigan, USA.

^dAgilent Technologies, Wilmington, Delaware, USA.

^eRoche Diagnostics, Indianapolis, Indiana, USA.

Firocoxib plasma concentrations

On Day 0 of each treatment period, prior to administration of either firocoxib preparation, plasma firocoxib concentrations were below the limit of detection in all treatment group horses. There was no significant difference in plasma concentrations of firocoxib between firocoxib treatment groups ($P = 0.70$), or firocoxib preparation $\times$ time of treatment interaction ($P = 0.52$), but there was a significant effect of time of treatment ($P < 0.001$) (Table 1). Plasma firocoxib concentrations were significantly greater on Day 7 one hour after dosing for horses receiving either preparation, as compared to Day 7 immediately prior to dosing (Table 1).

Serum creatinine, total protein, and albumin

There were no significant differences in serum creatinine, total protein, or albumin concentrations over time or between treatment groups. For horses receiving firocoxib, the serum creatinine values were within normal reference range for all horses on Day 0 and Day 7 for each treatment period (Table 2). Similarly, for both treatment periods, serum total protein and albumin concentrations did not fall below the minimum reference values established by the laboratory for all horses receiving firocoxib on Day 0 or Day 7 (Table 2).

Discussion

To ensure efficacy of both preparations in the study herein, a well-established pharmacodynamic effect of COX2 inhibitors was measured: inhibition of LPS-induced PGE₂ production [2,12,14]. If the drug is absorbed *in vivo*, then LPS stimulation of whole blood collected from horses receiving the COX2 inhibitor should reflect the level of active drug in the blood at the time of testing in the *ex vivo* model. The times for blood collection for determination of *ex vivo* LPS-induced PGE₂ production and for firocoxib plasma concentrations in this study were based on the report that administration of the paste formulation of firocoxib reaches a steady state plasma concentration by the seventh daily oral dose in horses, with peak plasma concentrations of firocoxib achieved approximately one hour after dosing for 7 days [7]. In reality, the 57 mg canine chewable tablet cannot be accurately divided to accommodate exact dosing, thus both products were given daily to all horses at an equivalent total dose of 57 mg. The mean and range in body weights of the horses included in this study were 575 kg and 532 to 614 kg, respectively. At a recommended dose of 0.1 mg firocoxib/kg body weight, some of the horses were either slightly under or over dosed by a maximum of 4 mg of firocoxib. However, each horse received 57 mg of each product in a cross over design, thus enabling direct comparison between preparations across a close range of body weights. To mimic the field situation of common oral dosing practices used by most horse owners, feed was not withheld prior to daily dosing and drugs were administered per os and not intragastric.[7]

Because of the inherent variability often encountered with LPS activated *ex vivo* blood preparations, a group of healthy horses on the same pasture were included as no drug treatment controls for 7 days during both drug treatment trial periods (i.e. each drug cross over period) though a placebo treatment was not included in this group. Compared to baseline values on Day 0, *ex vivo* LPS-induced PGE₂ production did not significantly change in the control horse group, whereas horses in both treatment groups experienced a significant decrease in *ex vivo* LPS-induced PGE₂ production over the same time period. Furthermore, there was no significant difference detected in PGE₂ levels between treated groups at either time tested on the seventh day of treatment. Thus the first hypothesis of this study was supported by our data in that when given orally to horses, the canine chewable preparation of firocoxib is as effective as the equine paste formulation at inhibiting LPS-induced PGE₂ production in an *ex vivo* model.

For additional credence to the premise that LPS stimulation of treated horses' blood should reflect the level of drug in the blood at the time of testing in the *ex vivo* model, blood concentrations of firocoxib were measured on Day 7. Supporting our second hypothesis of this study, there were no significant differences between the canine chewable and equine paste formulation groups in plasma firocoxib concentrations immediately prior to dosing or one hour after dosing on Day 7. Furthermore, plasma firocoxib concentrations for the canine chewable preparation (88 ± 9 ng/ml, SE) and for the equine paste formulation (83 ± 7 ng/ml, SE) were similar to that previously reported at steady state (104 ± 33 ng/ml, SD) in horses given the paste formulation for 7 days [7]. In an *in vitro* study in horses, the whole blood concentration of firocoxib needed to inhibit 80% of LPS-induced PGE₂ production was estimated to be 67 ng/ml blood [7]. Given that the plasma volume in horses is about 65% that of whole blood, this would be equivalent to approximately 103 ng firocoxib/ml plasma. In the current study, plasma firocoxib concentrations for both preparations reached approximately 110 ng/ml plasma at one hour after dosing on Day 7. At that same blood collection time, the mean decrease in LPS-induced PGE₂ production was 71 to 79%, relative to baseline before initiation of therapy, for horses receiving either drug preparation. Given the differences in study design, the striking similarity in the degree of prostaglandin inhibition by firocoxib between these studies is remarkable.

