Comparison of the Effects of Ketoprofen and Flunixin Meglumine on the *in vitro* Response of Equine Peripheral Blood Monocytes to Bacterial Endotoxin

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ABSTRACT

The purpose of this study was to investigate the *in vitro* effects of flunixin meglumine, a cyclo-oxygenase inhibitor, and ketoprofen, a reported cyclo-oxygenase and lipoxygenase inhibitor, on the synthesis of cyclo-oxygenase end-products thromboxane B_2 and prostaglandin E_2, lipoxygenase derived 12-hydroxyeicosatetraenoic acid, tumor necrosis factor and tissue factor. Six adult horses were each randomly administered flunixin meglumine (1.1 mg/kg) or ketoprofen (2.2 mg/kg) intravenously every 12 hours with the drug treatments separated by two weeks. Blood samples were obtained prior to initiating treatment, the last day of treatment and for two consecutive days after the termination of treatment for measurement of serum concentrations of thromboxane B_2 as well as isolation of peripheral blood monocytes. Quantitation of unstimulated, endotoxin- and calcium inophore-induced synthesis of thromboxane B_2, prostaglandin E_2, 12-hydroxyeicosatetraenoic acid, tumor necrosis factor and tissue factor by peripheral blood monocytes was performed *in vitro*. Both flunixin meglumine and ketoprofen significantly decreased serum concentrations of thromboxane B_2 demonstrating *in vivo* cyclo-oxygenase inhibition. There were no significant differences between drug treatment groups in the *in vitro* production of thromboxane B_2, prostaglandin E_2, 12-hydroxyeicosatetraenoic acid, tumor necrosis factor or tissue factor. This study does not identify significant differences between the effects of flunixin meglumine and ketoprofen.

RÉSUMÉ

Le but de cette étude était d'évaluer les effets *in vitro* de la méglumine de flunixine, un inhibiteur de la cyclo-oxygénase, et du kétoprophène, un inhibiteur de la cyclo-oxygénase et de la lipo-oxygénase, sur la synthèse de deux métabolites de la cyclo-oxygénase, la thromboxane B_2 et la prostaglandine E_2, sur l'acide 12-hydroxyéicosatétraénoique, une substance dérivée de la lipo-oxygénase, sur le facteur nécrosant tumoral (FNT) et le facteur tissulaire. On a administré à six chevaux, alternativement, de la méglumine de flunixine (1.1 mg/kg IV Q 12 heures) ou du kétoprophène (2.2 mg/kg IV BID Q 12 heures). L'ordre de traitement pour chaque cheval a été décidé d'une façon aléatoire et une période de deux semaines a séparé l'administration des deux médicaments. Des échantillons sanguins ont été obtenus avant le début du traitement, la dernière journée du traitement, et les deux jours suivant la fin du traitement afin de mesurer la concentration sérique de thromboxane B_2 et d'isoler les monocytes sanguins. L'évaluation de la synthèse de thromboxane B_2, de prostaglandine E_2, d'acide 12-hydroxyéicosatétraénoique, du TNF et du facteur tissulaire a été mesurée *in vitro* à partir de monocytes sanguins non-stimulés ou exposés à de l'endotoxine ou à un ionophore calcique. La méglumine de flunixine et le kétoprophène ont diminué d'une façon significative la concentration sérique de thromboxane B_2, démontrant l'inhibition in vivo de la cyclo-oxygénase. Aucune différence significative de production *in vitro* de prostaglandie E_2, d'acide 2-hydroxyéicosatétraénoique, du TNF et du facteur tissulaire n'a été démontrée entre les deux traitements. Cette étude n'a pas identifié de différence significative entre les effets de la méglumine de flunixine et le kétoprophène. (*Traduit par Dr Jean-Pierre Lavoie*)

INTRODUCTION

Endotoxemia is an important cause of patient morbidity and mortality in horses. Gastrointestinal disorders that cause colic and neonatal septicemia are the most important precipitating disease processes. Endotoxin induces synthesis of inflammatory mediators and initiation of intravascular coagulation (1). Previous *in vivo* studies have shown that many of the mediators that account for the adverse effects of endotoxemia are cyclo-oxygenase-derived metabolites of arachidonic acid (2,3). Consequently, over the past ten years nonsteroidal anti-inflammatory drugs, such as flunixin meglumine, have become a mainstay in the treatment of equine colic and endotoxemia in clinical practice (4,5).

