

Short-term administration of flunixin meglumine or firocoxib does not alter viscoelastic coagulation profiles in healthy horses

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doi.org/10.2460/javma.22.08.0367

OBJECTIVE

To evaluate the effect of the cyclooxygenase-2-selective NSAID firocoxib, compared to the nonselective NSAID flunixin meglumine on viscoelastic coagulation parameters in healthy horses.

ANIMALS

12 healthy adult mixed-breed horses.

PROCEDURES

Following a crossover protocol, horses were administered flunixin meglumine (1.1 mg/kg, IV, q 12 h for 5 days), allowed a 6-month washout period, and then administered firocoxib (0.3 mg/kg, PO, once, then 0.1 mg/kg, PO, q 24 h for 4 days). Omeprazole (1 mg/kg, PO, q 24 h) was administered concurrently with each NSAID. Viscoelastic coagulation profiles and traditional coagulation parameters (prothrombin time, partial thromboplastin time, and fibrinogen) were measured before and after each treatment.

RESULTS

Viscoelastic coagulation parameters were within reference intervals before and after both treatments. There was a statistically significant difference between treatments for amplitude at 10 minutes after clot time ($P = .02$) and maximum clot formation ($P = .02$); however, the magnitude of change was not clinically significant.

CLINICAL RELEVANCE

Short-term administration of flunixin meglumine and firocoxib did not result in significant alteration of viscoelastic coagulation profiles in healthy horses. However, clinicians should be aware of possible coagulopathy secondary to NSAID administration with long-term use or critical illness, and further study is indicated.

NSAIDs are commonly used in equine practice to treat pain, inflammation, and fever. Effects of NSAIDs are mediated by inhibition of the cyclooxygenase (COX) cascade, which consists of 2 isoforms, COX-1 and COX-2. NSAIDs have differential selectivity for COX-1 or COX-2, depending on the drug, dose, route, and timing of administration. Preferential inhibition of the induced isoform, COX-2, is thought to maintain anti-inflammatory properties while mitigating detrimental inhibition of COX-1. Firocoxib (Equioxx), the COX-2-selective NSAID licensed for use in equids in the US, has a high COX-2:COX-1 selectivity ratio in horses compared to less selective NSAIDs such as phenylbutazone and flunixin meglumine.¹ While COX-2-selective NSAIDs (coxibs) have been regarded as safer than nonselective NSAIDs, multiple homeostatic functions of COX-2 have been identified that may cause additional undesirable effects when inhibited.²⁻⁵

Beyond mediating pain and inflammation, the COX isoenzymes play an important role in coagulation homeostasis. COX-1 has procoagulant effects though production of thromboxane, which promotes vasoconstriction and platelet aggregation.⁶⁻⁸ COX-2 exerts anticoagulant effects through the synthesis of prostacyclin, which causes vasodilation and prevents platelet aggregation.^{2,9,10} Selective inhibition of COX-2 may disrupt the homeostatic balance between pro- and anticoagulant effects of COX-1 and COX-2. Thrombotic events have been associated with coxib administration in humans,¹¹⁻¹⁶ and adverse cardiovascular events such as heart attack and stroke have resulted in withdrawal of 2 human coxibs from the market.^{7,17,18}

While thromboembolic diseases like myocardial infarction and stroke are not commonly encountered in horses, the presence of coagulopathy in critically ill horses is well documented.^{19,20} Clinical consequenc-

es of coagulopathy in horses include disseminated intravascular coagulation, jugular thrombophlebitis, and laminitis, which all contribute to multiple organ dysfunction, morbidity, and mortality. Clinical trials comparing COX-2-selective and nonselective NSAIDs in critically ill equine patients have not reported significant differences in the incidence of jugular thrombosis.²¹⁻²³ However, these trials have not directly assessed coagulation parameters, and it remains possible that COX-2-selective NSAIDs induce subclinical hypercoagulability in horses. Considering the documented thrombotic events with coxib use in humans, and as COX-2-selective NSAIDs become increasingly popular for use in critically ill horses, it is important to investigate whether these medications alter coagulation homeostasis in horses.