Lastly, although crude proxies of renal function and colonic or renal protein loss, serum creatinine, total protein, and albumin concentrations did not significantly change during the study period in either treatment group or across treatment groups. With the plasma concentrations of firocoxib being well within the therapeutic window at steady state level for both preparations, it seems likely that the canine chewable product would be as "safe" as the paste formulation in horses when dosed at approximately 0.1 mg firocoxib/mg body weight.

In conclusion, the current study demonstrated that when given orally to healthy horses for 7 days, the canine chewable preparation and the equine paste formulation of firocoxib have near identical steady state plasma firocoxib concentrations and are equally effective at inhibiting LPS-induced PGE₂ production in an *ex vivo* model. These results encourage further investigation into the approval of a formulation similar to the canine tablet for use in horses. Despite the lack of difference in efficacy or firocoxib plasma concentrations between the 2 drug preparations in this study, animal owners and veterinarians should always use currently approved species-specific products. In the United States, the Animal Medicinal Drug Use Clarification Act of 1994 and the Extra-label Drug Use in Animal Act of 1996 [10] allows extra-label use of drugs in companion animals if such use does not pose a public health risk and its use takes place within the scope of a valid veterinarian-client-patient relationship. However, it is only when an approved product has been determined by the attending veterinarian to be clinically ineffective for the labeled use that another animal-approved drug may be legally used in an extra-label manner. Cost of the preparation is not listed as a legitimate reason for extra-label drug use. Veterinarians in the United States who deviate from the extra-label use laws do so illegally and risk becoming the target of unfavourable actions by the Food and Drug Administration which can range from warning to prosecution [15]. Similar limitations on the use of drugs licensed for other species exist in Great Britain and other countries. Because the canine chewable product is only available in either 57 or 220 mg tablets, veterinarians who can justify prescribing this product for use in horses must also be aware of the limitations of accurate dosing.

Acknowledgments

The authors would like to thank Ms. Devon Wochele for technical assistance.

Source of Funding

This study was funded by the University of Georgia's Provost's Summer Research Initiative and grant number 9T35OD010433-06, supporting EP, from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH). Contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH.