Recently, additional proinflammatory and procoagulant mediators, principally tissue factor and tumor necrosis factor, have been identified in endo-

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toxemic horses (6–10). These mediators are synthesized by cells of the mononuclear phagocyte system, and their synthesis may be altered by inhibition of cyclo-oxygenase activity. For instance, aspirin augments the generation of monocyte tissue factor activity by directing arachidonic acid metabolism away from cyclo-oxygenase and towards lipoxygenase (11). The subsequent increased production of at least one lipoxygenase product, 12-hydroxyeicosatetraenoic acid, augments the expression of tissue factor (11). Furthermore, the results of recent studies also have demonstrated that a cyclo-oxygenase product, prostaglandin E_2, down regulates the synthesis of tumor necrosis factor in response to stimulation with endotoxin (12). Thus, cyclo-oxygenase inhibitors, such as ibuprofen, result in increased synthesis of tumor necrosis factor (13).

With these recent findings in mind, we hypothesized that repeated administration of flunixin meglumine to horses would inhibit endotoxin-induced metabolism of arachidonic acid by cyclo-oxygenase and that such inhibition would result in 1) decreased production of thromboxane B_2 and prostaglandin E_2, 2) increased synthesis of tumor necrosis factor, 3) redirection of arachidonic acid towards lipoxygenase, 4) increased synthesis of 12-hydroxyeicosatetraenoic acid, and 5) increased production of tissue factor. Additionally, we proposed that administration of ketoprofen, a purported inhibitor of both cyclo-oxygenase and lipoxygenase (14,15), would 1) reduce the synthesis of thromboxane B_2, prostaglandin E_2, and 12-hydroxyeicosatetraenoic acid, 2) have no effect on tissue factor synthesis, and 3) increase synthesis of tumor necrosis factor.

MATERIALS AND METHODS

ANIMALS

Six healthy adult horses, housed in individual box stalls and fed grass hay, were used in this study. A crossover design was utilized to randomize the drug treatments in each horse. Two weeks elapsed between the two drug trials.

TREATMENT PROTOCOL

Jugular blood samples (100 mL) were obtained at 8:00 AM on day 1 before initiation of treatment. Either flunixin meglumine (1.1 mg/kg) (Banamine, Schering Plough Corporation, Kenilworth, New Jersey) or ketoprofen (2.2 mg/kg) (Ketofen, Fort Dodge Laboratories, Fort Dodge, Iowa) then was given IV every 12 hours for five consecutive days. The drugs were administered at manufacturer recommended dosages at intervals commonly utilized in equine practice. Subsequent blood samples were obtained at 8:00 AM on day 5 (12 hours before the last treatment), and at 8:00 AM on the two consecutive days after the termination of treatment (Days 6 and 7).

Two methods were used to test the effects of the drugs. Serum concentrations of thromboxane B_2, the stable metabolite of thromboxane A_2, increase markedly during the coagulation process. The administation of nonsteroidal anti-inflammatory drugs decreases the serum concentration of thromboxane B_2 through inhibition of either cyclo-oxygenase or thromboxane synthase. Therefore, we measured serum concentrations of thromboxane B_2 before and after administration of flunixin meglumine and ketoprofen.

The second method used to test the effects of the drugs involved quantitation of the constitutive (unstimulated) and endotoxin- and calcium ionophore (A23187)-induced synthesis of thromboxane B_2, prostaglandin E_2, 12-hydroxyeicosatetraenoic acid, tumor necrosis factor, and tissue factor by peripheral blood monocytes *in vitro*. Because the constitutive synthesis of these mediators *in vivo* is low, it is not valid to test the effects of these drugs without a stimulus. This *in vitro* method was selected to avoid the *in vivo* administration of endotoxin to the horses, and because the results of several recent studies indicate that cells of the mononuclear phagocyte system are pivotal in the body's reaction to endotoxin (9,10,16). A23187 was selected because it stimulates the cell by increasing intracellular calcium concentration through mechanisms distinct from the mechanisms involved during endotoxin stimulation.

SERUM THROMBOXANE B_2 CONCENTRATIONS

Generation of thromboxane B_2 by blood cells was stimulated by incubating whole blood in glass tubes at 37°C for 60 minutes. Serum was harvested and the concentration of immunoreactive thromboxane B_2 quantitated by radioimmunoassay (3).