The aim of this study was to evaluate the effect of the COX-2-selective NSAID firocoxib, compared to the nonselective NSAID flunixin meglumine on viscoelastic coagulation parameters in healthy horses. Our null hypothesis was that neither NSAID would result in a significant alteration of viscoelastic coagulation profiles in healthy horses.

Materials and Methods

Study design

Clinically healthy adult horses from the Veterinary Teaching Hospital herd were enrolled in a prospective crossover study. A priori power analysis was performed (95% confidence interval, 80% power), using a 2-sample *t* test accounting for paired samples, to detect a clinically relevant change in viscoelastic coagulation parameters (the magnitude of relevant change is specific to each parameter, based on 10% or greater deviation from standard normal reference values). Baseline means and SD were chosen from clinical data generated in our hospital, and the parameter with the most variable values between individuals was chosen, resulting in a sample size of 12 horses. All procedures were approved and performed with the oversight of the institutional animal care and use committee.

During treatment, horses were housed in individual stalls with timothy grass/alfalfa mixed hay fed twice daily and water available at all times. Horses were considered clinically healthy on the basis of complete physical examination, serum chemistry profile, and CBC. During each treatment week, horses were monitored with physical exams daily and attitude, appetite, manure production, and water consumption were recorded every 6 hours.

After collection of initial blood samples, flunixin meglumine was administered via intravenous catheter every 12 hours for 5 days. Post-treatment blood samples were collected, and horses were allowed a 6-month washout period. For the second treatment week, firocoxib (Equioxx) was administered with an initial loading dose of 0.3 mg/kg, PO, followed by 0.1 mg/kg, PO, every 24 hours for a total of 5 doses based on current best practice recommendations to achieve therapeutic concentrations.²⁴⁻²⁶ To mitigate risk of gastric ulceration, omeprazole (Gastrogard; 1

mg/kg, PO, q 24 h) was administered to all horses during both treatment weeks.

Hematologic monitoring

Blood was collected prior to the start of each protocol and following the last dose of NSAID (12 hours after final dose of flunixin meglumine and 24 hours after final dose of firocoxib) for coagulation testing as well as markers of hydration and renal function. For coagulation testing, whole blood samples were obtained by direct venipuncture of a jugular vein using an 18-gauge needle and 3-mL syringe for coagulation testing using a point-of-care viscoelastic coagulation monitor (VCM Vet; Entegri-on Inc). Briefly, blood was placed in prewarmed test cartridges within 4 minutes of collection and loaded in the VCM Vet device. The viscoelastic coagulation profile included clot time (CT), clot formation time (CFT), α angle, maximum clot formation (MCF), amplitude at 10 and 20 minutes after clot time (A10 and A20), and lysis index at 30 and 45 minutes after clot time. Remaining blood was processed in sodium citrate tubes, and sodium citrate anticoagulated plasma samples were stored at -20°C for traditional coagulation profile analysis (prothrombin time [PT], partial thromboplastin time [PTT], and fibrinogen).

Statistical analysis

All statistical analysis was completed using R version 4.1.2 in RStudio version 1.3.1093.²⁷ Data were visualized using the package ggpubr version 0.4.0.²⁸ All analyses were performed using 2-sided tests of hypotheses; $P < .05$ was the criterion for statistical significance. Normality was assessed by visual evaluation of histogram and QQ plots as well as the Shapiro-Wilk test. Data were not normally distributed; therefore, nonparametric methods were used. Descriptive statistics were reported as median and interquartile (25th to 75th percentile) range (lower quartile, upper quartile). A Wilcoxon signed rank test was used to assess differences in coagulation parameters between treatments (flunixin meglumine and firocoxib) and over time (pre- and post-treatment).