References

1. Dubois R, Abramson S, Grofford L, Gupta R, Simon L, Van de Putte L, Lipsky P. Cyclooxygenase in biology and disease. *FASEB J*. 1998; 12:1063–1073. [PubMed: 9737710]
2. Hinz B, Brune K. Cyclooxygenase 2: 10 years later. *J Pharm Exp Therapeutics*. 2001; 300:367–375.
3. Doucet MY, Bertone AL, Hendrickson D, Hughes F, MacAllister C, McClure S, Reinemeyer C, Rossier Y, Sifferman R, Vrins AA, White G, Kunkle B, Alva R, Romano D, Hanson PD. Comparison of efficacy and safety of paste formulations of firocoxib and phenylbutazone in horses with naturally occurring osteoarthritis. *J Amer Vet Med Assoc*. 2008; 232:91–97. [PubMed: 18167116]
4. Cook V, Meyer C, Campbell N, Blikslager A. Effect of firocoxib or flunixin meglumine on recovery of ischemia-injured equine jejunum. *Amer J Vet Res*. 2009; 70:992–1000. [PubMed: 19645580]
5. Koene M, Goupil X, Kampmann C, Hanson PD, Denton D, Pollmeier MG. Field trial validation of the efficacy and acceptability of firocoxib, a highly selective Cox-2 Inhibitor, in a group of 96 lame horses. *J Equine Vet Sci*. 2010; 30:237–243.
6. Kvaternick V, Pollmeier M, Fischer J, Hanson PD. Pharmacokinetics and metabolism of orally administered firocoxib, a novel second generation coxib, in horses. *J Vet Pharmacol Ther*. 2007; 30:208–217. [PubMed: 17472652]
7. Letendre L, Tessman R, McClure S, Kvaternick V, Fischer J, Hanson P. Pharmacokinetics of firocoxib after administration of multiple consecutive daily doses to horses. *Amer J Vet Res*. 2008; 69:1399–1405. [PubMed: 18980421]
8. McCann M, Andersen D, Brideau C, Black D, Zhang H, Hickey G. In vitro activity and in vivo efficacy of a novel COX-2 inhibitor in the horse. *J Vet Intern Med*. 2002; 16:789.
9. Goodman L, Torres B, Punke J, Reynolds L, Speas A, Ellis A, Budsberg S. Effects of firocoxib and tepoxalin on healing in a canine gastric mucosal injury model. *J Vet Intern Med*. 2009; 23:56–62. [PubMed: 19175721]
10. Department of Health and Human Services. Department of Health and Human Services FaDA. FaDA 61 FR 25601 Extra label drug use in animals 21 CFR Part 530. Office of the Federal Register, National Archives and Records Administration; 1996. p. 25106-25118.
11. Donovan D, Jackson C, Colahan P, Norton N, Hurley D. Exercise induced alterations in proinflammatory cytokines and prostaglandin F2 alpha in horses. *Vet Immunol Immunopathol*. 2007; 118:263–269. [PubMed: 17617470]
12. Davis J, Marshall J, Papich M, Blikslager A, Campell N. The pharmacokinetics and in vitro cyclooxygenase selectivity of deracoxib in horses. *J Vet Pharm Therap*. 2010; 34:12–16.
13. Kvaternick V, Malinski T, Wortmann J, Fischer J. Quantitative HPLC-UV method for the determination of firocoxib from horse and dog plasma. *J Chromatogr B*. 2007; 854:313–319.
14. Brideau C, Van Staden C, Chang C. In vitro effects of cyclooxygenase inhibitors in whole blood of horses, dogs, and cats. *Amer J Vet Res*. 2001; 62:1755–1760. [PubMed: 11703020]
15. Extralabel drug substitutions runs afoul of law. *J Amer Vet Med Assoc*. 2010:1275. [PubMed: 21118013]

Fig 1. Lipopolysaccharide-induced PGE₂ concentrations (mean $\pm$ SE) in whole blood in control horses (n = 10) receiving no treatments over 7 days.

Fig 2.

Ex vivo lipopolysaccharide-induced PGE₂ concentrations (mean $\pm$ SE) in whole blood in 8 horses receiving 2 different firocoxib preparations orally for 7 days. a = significantly different from the Day 0 value (before initiation of treatment) within the same treatment group. b = significantly different from both the Day 0 and the Day 7 immediately prior to dose values within the same treatment group.

Table 1

Plasma firocoxib concentrations (mean $\pm$ SE) * in 8 adult horses treated for 7 days with 2 different firocoxib preparations.

	Canine Chewable Tablet		Equine Paste Formulation	
	Day 7 Immediately Before Dosing	Day 7 One Hour After Dosing	Day 7 Immediately Before Dosing	Day 7 One Hour After Dosing
Plasma Firocoxib Concentration (ng/ml)	87.9 $\pm$ 9.1	109.2 $\pm$ 7.1 ^a	83.1 $\pm$ 7.6	110.9 $\pm$ 10.4 ^a

* Firocoxib was not detectable in the plasma of any horse prior to dosing on Day 0.

^a significantly different from corresponding firocoxib preparation on Day 7 before dosing value.

Table 2

Serum creatinine, serum total protein, and albumin concentrations (mean $\pm$ SE) in 8 adult horses treated for 7 days with 2 different firocoxib preparations.

	Canine Chewable Tablet		Equine Paste Formulation	
Parameter	Day 0	Day 7	Day 0	Day 7
Creatinine (mg/dl)	1.2 $\pm$ 0.06	1.1 $\pm$ 0.2	1.2 $\pm$ 0.06	1.1 $\pm$ 0.05
Total Protein (gm/dl)	7.0 $\pm$ 0.1	6.6 $\pm$ 0.7	6.8 $\pm$ 0.3	6.6 $\pm$ 0.2
Albumin (gm/dl)	3.4 $\pm$ 0.07	3.2 $\pm$ 0.2	3.3 $\pm$ 0.1	3.1 $\pm$ 0.06