ISOLATION OF PERIPHERAL BLOOD MONOCYTES

Blood was collected into 3.8% sodium citrate (1 part sodium citrate: 9 parts blood). Leukocyte-rich plasma was layered over 60% Percoll in Roswell Park Memorial Institute (RPMI)-1640 media (Whitaker MA Bioproducts, Walkersville, Maryland) and centrifuged to yield a mononuclear cell band. The mononuclear cells, suspended in 1 mL RPMI-1640, were incubated in sterile polystyrene tubes at 37°C in 5% CO_2 for two hours to permit adherence of monocytes. Nonadherent cells were removed by three washes with phosphate buffered saline. The adherent cell population consisted of >85% monocytes as determined by nonspecific esterase stain.

STIMULATION OF MONOCYTES

Monocytes were covered with 1 mL of RPMI-1640 media containing either nothing (no stimulus), *Escherichia coli* O55:B5 endotoxin (1 μg/mL) (List Biological Laboratory, Campbell, California), or the calcium ionophore, A23187 (5 μg/mL) (Sigma Chemical Company, St. Louis, Missouri). All tubes were incubated at 37°C, 5% CO_2 in air for six hours. At the end of the six hours, the supernatant from each tube was harvested and stored frozen at −70°C until assayed for immunoreactive thromboxane B_2, prostaglandin E_2, and 12-hydroxyeicosatetraenoic acid. Samples for quantitation of 12-hydroxyeicosatetraenoic acid were collected on day 1 (before treatment) and on day 5.

TISSUE FACTOR ACTIVITY

Following the six hours of incubation, monocytes were washed with phosphate buffered saline, overlaid with 500 μL of phosphate buffered saline, and then frozen at −70°C until assayed for tissue factor activity. Adherent cells were thawed, sonicated, and assayed for their ability to accelerate clotting times of pooled citrated equine plasma (9,10). Briefly, 0.1 mL of cell lysate was incubated for three minutes at 37°C with 0.1 mL citrated plasma. Coagulation was initiated upon the addition of 0.1 mL

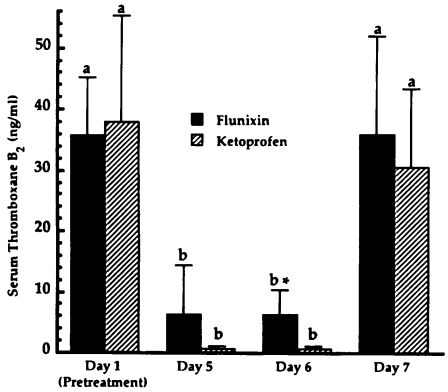

Fig. 1. Serum thromboxane B_2 concentrations before, during and after administration of flunixin meglumine or ketoprofen to six horses. Results are mean ± SD. Means within the same treatment groups with different letters are significantly different ($p < 0.05$). *Significantly different from the day 6 value for the ketoprofen treated horses.

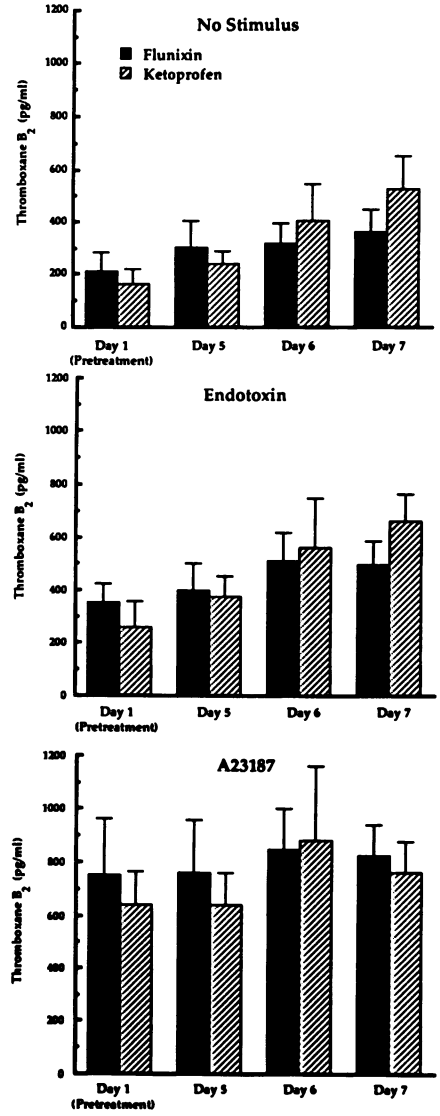

Fig. 2. Concentrations of thromboxane B_2 in the supernatants of peripheral blood monocytes exposed either to no stimulus, endotoxin or A23187. Results are mean ± SD. Cells from both flunixin meglumine and ketoprofen groups exposed to endotoxin or A23187 produced significantly more thromboxane B_2 than cells exposed to no stimulus ($p < 0.05$).