Results

Horses

All horses completed the study without complications. The study population consisted of 9 mares, 2 geldings, and 1 stallion with ages ranging from 6 to 22 years (median, 14.5 years). A variety of breeds were represented, including 3 Arabian horses, 2 Standardbreds, 2 Thoroughbreds, 2 Quarter Horses, an Appaloosa, a Missouri Fox Trotter, and a Tennessee Walker. Physical examination parameters remained within normal limits, and there was no change in attitude, appetite, or manure production throughout the duration of the study.

Hematologic monitoring

CBC and selected biochemical parameters remained within normal limits throughout the study in all horses. Coagulation parameters are summarized (**Table 1**).

Table 1—Values of viscoelastic and traditional coagulation parameters before (pre) and after (post) treatment with firocoxib and flunixin meglumine, presented as median (quartile 1, quartile 3). Results of Wilcoxon rank sum test over time (*P* value pre-post) and between treatments (*P* value pre-pre and *P* value post-post).

Parameter	Firocoxib			Flunixin meglumine			Between treatments		Ref
	Pre	Post	<i>P</i> value pre-post	Pre	Post	<i>P</i> value pre-post	<i>P</i> value pre-pre	<i>P</i> value post-post	
CT (s)	904.5 (776.8, 1078.3)	1049 (972.3, 1149)	0.2	870 (708.8, 933.8)	916 (838.8, 996.5)	0.6	0.3	0.3	536–1270.1
CFT (s)	318.5 (275.8, 398)	380.5 (340.8, 437)	0.1	283.5 (253, 349)	327.5 (285.5, 388)	0.5	0.3	0.3	122.5–574
Alpha (degrees)	34.5 (28.8, 42.3)	28.5 (25.3, 30.5)	0.1	35.9 (30.8, 39.7)	31.8 (26.3, 35.9)	0.5	> 0.9	0.5	11.6–50.5
A10 (VCM units)	16.5 (15, 21)	18 (14.8, 20.3)	0.6	22.7 (18.2, 23.8)	19.7 (16.5, 23.2)	0.3	0.009**	0.3	8.9–27.1
A20 (VCM units)	24.5 (19.8, 26.5)	25.5 (23, 27.3)	0.6	29.7 (26.9, 31.1)	30.1 (25.1, 34.4)	0.9	0.007**	0.02*	17.8–35.5
MCF (VCM units)	25 (20.8, 27)	27 (24, 28)	0.6	30.7 (27.6, 31.8)	30.9 (26.1, 32.7)	0.9	0.002**	0.02*	17.2–38.6
LI30 (%)	100 (99, 100)	100 (99, 100)	> 0.9	99.9 (99.2, 100)	99.8 (99.1, 99.9)	> 0.9	0.4	0.7	97.5–101.8
LI45 (%)	91 (90.3, 92.5)	92 (88, 92.5)	> 0.9	91.0 (90.5, 97.0)	91.2 (89.6, 94.3)	> 0.9	0.6	> 0.9	82.2–101.5
PT (s)	12.4 (11.9, 12.8)	12 (11.9, 12.15)	0.1	19.6 (17.4, 21.9)	16.7 (15.6, 19.1)	0.04*	< 0.001***	< 0.001***	8.0–15.0
PTT (s)	43.5 (39.3, 45.6)	42.3 (40.4, 43.5)	0.6	68 (64.7, 90.0)	65.5 (55.2, 88.6)	0.2	< 0.001***	< 0.001***	33.0–47.0
Fibrinogen (mg/dL)	163 (145.3, 213.3)	174.5 (144, 207.5)	> 0.9	164 (138.5, 184.5)	185 (166.8, 220.3)	0.03*	0.5	0.05	125–262

A10 = Amplitude at time 10. A20 = Amplitude at time 20. Alpha = Alpha angle. CFT = Clot formation time. CT = Clot time. LI30 = Clot lysis at time 30. LI45 = Clot lysis at time 45. MCF = Maximum clot formation. PT = Prothrombin time. PTT = Partial thromboplastin time. REF = Institutional reference range.

P* < .05. *P* < .01. ****P* < .001.