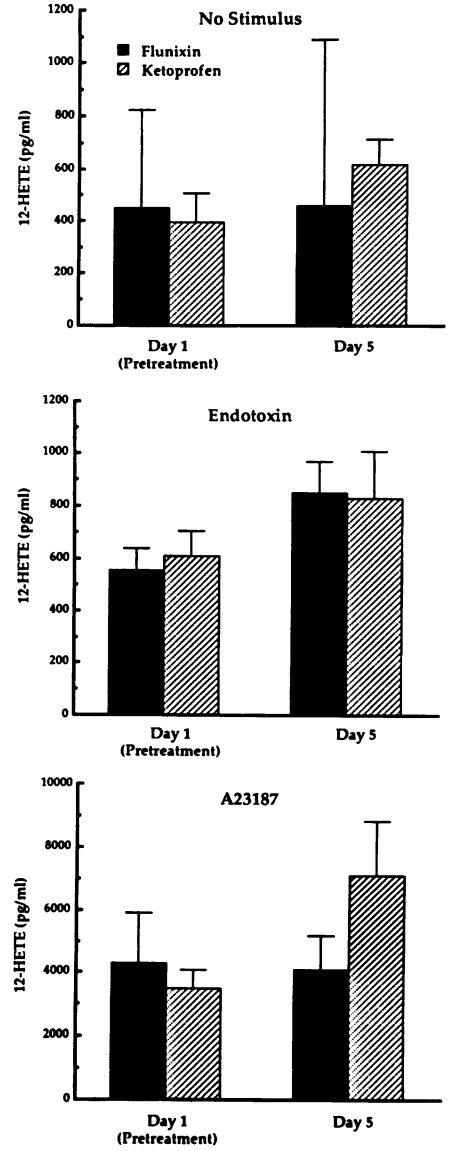

Fig. 3. Concentrations of 12-hydroxyeicosatetraenoic acid (12-HETE) in the supernatants of peripheral blood monocytes exposed either to no stimulus, endotoxin or A23187. Results are mean ± SD. Cells exposed to A23187 synthesized significantly more 12-HETE that either the unstimulated cells or the cells exposed to endotoxin ($p < 0.05$).

25 mM $CaCl_2$, and the clotting time was determined photometrically (Organon Teknika, Tessup, Maryland) in duplicate. The clotting times were compared to a standard curve using known dilutions of equine brain thromboplastin (9). Tissue factor activity was expressed as % thromboplastin × 100.

TUMOR NECROSIS FACTOR ACTIVITY

Tumor necrosis factor (TNF) activity was quantitated by a modified bioassay using the murine fibrosarcoma cell line WEHI 164 clone 13 as previously described (16). Briefly, WEHI 164 clone 13 cells were cultured in RPMI-1640 media supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, Utah). Cells in the log phase of growth were removed from flasks with trypsin-EDTA, then plated (7×10^4 cells per well) in 96-well microtiter plates. Dilutions of recombinant human TNF (Genzyme, Boston, Massachusetts) or monocyte supernatant were then added to the plates in triplicate. The cells were then incubated for 18 hours at 37°C and 5% CO_2 in air. Cell survival was estimated colorimetrically by addition of tetrazolium salt (MTT) (M2128, Sigma Chemical Company) in phosphate buffered saline. After cell incubation with MTT for four hours, 150 µL of solution was aspirated from each well and replaced by 100 µL of isopropanol containing 0.04 N HCl. Plates were read spectrophotometrically at 570 nm using an automated microplate reader. Results are reported as pg/mL TNF, using human recombinant TNF to construct a standard curve.

ANALYSIS OF DATA

All data were analyzed with Hartley's test for equal variance and then compared by a one-factor analysis of variance (ANOVA). Once it was determined that there were no significant differences between day 1 (pretreatment) values for the two trials (flunixin meglumine or ketoprofen), these data were grouped. Data subsequently were analyzed using ANOVA and means of interest compared with Scheffe's test. Significance was set at $p < 0.05$.

RESULTS

Serum concentrations of thromboxane B_2 after administration of flunixin meglumine or ketoprofen are depicted in Fig. 1. Both compounds significantly decreased the synthesis of thromboxane B_2 by whole blood cells on days 5 and 6. On day 6 there was

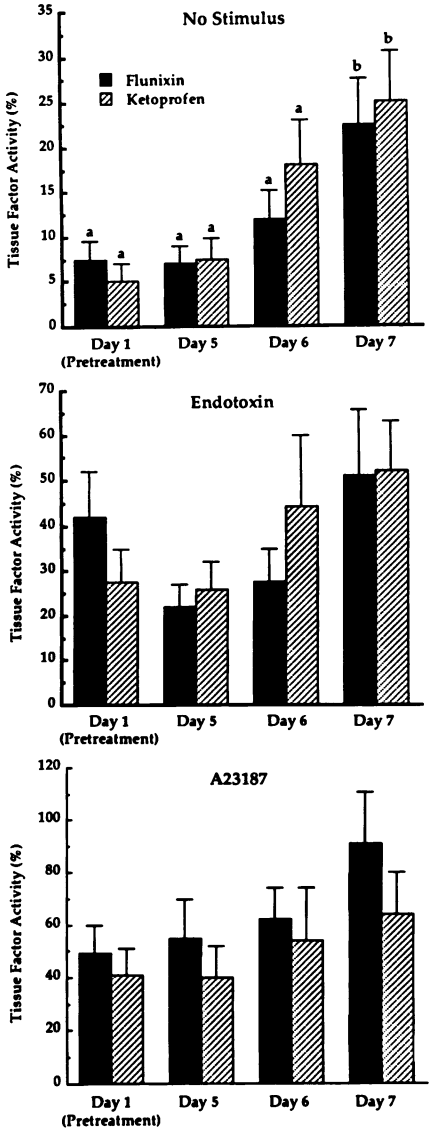

Fig. 4. Expression of tissue factor activity by lysates of peripheral blood monocytes exposed either to no stimulus, endotoxin or A23187. Results are mean ± SD. Means within the same treatment groups with different letters are significantly different ($p < 0.05$). Endotoxin and A23187 caused a significant increase in the tissue factor activity of monocytes from both drug treatment groups, as compared to cells exposed to no stimulus.

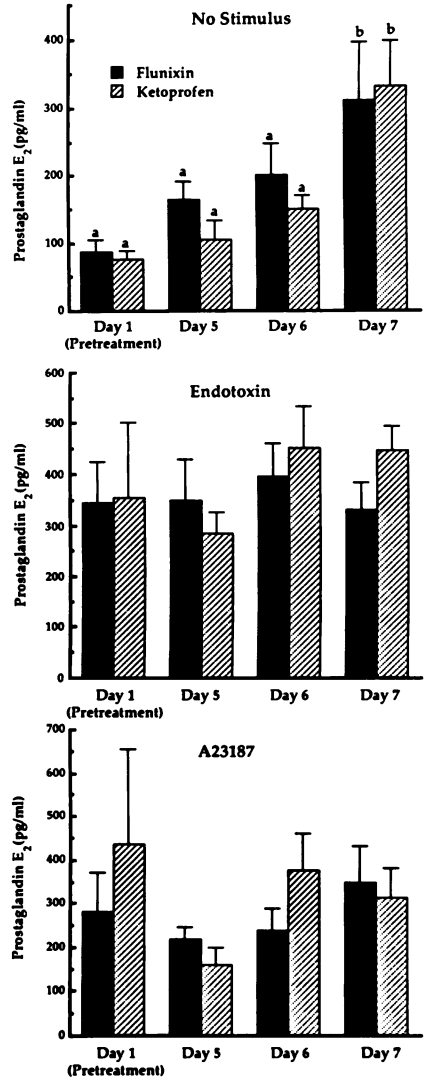

Fig. 5. Concentrations of prostaglandin E_2 in the supernatants of peripheral blood monocytes exposed either to no stimulus, endotoxin or A23187. Results are mean ± SD. Means within the same treatment groups with different letters are significantly different ($p < 0.05$). Except on day 7, concentrations of prostaglandin E_2 in supernatants of cells exposed to endotoxin or A23187 were greater than values for the unstimulated cells.