With a few individual exceptions, viscoelastic coagulation parameters were within reference intervals before and after treatment for both flunixin meglumine and firocoxib. There was a statistically significant difference in A20 between flunixin meglumine and firocoxib both before (*P* = .007) and after treatment (*P* = .02). There was also a statistically significant difference in MCF between flunixin meglumine and firocoxib both before (*P* = .002) and after treatment (*P* = .02). There was a significant difference in A10 between firocoxib and flunixin meglumine before treatment (*P* = .009) but not after (*P* = .3).

Both PT and PTT were increased above the reference range in all horses before and after treatment with flunixin meglumine, resulting in a significant difference between flunixin meglumine and firocoxib before and after treatment (all *P* < .001). Fibrinogen remained within normal limits for all horses throughout the study. There was a statistically significant increase in fibrinogen following treatment with flunixin meglumine (*P* = .03), and post-treatment fibrinogen was significantly different when horses received flunixin meglumine compared to firocoxib (*P* = .04).

Discussion

The findings of this study failed to reject the null hypothesis that neither flunixin meglumine nor firocoxib would result in a significant alteration of viscoelastic coagulation profiles in healthy horses. While there was a statistically significant difference over time and between treatments for 2 parameters (A20 and MCF), A20 and MCF represented clot firmness (at 20 minutes and maximum firmness before clot lysis began, respectively), with higher values indicating better clot quality. Both parameters were higher in during treatment with flunixin meglumine than firocoxib, suggesting stronger clot formation. However, values for both parameters remained within reference intervals and the magnitude of difference was unlikely to be clinically significant.

Both PT and PTT were significantly increased in samples obtained pre- and post-treatment with flunixin meglumine. However, it is suspected that this was an artifact secondary to prolonged storage of citrated plasma from the flunixin meglumine treatment week, as no horses showed signs of coagulopathy during the study. Storage of citrated plasma at -20 °C for ≥ 6 months has been shown to artifactually increase both PT and PTT.²⁹ Due to limitations in the design of this crossover study, there was prolonged storage of the plasma from the flunixin meglumine treatment period, likely resulting in increased PT and PTT results.

While there was a statistically significant increase in fibrinogen following treatment with flunixin meglumine, the magnitude of this change was not clinically significant. A single individual was above the upper end of the reference range (fibrinogen, 281 mg/dL; normal, 125 to 262 mg/dL); all other individuals were within normal limits.

Horses in this study were administered omeprazole prophylactically to mitigate the risk of gastric ulceration with NSAID administration. An association between omeprazole and drug-induced coagulopathy in human patients has recently been identified in postmarketing data.³⁰ However, a causative relationship has not been established. To the authors' knowledge, omeprazole-associated coagulopathy has not been reported in horses, and it is unlikely that the effect of flunixin meglumine and firocoxib on coagulation would have differed in the absence of omeprazole.

The findings reported here are limited to systemically healthy horses, as the relationship between COX inhibition and coagulation homeostasis may differ in horses with ongoing inflammation or predisposed to coagulopathy. NSAID administration was limited to short term (5 days), and it is possible that prolonged treatment would have further impact on coagulation homeostasis. Additional investigation in

a large population including clinically ill horses and horses on long-term firocoxib should be considered. Due to drug availability, flunixin meglumine was administered IV while firocoxib was administered PO, which may have confounded study outcomes.

In conclusion, short-term administration of flunixin meglumine and firocoxib to healthy adult horses did not result in significant alteration of viscoelastic coagulation profiles. However, clinicians should be aware of coagulopathy-associated adverse effects of COX-2-selective NSAIDs in other species, and further study is warranted.

Acknowledgments

Financial support for this study was from the Cooperative State Research Service, USDA, under Project No. 1433 and University of Illinois Companion Animal Memorial Fund, Dr. Robert G. Kern Endowment for Veterinary Medicine. The authors thank Entegriion Inc for providing the VCM Vet devices and cartridges used in the study. Funding sources did not have any involvement in the study design, data analysis and interpretation, or writing and publication of the manuscript.

The authors declare that there were no conflicts of interest.

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