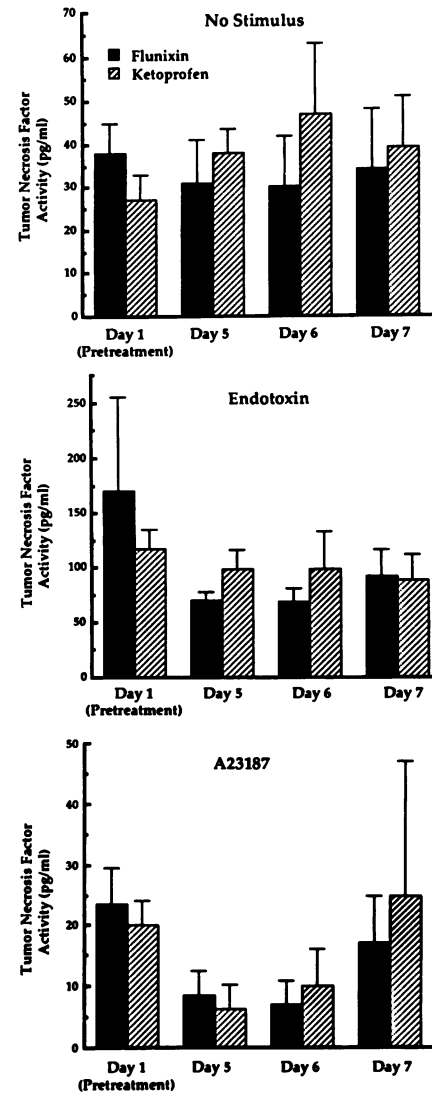

Fig. 6. Concentrations of tumor necrosis factor activity in the supernatants of peripheral blood monocytes exposed either to no stimulus, endotoxin or A23187. Results are mean ± SD. Supernatants of monocytes exposed to endotoxin contained significantly more TNF activity than those from the no stimulus or A23187 groups ($p < 0.05$).

significantly less thromboxane B_2 in the serum from horses administered ketoprofen than horses administered flunixin meglumine.

Concentrations of thromboxane B_2 produced by isolated peripheral blood monocytes are depicted in Fig. 2. Cells from both drug treatment groups exposed to endotoxin or A23187 produced significantly more thromboxane B_2 than did the unstimulated cells. Although there was no significant effect of either flunixin meglumine or ketoprofen, there was a trend towards increasing concentrations of thromboxane B_2 in the supernatant of cells isolated after termination of treatment (days 6 and 7). This was most evident for the unstimulated cells obtained on day 7 from the horses administered ketoprofen ($p = 0.07$).

Figure 3 depicts the concentrations of 12-hydroxyeicosatetraenoic acid in supernatants of monocytes exposed to no stimulus, endotoxin or A23187. All cells exposed to A23187 synthesized significantly more 12-hydroxyeicosatetraenoic acid than either the unstimulated cells or the cells exposed to endotoxin. Although there was no significant effect of either drug on the synthesis of 12-hydroxyeicosatetraenoic acid, there was a trend towards an increaes by both endotoxin- and A23187-stimulated cells isolated on day 5 from horses administered ketoprofen ($p = 0.08$).

Expression of tissue factor activity by isolated monocytes is depicted in Fig. 4. Endotoxin and A23187 caused a significant increase in the tissue factor activity of monocytes from both

drug treatment groups as compared to the unstimulated cells. The tissue factor activity of unstimulated cells from both groups was increased on day 6 and was significantly different from day 1 values on day 7. There was no effect of either drug on endotoxin- or A23187-induced tissue factor activity.

Concentrations of prostaglandin E_2 in monocyte supernatants are presented in Fig. 5. Except on day 7, concentrations of prostaglandin E_2 in supernatants of cells exposed to endotoxin or A23187 were greater than values for the unstimulated cells. The synthesis of prostaglandin E_2 by unstimulated cells from both treatment groups was increased on day 5 and was significantly different from day 1 values on day 7. There was no effect of either drug on endotoxin- or A23187-induced synthesis of prostaglandin E_2.

Figure 6 depicts TNF activity in the supernatants of monocytes exposed to no stimulus, endotoxin or A23187. Supernatants of monocytes exposed to endotoxin contained significantly more TNF activity than cells exposed to no stimulus or A23187. There was no significant effect of either drug on the synthesis of tumor necrosis factor activity.

DISCUSSION

The results of this study confirm that both flunixin meglumine and ketoprofen have activity against cyclo-oxygenase activity *in vivo*. This was evidenced by the significant decrease in serum thromboxane B_2 concentrations at the end of the treatment period. This effect has been demonstrated previously for flunixin meglumine (17) and was used to confirm that both drugs were pharmacologically active when administered twice daily. Inhibition of serum thromboxane B_2 synthesis was greatest for ketoprofen, indicating more profound inhibition of cyclo-oxygenase activity.

In contrast, neither the endotoxin- nor the A23187-stimulated synthesis of thromboxane B_2 by isolated monocytes appeared to be affected by the administration of either drug. This was an unexpected finding in light of the profound effect of both drugs on serum thromboxane B_2 concentrations. It is feasible that the methods used to isolate monocytes (density gradient centrifugation followed by suspension in cell culture media) allowed the drugs to be displaced from cyclo-oxygenase thereby permitting the isolated cells to respond to the stimuli. Alternatively, the concentrations of endotoxin and A23187 used in this study may have been sufficient to overshadow a moderate decrease in cyclo-oxygenase activity.

The synthesis of thromboxane B_2 by monocytes isolated from the horses after termination of treatment appeared to be different from their baseline values. This was especially evident for the unstimulated cells isolated from the horses administered ketoprofen and less so for the cells from these horses exposed to endotoxin (Fig. 2). It is possible that cyclo-oxygenase activity may be enhanced once inhibition has been removed and that this enhancement is only evident when the cells are mildly or moderately stimulated by the isolation procedure alone (unstimulated cells) or exposed to endotoxin. This effect was not evident when the cells were maximally stimulated with A23187.

Incubation of monocytes with endotoxin caused an increase in supernatant TNF activity. There was, however, no significant effect of either flunixin meglumine or ketoprofen on this response. Although these findings were contrary to our hypothesis, they were consistent with those of an *in vivo* study in which people were administered endotoxin after being treated with ibuprofen (13). The results of a recent report indicate that administration of aspirin decreased the synthesis of tumor necrosis factor activity by activated peritoneal macrophages (18). These disparate findings suggest that the control of TNF synthesis may be dependent upon the species and activation status of the mononuclear cells.

In addition to being metabolized by cyclo-oxygenase to the prostaglandins, thromboxanes, and prostacyclin, arachidonic acid also serves as the substrate for generation of hydroxyeicosatetraenoic acids and leukotrienes by the lipoxygenase enzymes (19). There is ample evidence that certain products of 5-lipoxygenase activity, leukotrienes A_4, B_4, C_4, D_4 and E_4, are involved in the pathogenesis of adult respiratory distress syndrome, inflammatory bowel disease, asthma, and several other allergic syndromes in people (20). Furthermore, there is convincing evidence to implicate these products as important mediators of endotoxic shock (21). In the present study we elected to quantitate 12-hydroxyeicosatetraenoic acid, a product of 12-lipoxygenase activity. Stimulation of equine peripheral blood monocytes with the calcium ionophore increased the concentration of 12-hydroxyeicosatetraenoic acid in the cell supernatants six to sevenfold over that of unstimulated monocytes. There was no significant effect of either drug on the synthesis of 12-hydroxyeicosatetraenoic acid by the peripheral blood monocytes, though there was a trend towards an increase by endotoxin- and calcium ionophore-stimulated cells isolated from horses administered ketoprofen (Fig. 3). Because ketoprofen may selectively inhibit 5-lipoxygenase and cyclo-oxygenase activities, this finding may reflect a redirection of arachidonic acid metabolism to 12-lipoxygenase. Ketoprofen has been shown to be less toxic than either flunixin meglumine or phenylbutazone in the equine species (22). A possible explanation for this reduction in toxicity may be related to the ability of ketoprofen to partially inhibit the lipoxygenase pathway and production of leukotrienes. Further studies are necessary to determine the effect of ketoprofen on the equine arachidonic acid cascade.

There were significant increases in the concentration of PGE_2 in the cell supernatants and enhanced expression of tissue factor activity by the lysates of the unstimulated cells isolated two days following termination of treatment. This effect was evident for cells from horses treated with either drug. Previous studies in our laboratory have failed to identify this magnitude of change in tissue factor activity expressed by monocytes isolated from nontreated healthy horses on successive days (10). The association between increasing concentrations of PGE_2 and 12-hydroxyeicosatetraenoic acid and enhanced expression of tissue factor activity has been identified previously in other species (11,23). Taken together, these results suggest

that the repeated administration of nonsteroidal anti-inflammatory drugs may enhance the constitutive thrombogenic potential of equine monocytes. The results of the present study do not identify significant differences between the effects of flunixin meglumine and ketoprofen.

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REFERENCES

1. **JOHNSTONE IB.** The prognostic value of hemostatic testing in horses with colic. In Proceedings of The Second Equine Colic Research Symposium, 1985: 186–188.
2. **BOTTOMS GD, FESSLER JF, ROESEL OF.** Endotoxin-induced hemodynamic changes in ponies: Effects of flunixin meglumine. Am J Vet Res 1981; 42: 1514–1518.
3. **MOORE JN, GARNER HE, SHAPLAND JE, HATFIELD DG.** Prevention of endotoxin-induced arterial hypoxemia and lactic acidosis with flunixin meglumine in the conscious pony. Equine Vet J 1981; 13: 95–98.
4. **MOORE JN, HARDEE MM, HARDEE GE.** Modulation of arachidonic acid metabolism in endotoxic horses: Comparison of flunixin meglumine, phenylbutazone and a selective thromboxane synthetase inhibitor. Am J Vet Res 1986; 47: 110–113.
5. **SEMRAD SD, HARDEE GE, HARDEE MM, MOORE JN.** Low dose flunixin meglumine: Effects on prostaglandin production and clinical signs induced by experimental endotoxemia. Equine Vet J 1987; 19: 201–206.
6. **EDWARDS RL, RICKLES FR.** Macrophage procoagulants. Prog Hemost Thromb 1984; 7: 183–209.
7. **HETLAND O, BOROLD AB, HOLME R.** Thromboplastin (tissue factor) in plasma membranes of human monocytes. Biochem J 1985; 228: 735–743.
8. **LYBERG T.** Clinical significance of increased thromboplastin activity on the monocyte surface: A brief review. Haemostasis 1988; 71: 1–8.
9. **HENRY MM, MOORE JN.** Endotoxin-induced procoagulant activity in equine peripheral blood monocytes. Circ Shock 1988; 26: 297–310.
10. **HENRY MM, MOORE JN.** Clinical relevance of monocyte procoagulant activity in horses with colic. J Am Vet Med Assoc 1991; 198: 843–848.
11. **LORENZET R, NIEMETZ J, MARCUS AJ.** Enhancement of mononuclear procoagulant activity by platelet 12-hydroxyeicosatetraenoic acid. J. Clin Invest 1986; 78: 418–423.
12. **KUNKEL SL, SPENGLER M, MAY MA, SPENGLER R, LARRICK J, REMICK D.** Prostaglandin E_2 regulates macrophage-derived tumor necrosis factor gene expression. J Biol Chem 1988; 263: 5380–5384.
13. **MICHIE HR, MANOGUE KR, SPRIGGS DR, REVHAUG A, O'DWYER S, DINARELLO CA, CERAMI A, WOLFF SM, WILMORE DW.** Detection of circulating tumor necrosis factor after endotoxin administration. N Engl J Med 1988; 318: 1481–1486.
14. **WALKER JL.** Interrelationships of SRS-A production and arachidonic acid metabolism in human lung tissue. Adv Prostaglandin Thromboxane Leukot Res 1980; 6: 115–120.
15. **DAWSON W, BOOT JR, HARVEY J, WALKER JR.** The pharmacology of benoxaprofen with particular reference to effects on lipoxygenase products formation. Eur J Rheumatol Inflamm 1982; 5: 61–68.
16. **MORRIS DD, MOORE JN, FISCHER K, TARLETON RL.** Endotoxin-induced tumor necrosis factor activity production by equine peritoneal macrophages. Circ Shock 1990; 30: 229–236.
17. **SEMRAD SD, HARDEE GE, HARDEE MM, MOORE JN.** Flunixin meglumine given in small doses: Pharmacokinetics and prostaglandin inhibition in healthy horses. Am J Vet Res 1985; 46: 2474–2479.
18. **MARCINKIEWICZ J.** In vitro cytokine release by activated murine peritoneal macrophages: Role of prostaglandins in the differential regulation of tumor necrosis factor alpha, interleukin 1, and interleukin 6. Cytokine 1991; 3: 327–332.
19. **LEWIS RA, AUSTEN KF.** The biologically active leukotrienes: Biosynthesis, metabolism, receptors, functions, and pharmacology. J Clin Invest 1984; 73: 889–897.
20. **LEWIS RA, AUSTEN KF, SOBERMAN RJ.** Leukotrienes and other products of the 5-lipoxygenase pathway. N Engl J Med 1990; 323: 645–655.
21. **LI EJ, COOK JA, WISE WC, JACKSON WT, HALUSHKA PV.** Effect of LTB_4 receptor antagonists in endotoxic shock in the rat. Circ Shock 1991; 34: 385–392.
22. **MacALLISTER CG, MORGAN SJ, BORNE AT, POLLET RA.** Comparison of adverse effects of phenylbutazone, flunixin meglumine, and ketoprofen in horses. J Am Vet Med Assoc 1993; 202: 71–77.
23. **SITRIN R, KALTREIDER H, GOLDYNE M.** Prostaglandin E is required for the augmentation of procoagulant activity of LPS-stimulated rabbit alveolar macrophages. J Immunol 1984; 132: 867–871.